Blood Agar Base (Infusion Agar)

Intended Use

Blood Agar Base is a non-selective general-purpose medium to which sterile blood may be added for use in isolation and cultivation of Streptococci, and other fastidious pathogenic organisms like *Neisseria* etc. It is also used for detection of haemolytic activity.

Summary

Without addition of blood the medium may be employed as a Nutrient Agar, or as a medium for the short-term maintenance of stock cultures. With added blood or serum, the medium is suitable for the cultivation of many fastidious organisms as well as determination of haemolytic reactions, which is an important diagnostic criterion for organisms like Streptococci, Staphylococci, etc. However, haemolytic reactions depend on the animal blood used. Group A Streptococci gives best results on sheep blood. *Haemophilus haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* on horse blood. Blood Agar Base is specified in standard methods for food testing and is included in the Bacteriological Analytical Manual for testing of cosmetics.

Principle

Heart muscle infusion, pancreatic digest of casein and yeast extract provide nitrogen, carbon and other growth factors. Sodium chloride maintains the osmotic balance. Supplementation with blood provides additional growth factors for fastidious organisms and is the basis for determining haemolytic reactions.

Formula*

Ingredients	**g/L**
Heart Muscle, Infusion from (solids)	2.0
Pancreatic Digest of Casein	13.0
Yeast Extract	5.0
Sodium Chloride	5.0
Agar	15.0
Final pH (at 25°C)	7.3 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability

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

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 40.00 g of the powder in 1000 mL of purified / distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. For preparation of blood agar, cool the base to 45°C-50°C and aseptically add 5% v/v sterile, defibrinated blood. Mix well.

Quality Control

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

Prepared Appearance: Basal Medium: Light yellow to amber coloured, slightly opalescent gel. With addition of 5% defibrinated blood - Cherry red, opaque gel forms in petridishes.

Cultural Response: Growth is observed after an incubation at 35°C±2°C for 48 hours under anaerobic condition.

Organism (ATCC)	Growth	Haemolysis
Streptococcus pyogenes Strain Bruno (19615)	Good	Beta
Staphylococcus aureus subsp. *aureus* (25923)	Good	Beta
Streptococcus pneumoniae (6305)	Good	Alpha
Candida albicans 3147 (10231)	Good	No Haemolysis
Listeria monocytogenes strain Li 23 (19114)	Good	Beta

Interpretation of Results

1. Colony morphology of some organisms on Blood Agar containing 5% sheep blood:
2. Haemolytic Streptococci may appear as opaque or translucent, greyish, small or large, matt or mucoid colonies, surrounded by a zone of haemolysis.
3. Pneumococci usually appear as very flat, smooth, translucent, greyish and sometimes mucoid colonies surrounded by a
1. narrow zone of alpha (green) haemolysis.
4. Staphylococci appear as opaque, white to golden yellow colonies with or without zones of beta haemolysis.
5. *Listeria* may form small zones of beta haemolysis.
6. Other organisms of clinical significance may also grow on this medium.

Performance and Evaluation

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

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. The animal blood used (horse or sheep) and the incubation conditions (aerobic or anaerobic) affect the haemolytic reactions of organisms on this medium.
2. Colonies of *Haemophilus haemolyticus* are beta haemolytic on horse and rabbit blood agar and therefore must be distinguished from colonies of beta haemolytic Streptococci. The use of sheep blood has been recommended to obviate this problem since sheep blood does not support the growth of *H. haemolyticus*.

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. Downes and Ito (ed.) 2001, Compendium Of Methods For The Microbiological Examination Of Foods, 4th edition, APHA Washington DC.
2. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.
3. Atlas. 1993. Handbook of microbiological media. CRC Press, Boca Raton, Fla.
4. Ruoff, Whiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
5. Forbes, Sahm and Weissfeld (ed.). 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201020200100	Dehydrated Culture Media	100 g
201020200500	Dehydrated Culture Media	500 g
201020202500	Dehydrated Culture Media	2.5 k