Tryptose Blood Agar Base

Intended Use

Tryptose Blood Agar Base is used with blood in isolating, cultivating and determining the hemolytic reactions of fastidious microorganisms.

Summary and Explanation

Investigations of the nutritive properties of tryptose demonstrated that culture media prepared with this peptone were superior to the meat infusion peptone media previously used for the cultivation of *Brucella*, streptococci, pneumococci, meningococci and other fastidious bacteria. Casman[1,2] reported that a medium consisting of 2% tryptose, 0.3% beef extract, 0.5% NaCl, 1.5% agar and 0.03% dextrose equaled fresh beef infusion base with respect to growth of organisms. The small amount of carbohydrate was noted to interfere with hemolytic reactions, unless the medium was incubated in an atmosphere of carbon dioxide.

Tryptose Blood Agar Base is a nutritious infusion-free basal medium typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, this base can be used as a general-purpose medium. Tryptose Blood Agar Base is included in the FDA *Bacteriological Analytical Manual* (pH adjusted to 6.8 ± 0.2).[3]

Principles of the Procedure

Tryptose is the source of nitrogen, carbon and amino acids in Tryptose Blood Agar Base. Beef extract provides additional nitrogen. Sodium chloride maintains osmotic balance. Agar is the solidifying agent.

Supplementation with 5-10% blood provides additional growth factors for fastidious microorganisms and is used to determine hemolytic patterns of bacteria.

Formula

Difco™ Tryptose Blood Agar Base

Approximate Formula* Per Liter		
Tryptose	10.0	g
Beef Extract	3.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 33 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium cooled to 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable typical control cultures.

User Quality Control

Identity Specifications

Difco™ Tryptose Blood Agar Base

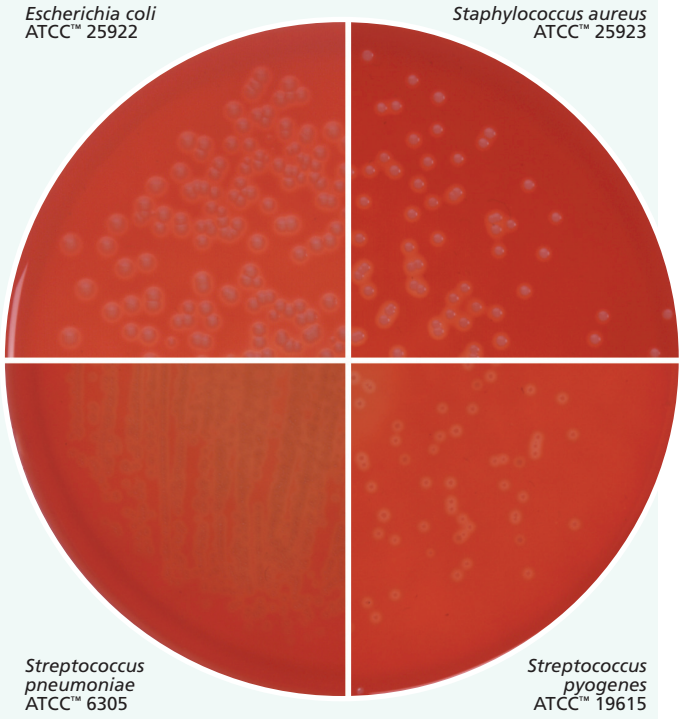
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Plain – Light amber, slightly opalescent. With 5% sheep blood – Cherry red, opaque.
Reaction of 3.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Tryptose Blood Agar Base

Prepare the medium per label directions without (plain) and with 5% sterile defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C for 18-48 hours (blood plates under 5-10% CO_2).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB	HEMOLYSIS 18-48 HR
Escherichia coli	25922	10^2-10^3	Good	Good	Beta
Neisseria meningitidis	13090	10^2-10^3	None to poor	Good	None
Staphylococcus aureus	25923	10^2-10^3	Good	Good	Beta
Streptococcus pneumoniae	6305	10^2-10^3	Fair to good	Good	Alpha
Streptococcus pyogenes	19615	10^2-10^3	Fair to good	Good	Beta



Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions of both oxygen-stable and oxygen-labile streptolysins.[4]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.
3. Examine plates for growth and hemolytic reactions after 18-24 and 48-hour incubation.

Expected Results

Four different types of hemolysis on blood agar media can be described:[5]

a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish discolorization of the medium.
b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.
d. Alpha-prime (ά)-hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Blood Agar Base Media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
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.[4]
3. Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar, and must be distinguished from colonies of beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[6]
4. The atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[3] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.
5. Hemolytic patterns may vary with the source of animal blood or type of base medium used.[4]

References

1. Casman. 1942. J. Bacteriol. *43*:33.
2. Casman. 1947. Am. J. Clin. Pathol. *17*:281.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
4. 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.
5. Isenberg. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.

Availability

Difco™ Tryptose Blood Agar Base

BAM

Cat. No.	223220	Dehydrated – 500 g
	223210	Dehydrated – 2 kg