BLOOD AGAR (TSA w/ 5% Sheep Blood)

INTENDED USE

Remel Blood Agar (TSA w/ 5% Sheep Blood) is a solid medium recommended for use in qualitative procedures for the cultivation of a wide variety of microorganisms and visualization of hemolytic reactions.

SUMMARY AND EXPLANATION

Tryptic Soy Agar is based on the soybean casein digest agar formula described in the U.S. Pharmacopeia.[1] Blood is incorporated in the medium to facilitate growth of more fastidious bacteria and to observe hemolytic reactions. The absence of carbohydrates and reducing sugars permits the demonstration of hemolysis which is an important differentiating characteristic for bacteria, especially *Streptococcus* species.

PRINCIPLE

Blood Agar (TSA w/ 5% Sheep Blood) contains casein and soy peptones which provide nitrogen, amino acids, and peptides necessary for bacterial growth. Sodium chloride supplies essential electrolytes and maintains osmotic equilibrium. Sheep blood enriches the medium by providing essential growth factors and allows hemolytic reactions to be demonstrated. Group B streptococci produce a protein-like compound called the CAMP factor which acts synergistically with beta toxin, produced by some strains of *Staphylococcus aureus*.[2] This reaction occurs when a streak of β-lysin-producing *S. aureus* is inoculated perpendicular to a streak of group B *Streptococcus* resulting in an area of complete lysis in the shape of an arrowhead or crescent. Blood Agar (TSA w/ 5% Sheep Blood) is suitable for performing the CAMP test.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	15.0 g	Sheep Blood	5 %
Sodium Chloride	5.0 g	Agar	15.0 g
Soy Peptone	5.0 g	Demineralized Water	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If material is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate plate aerobically, or in 5-10% CO_2, for 18-24 hours at 33-37°C.
4. Examine plate for typical colony morphology and hemolytic reactions.

QUALITY CONTROL

All lot numbers of Blood Agar (TSA w/ 5% Sheep Blood) have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[3] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Enterococcus faecalis ATCC® 29212	Ambient, 18-24 h @ 33-37°C	Growth, gamma hemolysis
**Escherichia coli* ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Growth
Listeria monocytogenes ATCC® 7646	Ambient, 18-24 h @ 33-37°C	Growth, beta hemolysis
Moraxella catarrhalis ATCC® 25238	CO_2, 18-24 h @ 33-37°C	Growth
**Staphylococcus aureus* ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Growth
Streptococcus agalactiae ATCC® 12386	Ambient, 18-24 h @ 33-37°C	Growth, beta hemolysis
**Streptococcus pneumoniae* ATCC® 6305	CO_2, 18-24 h @ 33-37°C	Growth, alpha hemolysis
**Streptococcus pyogenes* ATCC® 19615	Ambient, 18-24 h @ 33-37°C	Growth, beta hemolysis
CAMP Test**		
**Streptococcus agalactiae* ATCC® 12386	Ambient, 18-24 h @ 33-37°C	Arrowhead-shaped zone of increased beta hemolysis
**Streptococcus pyogenes* ATCC® 19615	Ambient, 18-24 h @ 33-37°C	No arrowhead formation

*CLSI recommended organism
**CAMP Test performed with *S. aureus* ATCC® 33862

BIBLIOGRAPHY

1. The United States Pharmacopeia. 2006. 24th ed., Sup. 2., 29th rev. United States Pharmacopeial Convention, Rockville, MD.
2. Christie, R., N.E. Atkins, and E. Munch-Peterson. 1944. Aust. J. Exp. Biol. 22:197-200.
3. Clinical and Laboratory Standards Institute (CLSI). 2004. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3rd ed. M22-A3. CLSI, Wayne, PA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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