



Technical Data

HiEncap™ Blood Agar Base(HiEncap™ Infusion Agar)

EC073D

HiEncap™ Blood Agar Base is recommended for isolation and cultivation of various fastidious pathogenic microorganisms after addition of blood.

Composition**

| Ingredients | Gms / Litre |
|---------------------------|-------------|
| Beef heart, infusion from | 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

Directions

Each capsule contains 20 grams medium. Suspend 1 capsule in 500 ml (2 capsules in 1000 ml) distilled or purified 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 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 with added phenolphthalein phosphate (1) for the detection of phosphate producing Staphylococci, with added salt and agar for assessment of surface contamination on equipment and pig carcass (2) and to determine salinity range of marine *Flavobacteria* (3). It can also be used for preparation of *Salmonella* Typhi antigens (4). Blood Agar Base is recommended by APHA (5) and Standard Methods (6, 7) 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 animal blood used. Sheep blood gives best results for Group A Streptococci (8). 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* (9).

Quality Control

Appearance

Gelatin capsule containing cream to yellow coloured granular media

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.

Quantity

Each capsule contains 20 gms of medium sufficient for 500 ml media.

Reaction

Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

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

Cultural Response

| Organism | Inoculum (CFU) | Growth w/o blood | Recovery w/o blood | Growth with blood | Recovery with blood | Haemolysis |
|--|----------------|------------------|--------------------|-------------------|---------------------|------------|
| Cultural Response | | | | | | |
| <i>Neisseria meningitidis</i> ATCC 50-100 13090 | | 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

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

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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.
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