Technical Information

Blood Agar Base with low pH

Product Code: DM 1089

Application: - Blood Agar Base with low pH is recommended as a base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms like *Neissseria. Streptococci* etc.

Composition**

Ingredients	Gms / Litre
Beef heart, infusion from(Beef extract)	500.000
Tryptose	10.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Blood Agar Base is a highly nutritious medium commonly 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. If the culture medium is to be used without addition of blood, the pH should be adjusted to 7.2 to 7.4, since most bacteria can grow better in a slightly alkaline medium. The low pH of Blood Agar Base w/ low pH (pH 6.8) stabilizes the red blood corpuscles and favours the formation of clear zone of haemolysis [1]. Also it is fruitful for cultivation of *streptococci* and *pneumococci* Blood Agar Base media can be used after adding phenolphthalein phosphate [2] for the detection of phosphate producing staphylococci, after adding salt and agar for assessment of surface contamination on equipment and pig carcass [3] and to determine salinity range of marine *Flavobacteria* [4]. It can also be used for preparation of *Salmonella typhi* antigens [5].

Beef extract and tryptose provides carbon, nitrogen, amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. 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* [6]. 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* [7].

Methodology

Suspend 40 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of 5-7% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

Reaction
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH Range 6.60-7.00

Cultural Response/Characteristics
DM1089: Cultural characteristics observed with added 5% w/v sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth w/o blood	Recovery w/o blood	Growth with blood	Recovery with blood	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	Fair	40-50%	Luxuriant	>=70%	None
Staphylococcus aureus ATCC 25923	50-100	Good	50-70%	Luxuriant	>=70%	Beta
Staphylococcus epidermidis ATCC 12228	50-100	Good	50-70%	Luxuriant	>=70%	None
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

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days

Further Reading

1. Norton J. F., 1932, J. Lab. Clin. Med., 17:558-565.
2. Noble W. C., 1962, J. Clin, Pathol., 15:552.
3. Hansen N. H., 1962, J. Appl. Bacteriol., 25:46.
4. Hayes P. R., 1963, J. Gen. Microbiol., 30:1.
5. Schuber J. H., Edwards P. R. and Ramsere C. H., 1959, J. Bacteriol., 77:648.
6. Snavely J. G. and Brahier J., 1960, Am. J. Clin. Pathol., 33:511.
7. 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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