

## Technical Information

### Blood Agar Base (Infusion Agar)

**Product Code: DM 1073**

**Application:** Blood Agar Base is recommended as a base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms like *Neisseria*, *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)	7.3±0.2

\*\*Formula adjusted, standardized to suit performance parameters

#### Principle & Interpretation

Blood Agar Base 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.

Blood Agar Base media can be used <sup>(1)</sup> for the detection of phosphate producing *Staphylococci*, after adding phenolphthalein for assessment of surface contamination on equipment and pig carcass <sup>(2)</sup> and to determine salinity range of marine *Flavobacteria* <sup>(3)</sup> by adding salt agar. It can also be used for preparation of *Salmonella* Typhi antigens <sup>(4)</sup>. Blood Agar Base is recommended by APHA <sup>(5)</sup> and Standard Methods <sup>(6, 7)</sup> for testing of food samples.

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 type of animal blood used. Sheep blood gives best results for Group A Streptococci <sup>(8)</sup>. But sheep blood fails to support growth of *Haemophilus haemolyticus* since it is deficient in pyridine nucleotides. However when horse blood is used *H. haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* <sup>(9)</sup>

#### 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% 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 : 7.3±0.2

pH Range 7.10-7.50

### Cultural Response/Characteristics

DM1073: 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
<i>Neisseria meningitidis</i> ATCC 13090	50-100	Fair	40-50%	Luxuriant	>=70%	None
<i>Staphylococcus aureus</i> ATCC 25923	50-100	Good	50-70%	Luxuriant	>=70%	Beta
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	Good	50-70%	Luxuriant	>=70%	None
<i>Streptococcus pneumoniae</i> ATCC 6303	50-100	fair-good	40-50%	Luxuriant	>=70%	Alpha
<i>Streptococcus pyogenes</i> 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<sup>0</sup> in sealable plastic bags for 2-5 days

### Further Reading

1. Noble W. C., 1962, J. Clin. Pathol., 15:552.
2. Hansen N. H., 1962, J. Appl. Bacteriol., 25:46.
3. Hayes P. R., 1963, J. Gen. Microbiol., 30:1.
4. Schuber J. H., Edwards P. R. and Ramsere C. H., 1959, J. Bacteriol., 77:648.
5. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, DC.
6. U.S. Food and Drug Administration, 1995, Bacteriological Analytical Manual, 8th Ed., AOAC International, Gaithersburg, Md.
7. Atlas R. M., 1993, Handbook of Microbiology of Microbiological Media, CRC Press, Boca Raton, Fla.
8. Snavely J. G. and Brahier J., 1960, Am. J. Clin. Pathol., 33:511.
9. Murray P. R., Baron J. H., Jorgensen J. H., Tenover F. C., Tenover P. C., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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