

Blood Agar Base No. 2

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.

Summary

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.

Principle

MX Nutrients 3 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.

Formula*

| Ingredients | g/L |
|--------------------|-----------|
| Proteose Peptone | 15.0 |
| MX Nutrients 3# | 2.5 |
| Yeast Extract | 5.0 |
| Sodium Chloride | 5.0 |
| Agar | 12.0 |
| Final pH (at 25°C) | 7.4 ± 0.2 |

*Adjusted to suit performance parameters

#Equivalent to intended performance of Liver Digest

Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen

Clinical material: blood and other pathological material; food samples.

Specimen Collection and Handling

Ensure that all samples are properly labelled.

Follow appropriate techniques for handling samples as per established guidelines.

Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.

The samples must be stored and tested within the permissible time duration.

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

Directions

1. Suspend 39.50 g of the powder in 1000 mL purified / distilled water.
2. Heat to boiling to dissolve the powder completely.
3. Dispense into testtubes.
4. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes as per validated cycle.
5. For preparation of Blood Agar Base No.2, cool the base to 45°C-50°C and aseptically add 5% sterile, defibrinated blood. Mix well.
6. To prepare chocolate agar, add 10% sterile defibrinated blood to Blood Agar Base No.2 at 80°C Mix well.

Quality Control

Dehydrated Appearance: Light yellow to yellow coloured, homogenous free flowing powder

Prepared Appearance: Basal medium: Medium to dark amber coloured, slightly opalescent gel.

With addition of 5% defibrinated sheep blood: Cherry red opaque gel forms in petridishes.

With addition of 10% chocolatized sheep blood (Chocolate agar): Brown coloured, opaque gel forms in petridishes.

Cultural Response: Growth is observed after an incubation at 35°C ± 2°C for 18-48 hours with added CO₂.

| Organism (ATCC) | Growth | Haemolysis |
|--|--------|------------|
| <i>Haemophilus influenzae</i> (19418) | NA | NA |
| <i>Neisseria meningitidis</i> (13090) | Good | Gamma |
| <i>Streptococcus pyogenes</i> Strain Bruno (19615) | Good | Beta |
| <i>Streptococcus pneumoniae</i> (6306) | Good | Alpha |

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

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., Pfaffer M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

| Cat No. | Product description | Pack Size |
|----------------|----------------------------|------------------|
| 201020210100 | Dehydrated Culture Media | 100 g |
| 201020210500 | Dehydrated Culture Media | 500 g |

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|---|--|---|------------------------------|---|---|--|---|--|-----------|
|  Temperature Limit |  Manufacturer |  LOT | Batch Code |  Date of Manufacture |  This way up |  RO | Received on | | |
|  REF | Catalogue Number |  i | Consult Instructions for use |  S | Use-by Date |  HO | Hygroscopic keep container tightly closed |  OO | Opened on |

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Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.