

Blood Agar Base

REF. Pack size

600 01 100 100 gm

600 01 500 500 gm

Intended Use

Blood Agar Base is a medium to which blood may be added for use in isolation and cultivation of Streptococci, Staphylococci and other fastidious pathogenic organisms as Neisseria in throat and ear swab, sterile urine and CSF specimens. It is also used for determination of haemolytic activity.

Background

Blood Agar Base is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. It can also be used as general-purpose media without the addition of blood. The medium is suitable for the cultivation of many fastidious organisms as well as determination of haemolytic reactions, which is an important diagnostic criteria for organisms like streptococci, Staphylococci.

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.

Components gm/Litre

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

Preparation, Storage and Stability

Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Store the prepared medium at 2-8°C

Procedure

1. Suspend 40 g of the powder in 1 L distilled water and mix well.
2. Heat with frequent agitation and boil for one minute to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C for 15 minutes.
4. To prepare Blood Agar, cool the base to 45-50°C and aseptically add 5% v/v sterile, defibrinated blood.
5. Mix well and pour into sterile petri plates.

Quality Control

Appearance

1- Dehydrated Appearance : Cream to yellow coloured, homogeneous, free flowing powder.

2- Prepared Appearance :

Basal medium : Light amber coloured slightly opalescent gel.
With addition of defibrinated blood : Cherry red opaque gel

3- Cultural Response : after 18-48 hours at 30-35°C (As per pharmacopeia) or 35± 2°C for clinical specimens

Organisms	Recovery plain	Recovery with blood
<i>Streptococcus pyogenes</i>	Good	Good, beta hemolysis
<i>Streptococcus pneumoniae</i>	Good	Good, Alpha hemolysis
<i>Candida albicans</i>	-	Good, no hemolysis
<i>Pseudomonas aeruginosa</i>	Good	-
<i>Staphylococcus aureus</i>	Good	Good, beta hemolysis
<i>Listeria monocytogenes</i>	-	Good, beta hemolysis

Interpretation of the results

Colony morphology of some organisms on Blood Agar containing 5% sheep blood:

1. Haemolytic streptococci may appear as opaque or translucent, greyish, small or large, matt or mucoid colonies, surrounded by a zone of haemolysis.
2. Pneumococci usually appear as very flat, smooth, translucent, greyish and sometimes mucoid colonies surrounded by a narrow zone of alpha (green) haemolysis.
3. Staphylococci appear as opaque, white to golden yellow colonies with or without zones of beta haemolysis.
4. Listeria may form small zones of beta haemolysis.

Other organisms of clinical significance may also grow on this medium.

Precautions

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.

Bibliography

1. Noble W. C., 1962, J. Clin. Pathol., 15:552.
2. Hansen N. H., 1962, J. Appl. Bacteriol., 25:46.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition