# Technical Information

## Blood Agar Base No. 2 with 1.2% Agar

## Product Code: DM 1834A

**Application:** - Blood Agar Base No. 2 with 1.2% Agar is especially devised to permit the maximum recovery of 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 | 12.000 |
| Final pH ( at 25°C) | 7.4±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Principle & Interpretation

A fastidious organism is one which with complete nutritional requirements requires additional cellular building-block molecules for survival [1]. Blood Agar Base No. 2 w/ 1.2 % Agar 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 antibiotics supplements for the respective bacteria [2, 3]. *Brucella* cultures are highly infective and must be handled taking all biosafety precautions 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]. Also it can be used to prepare Chocolate Agar for the isolation of *Ha em ophilus* and *Neisseria* species. It is recommended by the American Food and Drug Administration for the preparation of blood agar using sheep blood [5].

This medium 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 [6]. 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 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 [7].

## Methodology

Suspend 19.75 grams of powder Media in 500 ml distilled water. Shake well & heat 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/v sterile 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 1.2% 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 3.95% w/v aqueous solution at 25°C. pH : 7.4±0.2

**pH range** 7.20-7.60

**Cultural Response/ characteristics**

DM 1834A: 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° 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. FDA Bacteriological Analytical Manual, 1992, 7th Ed., F.D.A. Washington, D.C.
6. Waterworth and Pamela M., 1955, Brit. J. Exp. Pathol., 36:186.
7. Murray P. R,, Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiolo gy, ASM, Washington, D.C.

## Disclaimer :

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