HI Agar

**Intended Use:**
Recommended for isolation and cultivation of a wide variety of fastidious organisms.

**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>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 40.0 grams in 1000 ml of 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. If desired 5% v/v sterile defibrinated blood may be added. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Fastidious organisms having exacting nutritional requirement could be cultivated on infusion media, as demonstrated by Huntoon (5). A liquid medium containing an infusion of meat was one of the first media used for the cultivation of bacteria. These infusion media need not be further supplemented by the addition of supplements for cultivation of fastidious bacteria (4). HI Agar, containing HM infusion B from (equivalent to infusion from beef heart) is used for the isolation and cultivation of a wide variety of fastidious organisms (2). HI Agar can also be used for the cultivation of *Vibrio* species (4,9). It can also be supplemented with glucose, horse serum and antibiotics for the cultivation a wide variety of organisms (2). It is used for mass cultivation of organisms for preparation of vaccines. On supplementation of blood, HI Agar can be used to study haemolytic reactions (8). This medium was used for isolation and enumeration of haemolytic Streptococci in milk (3). Tryptose and HM infusion B infusion provide nutritional requirements for the pathogenic bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

**Type of specimen**

Clinical samples - CSF, Blood, Pus, Urine, Feces samples; Food and dairy samples - Milk, meat samples

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (1,9).
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. Further biochemical and serological tests must be carried out for further identification.
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 yellow coloured, clear to slightly opalescent gel After addition of 5-7% w/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 : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added 5-7% 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>Staphylococcus aureus</em> subsp. aureus ATCC 25923 (00034*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;70%</td>
<td>luxuriant</td>
<td>&gt;70%</td>
<td>beta</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em> ATCC 13090</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;70%</td>
<td>luxuriant</td>
<td>&gt;70%</td>
<td>none</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> ATCC 6303</td>
<td>50-100</td>
<td>good</td>
<td>50-70%</td>
<td>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</td>
<td>50-70%</td>
<td>luxuriant</td>
<td>&gt;70%</td>
<td>beta</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;70%</td>
<td>luxuriant</td>
<td>&gt;70%</td>
<td>beta</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 (6,7).

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 related HiMedia™ 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.