SELECTIVE STREP AGAR, MODIFIED # 2 (7405)

Intended Use

Selective Strep Agar, Modified # 2 is used with blood for the selective isolation of group A streptococci.

Product Summary and Explanation

Group A streptococcal infections are the most common cause of bacterial pharyngitis in children 5 to 10 years old.[1] Selective Streptococcus Agar was developed by Roantree et al., for the isolation of group A beta-hemolytic streptococci. [2] Further improvement was made by adding yeast nucleic acid and maltose, promoting increased colony size and enhanced clarity of hemolytic zones produced by group A streptococci.[3,4] This modified formula also increased inhibition of *Staphylococcus* spp.

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Beef Extract. Maltose is added as an energy source, and along with Nucleic Acid promotes organism growth and enhanced hemolytic zone sizes produced by group A streptococci. Sodium Chloride maintains the osmotic balance of the medium. Neomycin Sulfate, Polymyxin B Sulfate, and other selective agents are used to inhibit *Staphylococcus* spp. Agar is the solidifying agent. In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β-hemolytic streptococci.[5] Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.[1]

Formula / Liter

Enzymatic Digest of Casein	10 g
Beef Extract	6.7 g
Maltose	0.25 g
Nucleic Acid	6 g
Sodium Chloride	5 g
Neomycin Sulfate	0.002 g
Polymyxin B Sulfate	0.024 g
Selective Agents	0.00289 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 43 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Do not overheat.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light beige.

Prepared Appearance: Prepared medium without blood is amber, and clear to trace hazy. With 5% sheep blood the medium is red and opaque.

Expected Cultural Response: Cultural response incubated aerobically at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Hemolysis
Escherichia coli ATCC® 25922	300 - 1000	Inhibited	---
Staphylococcus aureus ATCC® 25923	100 - 1000	Inhibited	---
Streptococcus pneumoniae ATCC® 6305	10 - 300	Good to excellent	Alpha hemolysis
Streptococcus pyogenes ATCC® 19615	10 - 300	Good to excellent	Beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.[1]
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

Results

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are three types of hemolysis on blood agar media described as:[6]

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and no change in the medium.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human, and rabbit blood agar and alpha-hemolytic on sheep blood agar.[1]
3. Atmosphere of incubation is known to influence hemolytic reactions of beta-hemolytic streptococci.[1] For optimal performance, incubate blood agar base media under increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

Packaging

Selective Strep Agar, Modified # 2	**Code No.**	**7405A**	**500 g**
		7405B	**2 kg**
		7405C	**10 kg**

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
2. **Roantree, R. J., L. A. Rantz, and E. J. Haines.** 1958. A medium containing nucleic acid, maltose, and antibiotics for the isolation of group A hemolytic streptococci. J. Lab. Clin. Med. **52**:496-500.
3. **Bernheimer, A. W., and M. Rodbart.** 1948. The effect of nucleci acids and of carbohydrates on the formation of streptolysin. J. Exper. Med. **88**:149-168.
4. **Schweiz Ztschr.** 1955. Allg. Path. **18**:278-287.
5. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
6. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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