Mannitol Lysine Agar

Intended Use:
Recommended for selective isolation of Salmonellae other than Salmonella Typhi and Salmonella paratyphi A.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>HM peptone B #</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.000</td>
</tr>
<tr>
<td>Mannitol</td>
<td>3.000</td>
</tr>
<tr>
<td>L-Lysine hydrochloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.0125</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.010</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 extract

Directions
Suspend 49.02 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Cool to 45-50°C.

Principle And Interpretation
Human Salmonella infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta (4). One of the most important criteria in the identification of Salmonella species is the production of hydrogen sulphide. Salmonella Typhi and Salmonella Paratyphi A can be differentiated from the rest of the Salmonella due to their inability to form hydrogen sulphide.

Mannitol Lysine Agar, formulated as described by Inoue et al (5) is used for the selective isolation of Salmonella species other than Salmonella Typhi and Salmonella Paratyphi A from different foods and faeces. Mannitol Lysine Agar may be used directly with the specimen or from an enrichment culture (1). Enrichment can be carried out in Modified Semisolid RV Medium (M1482). Mannitol Lysine Agar does not depend upon lactose fermentation and is therefore recommended for investigating lactose fermenting Salmonellae like Salmonella Arizonae. Further tests should be carried out for confirming Salmonella species.

Peptone, HM peptone B, yeast extract provide essential nutrients for the growth of Salmonella. Mannitol is the fermentable carbohydrate in the medium while L-lysine is the amino acid. Salmonellae grow as large purple colony with black center because of H2S production. Mannitol is fermented by organisms and the resulting acid stimulates lysine decarboxylation. This elevates the pH due to production of amines and promotes blackening. Sodium thiosulphate and ferric ammonium citrate help in H2S production. Atypical Salmonella strains do not produce H2S and form grey colonies. Brilliant green dye in the medium inhibits gram-positive and majority of gram-negative organisms. Mannitol Lysine Medium should be used in conjunction with Brilliant Green Agar, Modified (M016) or Bismuth Sulphite Agar (M027). Mannitol Lysine Medium can be directly inoculated with the specimen or the specimen can be first enriched in Modified Semisolid RV Medium Base (M1482). Atypical Salmonella will form a characteristic bulls eye due to less H2S production, which gets concentrated in the centre of the colony. Salmonella colonies will form purple black colonies. Presumptive Salmonella should be confirmed by biochemical tests.
**Type of specimen**
Clinical samples - Faeces

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3). 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. Presumptive Salmonella should be confirmed by biochemical tests.

**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**
Light yellow to greenish yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Yellow with purple coloured tinge clear to slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 4.9% w/v aqueous solution at 25°C. pH : 6.8±0.2

**pH**
6.60-7.00

**Cultural Response**
Cultural characteristics observed 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>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>$&gt;=10^4$</td>
<td>inhibited</td>
<td>0%</td>
<td>purple with black centre</td>
</tr>
<tr>
<td><em>Salmonella Paratyphi B</em> ATCC 8759</td>
<td>50-100</td>
<td>luxuriant</td>
<td>$&gt;=50%$</td>
<td>colourless with purple tinge, may have black centres</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em> ATCC 6539</td>
<td>50-100</td>
<td>None-poor</td>
<td>0-10%</td>
<td>purple with black centre</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>$&gt;=50%$</td>
<td>purple with black centre</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 50-100 (13076 (00030*))</td>
<td>luxuriant</td>
<td>$&gt;=50%$</td>
<td>purple with black centre</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 25923 (00034*)</td>
<td>$&gt;=10^4$</td>
<td>inhibited</td>
<td>0%</td>
<td>purple with black centre</td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

Please refer disclaimer Overleaf.
Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (2,3).

Reference

Revision : 03/ 2019

In vitro diagnostic medical device
CE Marking
Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
23 Vadhani Industrial Estate,
LBS Marg,Mumbai-86,MS,India

CE Partner 4U,Esdoornlaan 13, 3951
DB Maarn The Netherlands,
www.cepartner 4u.eu

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.