



**QUALITY CONTROL PROCEDURES**

**I INTRODUCTION**

Selective Streptococcus Agar is designed for the isolation of group A streptococci from respiratory sources.

**II PERFORMANCE TEST PROCEDURE**

1. Inoculate representative samples with broth cultures diluted to contain 10<sup>3</sup>–10<sup>4</sup> CFU/0.01 mL.
  - a. To each plate, add 0.01 mL of the dilution and streak for isolation.
  - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
  - c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates and Chocolate II Agar plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for amount of growth, inhibition, colony size and hemolytic reactions.
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Streptococcus pyogenes</i>	19615	Fair to heavy growth of pinpoint to very small colonies surrounded by zones of β-hemolysis.
* <i>Streptococcus pneumoniae</i>	6305	Fair to heavy growth; colonies surrounded by zones of α-hemolysis.
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)
<i>Neisseria subflava</i>	14799	Inhibition (partial to complete)

\*Recommended organism strain for User Quality Control.

**NOTE:** This medium is exempt from User QC testing according to CLSI M22-A3.

**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 7.3 ± 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**

Selective Streptococcus Agar is designed for the isolation of group A streptococci from respiratory sources.

**V SUMMARY AND EXPLANATION**

Roantree et al.<sup>1</sup> introduced a medium for isolation of group A beta-hemolytic streptococci. The medium enriched with yeast nucleic acid and maltose promoted increased colony size and enhanced clarity and sharpness of hemolytic zones produced by these organisms.<sup>2,3</sup>

**VI PRINCIPLES OF THE PROCEDURE**

Selective Streptococcus Agar is prepared from beef extract and casein peptone, which are relatively free of dextrose, permitting the addition of animal blood to detect hemolytic activity. The incorporation of the antimicrobial agents, neomycin and polymyxin B, provides suppression of normal throat flora for improved recovery of *Streptococcus pyogenes*.

**VII REAGENTS**

**Selective Streptococcus Agar**

Approximate Formula\* Per Liter Purified Water

Pancreatic Digest of Casein .....	10.0	g
Beef Extract .....	6.7	g
Sodium Chloride .....	5.0	g
Maltose .....	0.25	g
Agar .....	15.0	g
Nucleic Acid .....	6.0	g
Neomycin Sulfate .....	0.002	g
Polymyxin B Sulfate .....	200,000	units
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 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>4-7</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

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.<sup>8,9</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:** Selective Streptococcus Agar

**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, firm and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface, and streak the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs may be streaked onto the medium with a sterilized inoculating loop. Without resterilizing the loop, stab the agar two or three times in the area of heaviest inoculation. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Incubate the plates in an inverted position (agar side up) at 35–37°C for 18–48 h in a CO<sub>2</sub>-enriched atmosphere.

**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 18–48 h of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Group A streptococci (*S. pyogenes*) will appear as translucent or opaque, white to gray, small colonies surrounded by a zone of beta hemolysis. Most *Neisseria* species and gram-negative rods are inhibited.

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

## XI LIMITATIONS OF THE PROCEDURE

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>8-15</sup>

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

## XII PERFORMANCE CHARACTERISTICS

An in-house study was performed comparing Selective Streptococcus Agar to Group A Selective Strep Agar with 5% Sheep Blood (ssA™), Strep Selective II Agar and Trypticase Soy Agar with 5% Sheep Blood (TSA II) using 20 clinical isolates of group A *Streptococcus* with mixed flora.<sup>16</sup> For each of the 20 cultures, a cell suspension of mixed organisms equivalent to a 0.5 McFarland standard was prepared and a loopful of each suspension streaked onto each of the four media. The plates were incubated aerobically at 35 ± 2°C with CO<sub>2</sub> for 18–24 h after which they were read for recovery of group A *Streptococcus*. Two isolates of Group A *Streptococcus* were not recovered on any of the media. Of the remaining 18 isolates, 16 were equally recovered on all four media. Selective Streptococcus Agar failed to recover two isolates and TSA II failed to recover one isolate due to heavy growth of *Staphylococcus*. These isolates were recovered by the other two media.

A second in-house study was performed on the four media to determine differences in recovery of group A *Streptococcus* using the plate count method.<sup>16</sup> Ten pure cultures of Group A *Streptococcus* were diluted in sterile water to produce a final concentration of 100 CFU (colony-forming units)/plate. Each medium was inoculated by the spread plate method, incubated (as described above) and examined for colony count and colony size. Colony counts were comparable on all four media; however, colony size differed. Selective Streptococcus Agar, Strep Selective II Agar and TSA II media produced an average colony size of ~1.0 mm, whereas ssA medium produced an average colony size of ~0.5 mm.

## XIII AVAILABILITY

Cat. No.	Description
221934	BBL™ Selective Streptococcus Agar, Pkg. of 20 plates
221935	BBL™ Selective Streptococcus Agar, Ctn. of 100 plates

## XIV REFERENCES

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