Heart Infusion Agar, Modified (Revised as HI Agar, Modified)  

**Intended Use:**
Recommended for isolation and cultivation of fastidious pathogenic microorganisms like *Neisseria*, Streptococci etc. and for confirmation of diarrheagenic *Escherichia coli* in accordance with FDA BAM, 1998.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMH infusion from #</td>
<td>375.000</td>
</tr>
<tr>
<td>Soya peptone</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>7.3±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

# Equivalent to Heart muscle, infusion from

**Directions**
Suspend 37.5 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 20 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

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 incase of fastidious bacteria(2). Heart Infusion Agar, Modified is used in the isolation and cultivation of diarrheagenic *Escherichia coli* in accordance with FDA BAM, 1998 (1). *E.coli* is a Gram negative, facultatively anaerobic bacterium that is found as commensals in human intestine.

This medium is cited in BAM for primary screening in the conventional biochemical screening and identification of diarrheagenic *Escherichia coli*. Enrichment of the sample on BHI and TP broths is recommended as the first step in primary screening of diarrheagenic *E.coli*. This may induce the growth and proliferation of other members of *Enterobacteriaceae* including non lactose fermenting strains of *E.coli*. Hence additional tests may need to be performed for isolation. Transfer suspicious colonies to TSI agar, Heart Infusion Agar, Modified slants (M073F), Tryptone Broth, Arabinose Broth, and Urea Broth and incubate for 20 hr at 35°C. Organisms isolated on primary screening are processed for secondary screening and confirmed using genotypic, biochemical and serological reactions. On supplementation of blood, Heart Infusion Agar, Modified can be used to study haemolytic reactions (5). HMH infusion and soya peptone provide nutritional requirements for the pathogenic bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

**Type of specimen**
Food samples

**Specimen Collection and Handling**
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions :**
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 specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations :**
1. Due to nutritional variations certain strains may show poor growth.

**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% 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 : 7.3±0.2

**pH**
7.10-7.50

**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>Neisseria meningitidis ATCC 13090</td>
<td>50-100</td>
<td>fair</td>
<td>40-50%</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>none</td>
</tr>
<tr>
<td>Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)</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>Staphylococcus epidermidis ATCC 12228 (00036*)</td>
<td>50-100</td>
<td>good</td>
<td>50-70%</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>none</td>
</tr>
<tr>
<td>Streptococcus pneumoniae ATCC 6503</td>
<td>50-100</td>
<td>fair-good</td>
<td>40-50%</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>alpha</td>
</tr>
<tr>
<td>Streptococcus pyogenes ATCC 19615</td>
<td>50-100</td>
<td>fair-good</td>
<td>40-50%</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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

**Reference**

Revision : 02/ 2019

Disclaimer :
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