Blood HiCynth™ Agar Base No.2

MCD834

Blood HiCynth™ Agar Base No. 2 is specially devised to permit the maximum recovery of streptococci, pneumococci and other fastidious pathogenic microorganisms without interfering with their haemolytic reactions.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.3*	17.500
HiCynth™ Peptone No.5*	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

*Chemically defined peptones

Directions

Suspend 21.25 grams in 500 ml 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 7% v/v sterile defibrinated blood.

For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (FD005) to500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement - I (FD006) or Campylobacter Supplement - II (FD007) or Campylobacter Supplement - III (FD008) or Campylobacter Growth Supplement (FD009) to 500 ml sterile molten base.

For *Streptococcus* species: Add rehydrated contents of 1 vial of Strepto Supplement (FD031) to 500 ml sterile molten base. Mix well and pour into sterile Petri plates.

Principle And Interpretation

A fastidious organism is one with complete nutritional requirements, needing additional cellular building-block molecules in order to survive (1). Blood HiCynth™ Agar Base No. 2 is a highly nutritive medium prepared by replacing animal peptones in Blood Agar Base with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. Microorganisms producing haemolysin give visible haemolytic zones on this medium. It also serves as a differential medium for *Brucella* and *Campylobacter* species by adding different antibiotic supplements for the respective bacteria (2, 3). *Brucella* cultures are highly infective and must be handled with care. Incubate 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, where 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). HiCynth™ Peptone No.3 and HiCynth™ Peptone No.5 serves as a source of 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 serves as basis for determining haemolytic reactions. Haemolytic patterns may vary with the source of animal blood or type of base medium used (6).

Quality Control

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: Reddish brown 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
7.20-7.60

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Cultural Response				
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant	>=70%	none
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	>=70%	beta
Streptococcus pneumoniae ATCC 6303	50-100	good-luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%	beta

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference
1. Norton C. F., 1986, Microbiology, 2nd Edition, Addison-Wesley Publishing Company.
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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.

Revision : 00 / 2015