Blood Agar Base No. 2

M834

Intended use

Blood 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
Proteose peptone	15.000
HML 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

-Equivalent to Liver extract

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) to 500 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 Agar Base No. 2 is a highly nutritive medium. 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). HML extract and yeast extract helps enhance the growth and haemolytic 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 serves as basis for determining haemolytic reactions. Haemolytic patterns may vary with the source of animal blood or type of base medium used (6).

Type of specimen

Clinical material : blood and other pathological material ; food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10,11).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Addition of sheep blood is recommended to detect haemolysis. This medium does not support the growth of *H.haemolyticus*
2.Addition of Horse blood or rabbit blood to base medium supports growth of *H.haemolyticus* but resemble beta-haemolytic Streptococci and hence must be confirmed.
3. Haemolytic pattern varies with the source of blood used.

Performance and Evaluation

Performace of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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 : 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 : 7.4±0.2

pH
7.20 - 7.60

Cultural Response
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
Cultural Response						
Neisseria meningitidis ATCC 13090	50-100	fair	40-50%	luxuriant	>=70%	none
Staphylococcus aureus ATCC 25923	50-100	good	50-70%	luxuriant	>=70%	beta
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

Store below 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10,11).

Reference

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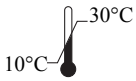
IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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