Streptococcus Selective Broth

For the selective growth of streptococci from clinical samples

Formula in grams per liter:

Casein Peptone	15,00	Soy Peptone	5,00
Sodium Chloride	4,00	Sodium Citrate	1,00
L-Cystine	0,20	Sodium Sulfite	0,20
Dextrose	5,00	Sodium Azide	0,20
Crystal Violet	0,0002		

Final pH: 7,4 ± 0,2 at 25 °C

Preparation:

Suspend 30,6 grams of the medium in a litre of distilled water. Heat with frequent agitation and boil for one minute. Dispense in 10 ml. amounts into screw-capped tubes and sterilize in the autoclave at 118°C (12 lbs. sp.) for 15 minutes. **DO NOT OVERHEAT**, or the medium will become too inhibitory.

Uses:

Clinical material, obtained by a swab of the nasal passage or pharynx, is inoculated into this selective medium and the tubes are incubated at 35°C for 18-24 hours in a normal atmosphere. If one wants to streak Blood Agar and/or Streptococcus Selective Agar with 5% sheep or rabbit blood, incubate these plates in a 5-10% CO2 atmosphere. CDC (Center for Disease Control, Atlanta, GA.) does not recommend the use of candle jars to generate CO2. It is recommended to inoculate the Blood Agar plates by the pour plate method (in thick plates) or to inoculate the plates with a streak and make several stabs with the loop and incubate in a normal atmosphere.

Many organisms such as saprophytic Neisseria, Staphylococcus, Haemophilus, non-hemolytic streptococci, and a certain number of enterobacteria will not grow or only scarcely, in this medium. The growth of streptococci can be determined by the formation of a granular precipitate in the bottom of the tube, with the liquid above clean or slightly turbid. At this point, perform a Gram stain and restreak on Blood Agar to purify the strain.

It is convenient to place bacitracin and optochin discs in the area of heavy inoculum on the Blood Agar plate and incubate for 18-24 hours at 35°C under the recommended conditions. It is important to remember that the discs are used for differentiation of streptococci and pneumococci and are not to be confused with antibiotic sensitivity discs of higher concentration.

Subculture the organism growing in the zone of inhibition from 10-18 mm. in diameter around the bacitracin disc into 2,5 ml. of the Streptococcus Selective Broth and incubate under the normal conditions. Perform a Gram stain and observe for formation of coccal chains. Perform the catalase and bile solubility tests on characteristic colonies taken from the Blood Agar Plate or from the growth obtained from the broth.

The presence of variable length chains of Gram-positive cocci inhibited by bacitracin in low concentration, catalase negative and insoluble in bile or bile salts, constitute a valid presumptive identification of Group A beta-hemolytic streptococci. The definitive identification of the streptococcal groups can be made by performing other biochemical tests such as esculin hydrolysis, pyruvate hydrolysis, etc. Also, serological typing, using Lancefield antisera methods, or easier or more conveniently, the techniques of coagglutination of Edwards and Larson can be performed.

Microbiological Tests:

Microorganisms	Growth
Escherichia coli ATCC 25922	Inhibited
Streptococcus faecalis ATCC 19433	Satisfactory
Streptococcus faecium ATCC 27270	Satisfactory