TM 041 – BLOOD AGAR BASE No. 2

INTENDED USE

For isolation, cultivation and detection of haemolytic activity of Streptococci, Pneumococci and other fastidious microorganisms.

PRODUCT SUMMARY AND EXPLANATION

A fastidious organism is one with complete nutritional requirements, needing additional cellular building-block molecules in order to survive. 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. *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. 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.

COMPOSITION

Ingredients	Gms / Ltr
Proteose peptone	15.000
Liver extract	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	15.000

PRINCIPLE

Liver 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 extract 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.

INSTRUCTION FOR USE

- Dissolve 21.25 grams in 500 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi 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 to 500 ml sterile molten base.
- For Campylobacter species: Add rehydrated contents of 1 vial of Campylobacter Supplement - I or Campylobacter Supplement - II or Campylobacter Supplement - III or Campylobacter Growth Supplement to 500 ml sterile molten base.
- For Streptococcus species: Add rehydrated contents of 1 vial of Strepto Supplement to 500 ml sterile molten base. Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance 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.
pH (at 25°C) : 7.4±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth w/o blood	Recovery w/o blood	Growth with blood	Recovery with blood	Haemolysis	Incubation Temperature	Incubation Period
Neisseria meningitidis	13090	50-100	Fair	20-30%	Luxuriant	>=70%	None	35-37°C	18-48 Hours
Staphylococcus aureus subsp. aureus	25923	50-100	Good	50-70%	Luxuriant	>=70%	Beta	35-37°C	18-48 Hours
Streptococcus pneumoniae	6303	50-100	Fair-good	20-40%	Luxuriant	>=70%	Alpha	35-37°C	18-48 Hours
Streptococcus pyogenes	19615	50-100	Fair-good	20-40%	Luxuriant	>=70%	Beta	35-37°C	18-48 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Waterworth and Pamela M., 1955, Brit. J. Exp. Pathol., 36:186.
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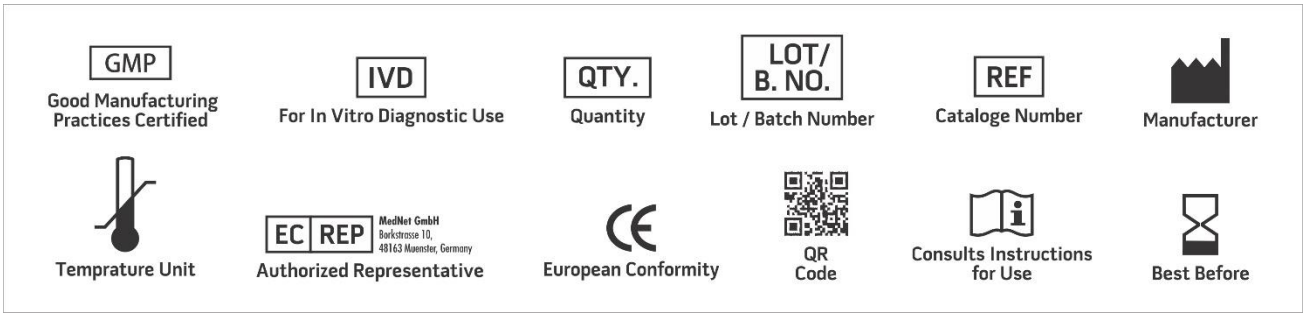
3. Norton C. F., 1986, Microbiology, 2nd Edition, Addison-Wesley Publishing Company
4. Skirrow M. B., 1977, B.M.J., ii: 9.
5. Snavely and Brahier, 1960, Am. J. Clin. Pathol., 33:511



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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