**Mannitol Lysine HiCynth™ Agar**

**MCD1071**

**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>HiCynth™ Peptone No.1*</td>
<td>12.000</td>
</tr>
<tr>
<td>HiCynth™ Peptone No.3*</td>
<td>5.000</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>3.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>5.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>4.000</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>1.000</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.0125</td>
</tr>
<tr>
<td>Agar</td>
<td>0.010</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>15.000</td>
</tr>
</tbody>
</table>

**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.

**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. Mannitol Lysine HiCynth™ Agar is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones.

HiCynth™ Peptone No.1, HiCynth™ Peptone No.3 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 HiCynth™ Agar should be used in conjunction with Brilliant Green HiCynth™ Agar Base, Modified (MCD016) or Bismuth Sulphite HiCynth™ Agar (MCD027). Mannitol Lysine HiCynth™ Agar 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.

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*Chemically defined peptones*

**Technical Data**

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Please refer disclaimer Overleaf.
**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;=10⁴</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>purple with black centre and 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</td>
<td>luxuriant</td>
<td></td>
<td>&gt;=50%</td>
<td>purple with black centre</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 25923 (00034*)</td>
<td>&gt;=10⁴</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 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

Revision : 00/2019