Technical Information

Blood Agar Base No. 2

Product Code: DM 1834

Application: - Blood Agar Base No. 2 is specially devised for the maximum recovery of streptococci, pneumococci and other fastidious pathogenic microorganisms without interfering with their haemolytic reactions.

Composition**

Ingredients	Gms / Litre
Proteose peptone	15.000
Liver extract	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

A fastidious organism is one that requires additional cellular building-block molecules for its survival [1]. Blood Agar Base No. 2 is a highly nutritive medium. Microorganisms producing haemolysin give visible zone of haemolysis on this medium. It also serves as a differential medium for *Brucella* and *Campylobacter* species by using different antibiotic supplements for the respective bacteria [2, 3]. Being highly infective *Brucella* culture must be handled with care. Best results are obtaind when plates are incubated preferably in 5-10% carbon dioxide atmosphere. Comparative studies of horse, rabbit and sheep blood showed that sheep blood gave the clearest and most reliable colony and haemolysis characteristics at both 24 and 48 hours of incubation [4]. It can be used to prepare Chocolate Agar for the isolation of *Haemophilus* and *Neisseria* species. It can also be used for primary isolation of *Haemophilus* species, when horse blood is used for enrichment. Better results are obtained by spreading half of the horse blood agar plate with 2 drops of 10% saponin [5]. Liver extract and yeast extract helps to enhance the growth and haemolysis reactions of fastidious organisms like Streptococci and Pneumococci. Proteose peptone serves as the nitrogen source while liver digest and yeast extract provide essential carbon, vitamin, nitrogen and amino acid sources. Sodium chloride maintains the osmotic equilibrium. Supplementation with blood (5-10%) provides additional growth factors and also basis for understanding haemolytic reactions. Haemolytic patterns may change according to the source of animal blood or type of base medium used [6].

Methodology

Suspend 21.25 grams of powder media in 500 ml distilled water. Shake well & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 40 - 50°C and aseptically add 7% v/vsterile defibrinated blood. For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (MS2005) to 500 ml sterile molten base. For *Campylobacter* species : Add rehydrated contents of 1 vial of Campylobacter Supplement - I (MS2006) or Campylobacter Supplement - II (MS2007) or Campylobacter Supplement - III (MS2008) or Campylobacter Growth Supplement (MS2009) to 500 ml sterile molten base. For *Streptococcus* species: Add rehydrated contents of 1 vial of Strepto Supplement (MS2031) to 500 ml sterile molten base. 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: Yellow 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 4.25% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH range 7.20-7.60

Cultural Response/Characteristics

DM1834: Cultural characteristics observed with added 5-7% sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant	>=70%	None
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	>=70%	Beta
Streptococcus pneumonia ATCC 6303	50-100	good-luxuriant	>=70%	Alpha
Streptococcus pyogenes ATCC 19615	50-100	good-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 C. F., 1986, Microbiology, 2nd Edition, Addison-Wesley Publishing Company.
2. Hunter D. and Kearns M., 1977, Brit. Vet. J., 133:486.
3. Skirrow M. B., 1977, B.M.J., ii: 9.
4. Snavely and Brahier, 1960, Am. J. Clin. Pathol., 33:511.
5. Waterworth and Pamela M., 1955, Brit. J. Exp. Pathol., 36:186.
6. Murray P. R,, Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.Donot use the products if it fails to meet specificatons for identity and performens parameters.