Blood Agar Base, Modified

M1989

Intended use

Recommended as a base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms.

Composition**

Ingredients	g / L
Tryptone	7.500
HM peptone #	2.500
Sodium chloride	8.000
L-Lysine	0.040
Potassium dihydrogen phosphate	0.250
Disodium hydrogen 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

Equivalent to Meat peptone

Directions

Suspend 33.64 grams in 1000 ml purified / 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 HM 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).

Type of specimen

Clinical material : faeces, pus, wound sample

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Addition of sheep blood is recommended to detect haemolysis. This medium does not support the growth of *H.haemolyticus.*

2.Addition of Horse blood or rabbit blood to base medium supports growth of *H.haemolyticus* but resemble beta-haemolytic Streptococci and hence must be confirmed.

3.Haemolytic pattern varies with the source of blood used.

4.Biochemical and serological tests must be performed for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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.

Organism	Inoculum (CFU)	Growth w/o blood	Recovery w/o blood	Growth with blood	Recovery with blood	Haemolysis
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	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 subsp. aureus ATCC 6538 (00032*)	50-100	good	50-70%	luxuriant	>=70%	beta
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good	50-70%	luxuriant	>=70%	gamma
Escherichia coli ATCC 8739 (00012*)	50-100	good	40-50%	luxuriant	>=70%	gamma

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

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.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual Clinical Microbiology, 11th Edition. Vol. 1

Revision : 03 / 2024

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IVD ***In vitro*** **diagnostic medical device**

30°C
10°C **Storage temperature**

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