Blood Agar Base with low pH

Intended use
Recommended as a base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms like *Neisseria, Streptococci* etc from clinical samples.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM infusion B from ●</td>
<td>500.000</td>
</tr>
<tr>
<td>Tryptose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters   
● Equivalent to Beef heart, infusion from

Directions
Suspend 40.00 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation
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. If the culture medium is to be used without addition of blood, the pH should be adjusted to 7.2 to 7.4, since most bacteria can grow better in a slightly alkaline medium. The low pH of Blood Agar Base w/ low pH (pH 6.8) stabilizes the red blood corpuscles and favors the formation of clear zone of haemolysis (7). Also it is advantageous for cultivation of Streptococci and Pneumococci Blood Agar Base media can be used with added phenolphthalein phosphate (6) for the detection of phosphate producing staphylococci, with added salt and agar for assessment of surface contamination on equipment and pig carcass (1) and to determine salinity range of marine Flavobacteria (2). It can also be used for preparation of *Salmonella* Typhi antigens (8).

HM infusion B from and tryptose provides carbon, nitrogen, amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. It also helps in visualizing the haemolytic reactions. However, haemolytic reactions depend on the animal blood used. Sheep blood gives best results for Group A Streptococci (9). But sheep blood fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However when horse blood is used *H. haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* (5).

Type of specimen
Clinical material: blood and other pathological material

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions
In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.
Limitations
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.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium: Light amber 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 4.0% w/v aqueous solution at 25°C. pH: 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed with added 5% w/v sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth (w/o blood)</th>
<th>Recovery (w/o blood)</th>
<th>Growth (with blood)</th>
<th>Recovery (with blood)</th>
<th>Haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>ATCC 50-100</td>
<td>fair-good</td>
<td>40-50%</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>none</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>subsp. aureus</td>
<td>ATCC 25923 (00034*)</td>
<td>50-100</td>
<td>good</td>
<td>50-70%</td>
<td>luxuriant</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>ATCC 12228 (00036*)</td>
<td>50-100</td>
<td>good</td>
<td>50-70%</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>ATCC 6303</td>
<td>50-100</td>
<td>fair-good</td>
<td>40-50%</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>ATCC 19615</td>
<td>50-100</td>
<td>fair-good</td>
<td>40-50%</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
</tbody>
</table>

Key : * - Corresponding WDCM numbers

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Please refer disclaimer Overleaf.
Reference


Disclaimer :
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other relatedHiMedia™publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.