SHEEP BLOOD AGAR

INTENDED USE

Sheep Blood Agar is used for the isolation, cultivation and detection of hemolytic activity of streptococci and other fastidious microorganisms.

SUMMARY AND EXPLANATION

In a study of viability of streptococci, Snavely and Brahier performed comparative studies of horse, rabbit and sheep blood with Blood Agar Base, and found that sheep blood gave the clearest and most reliable colony and hemolysis characteristics at both 24 and 48 hours.[1] In the course of the investigation, about 1,300 isolations of streptococci were made with Blood Agar Base containing 5% sheep blood.

PRINCIPLE

Peptone and yeast extract provide nitrogen, carbon, amino acids and vitamins in Blood Agar Base. Medium contains sodium chloride to maintain osmotic equilibrium and agar is the solidifying agent.

Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms, and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood.[2]

REAGENTS (FORMULA)

Proteose Peptone	15.0	g
Liver Digest	2.5	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g
Agar	12.0	g
Sheep Blood, Defibrinated	50.0	ml
Deionized Water	1000.0	ml

PROCEDURE

Use standard procedures to obtain isolated colonies from specimens. After streaking, stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.[2] Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2. Incubate plates at 35 ± 2°C for 18-24 hours.

EXPECTED RESULTS

Colonial morphology on blood agar containing 5% sheep blood is as follows:

1. Hemolytic streptococci may appear as translucent or opaque, grayish, small (1 mm), or large matte or mucoid (2-4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.) Approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.

2. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.

3. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.

4. *Listeria* may be distinguished by their rod shape in stains, and by motility at room temperature. Small zones of beta hemolysis are produced.

5. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Candida albicans ATCC 10231	35 ± 2°C for 18-24 hours	Growth, No Hemolysis
Staphylococcus aureus ATCC 25923	35 ± 2°C for 18-24 hours	Growth, Beta Hemolysis
Streptococcus pneumoniae ATCC 6305	35 ± 2°C for 18-24 hours	Growth, Alpha Hemolysis

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BIBLIOGRAPHY

1. Snavely and Brahier. 1960. Am. J. Clin. Pathol. 33:511.

2. Ruoff, Whiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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