Blood Agar Base,Modified

M1989

Blood Agar Base, Modified is recommended as a base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms.

Composition**

Ingredients	Gms / Litre
Tryptone	7.500
Meat peptone	2.500
Sodium chloride	8.000
L-Lysine	0.040
Monopotassium phosphate	0.250
Disodium phosphate	1.750
Sodium bisulphite	0.100
Agar	13.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.64 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Blood Agar Base, Modified is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. It can also be used as general-purpose media without the addition of blood.

Tryptone and Meat peptone provides carbon, nitrogen, amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Phosphates buffer the medium and Sodium bisulphite is a reducing agent.Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. It also helps in visualizing the haemolytic reactions. However, haemolytic reactions depend on the animal blood used. Sheep blood gives best results for Group A Streptococci (1). But sheep blood fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However when horse blood is used *H. haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* (2).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.35% Agar gel

Colour and Clarity of prepared medium
Basal medium : Light amber coloured clear to slightly opalescent gel. After addition of 5% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

Reaction
Reaction of 3.36% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added 5% w/v sterile defibrinated blood,after an incubation at 35-37°C for 48-72 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth w/o blood	Recovery w/o blood	Growth with blood	Recovery with blood	Haemolysis
Cultural Response						
Staphylococcus aureus ATCC 25923	50-100	good	50-70%	luxuriant	>=70%	beta
Streptococcus pneumoniae ATCC 6303	50-100	fair-good	40-50%	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	fair-good	40-50%	luxuriant	>=70%	beta
Staphylococcus aureus ATCC 6538	50-100	good	50-70%	luxuriant	>=70%	beta
Enterococcus faecalis ATCC 29212	50-100	good	50-70%	luxuriant	>=70%	gamma
Escherichia coli ATCC 8739	50-100	good	40-50%	luxuriant	>=70%	gamma

Storage and Shelf Life

Store below 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1.Snavely J. G. and Brahier J., 1960, Am. J. Clin. Pathol., 33:511.
2.Murray P. R., Baron J. H., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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