Blood Agar Base No. 2 with 1.2% Agar

**Intended Use:**
Recommended especially to permit the maximum recovery of fastidious pathogenic microorganisms without interfering with their haemolytic reactions.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>15.000</td>
</tr>
<tr>
<td>HML extract #</td>
<td>2.500</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>12.000</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 19.75 grams in 500 ml 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 7% v/v sterile defibrinated blood.

For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (FD005) to 500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement - I (FD006) or Campylobacter Supplement - II (FD007) or Campylobacter Supplement - III (FD008) or Campylobacter Growth Supplement (FD009) to 500 ml sterile molten base.

For *Streptococcus* species: Add rehydrated contents of 1 vial of Strepto Supplement (FD031) to 500 ml sterile molten base. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

A fastidious organism is one with complete nutritional requirements, needing additional cellular building-block molecules in order to survive (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 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 *Haemophilus* 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). HML 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 HML 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 (7).

**Type of specimen**

Clinical material : blood and other pathological material ; food samples
Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,10,11). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,12,13). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions
In Vitro diagnostic use only. 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, that 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 1.2% 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
7.20-7.60

Cultural Response
Cultural characteristics observed with added 5-7% 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</th>
<th>Recovery</th>
<th>Haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Neisseria meningitidis</em> ATCC 50-100</td>
<td>13090</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>none</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 50-100</td>
<td>25923 (00034*)</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>beta</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> ATCC 50-100</td>
<td>6303</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>alpha</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> ATCC 19615</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>beta</td>
</tr>
</tbody>
</table>

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life
Store below 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.

Please refer disclaimer Overleaf.
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 (10,11).

Reference
10. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition

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In vitro diagnostic medical device

CE Marking

Storage temperature

10°C - 30°C

Do not use if package is damaged

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