Mannitol Salt Agar MH118

Intended Use
Recommended for selective isolation of pathogenic Staphylococci from pharmaceutical products in accordance with Microbial Limit Test by harmonized method of USP/EP/BP/JP/IP.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone #</td>
<td>5.000</td>
</tr>
<tr>
<td>Tryptone ##</td>
<td>5.000</td>
</tr>
<tr>
<td>HM Peptone B ###</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>75.000</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>10.000</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>pH after sterilization (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

# Peptic digest of animal tissue
## Pancreatic digest of casein
### Equivalent to Beef extract

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 111.02 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 15 minutes or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Note: This product contains 7.5% Sodium chloride as one of its ingredients. On repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, therefore we strongly recommend storage in tightly closed containers in dry place away from bright light.

Principle And Interpretation
Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e. Staphylococcus aureus is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (13). Staphylococci have the unique ability of growing on a high salt containing media (11). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5% NaCl was studied by Chapman (3). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (13,1,4,6,14). The additional property of lipase activity of Staphylococcus aureus can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (7). It is also used in the performance of microbial limit tests for the selective isolation of Staphylococcus. The formulation is in accordance with the harmonization of USP/EP/BP/JP/IP (15,5,2,10,8).

The medium contains HM peptone B, tryptone and peptone which makes it very nutritious as they provide carbon, nitrogen compounds, long chain amino acids, vitamins and other essential growth factors and trace nutrients. Many other bacteria except Staphylococci are inhibited by 7.5% sodium chloride. Mannitol is the fermentable carbohydrate fermentation of which leads to acid production, detected by phenol red indicator.

S. aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of S. aureus are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of S. aureus should be confirmed by performing the coagulase test [tube or slide (13)]. Lipase activity of S. aureus can be detected by supplementing the medium with egg yolk emulsion

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Please refer disclaimer Overleaf.
A possible *S. aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth)(M002) (12). Few strains of *S. aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (12).

**Type of specimen**
Pharmaceutical samples; Clinical samples

**Specimen Collection and Handling**
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (15,5,2,10,8). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,6).

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 specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations**
1. This medium is a selective medium, some strains of *Staphylococcus aureus* may exhibit a delayed fermentation of mannitol.
2. Certain other bacteria are also mannitol fermenting other than *Staphylococcus*, therefore further biochemical testing is required for 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**
Light yellow to pink homogeneous free flowing powder

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

**Colour and Clarity of prepared medium**
Red coloured clear to slightly opalescent gel forms in Petri plates

**pH**
7.20-7.60

**Growth Promotion Test**
Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BP/IP, after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

**Growth promoting properties**
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for <=18 hours).

**Indicative properties**
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100cfu (at 30-35°C for 18-72 hours).

**Inhibitory properties**
No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100cfu (at 30-35°C for >= 72 hours).

Please refer disclaimer Overleaf.
Cultural Response

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Observed Lot value (CFU)</th>
<th>Recovery</th>
<th>Colour of colony</th>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Promoting + Indicative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp. aureus ATCC 6538 (00032</em>)*</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50%</td>
<td>yellow/white colonies surrounded by yellow zone</td>
<td>&gt;=18 hrs - 72 hrs</td>
</tr>
<tr>
<td><strong>Inhibitory</strong></td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
<td>yellow/white colonies</td>
<td>&gt;=72 hrs</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739 (00012*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Additional Microbiological testing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp. aureus ATCC 25923 (00034</em>)*</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50%</td>
<td>yellow/white colonies surrounded by yellow zone</td>
<td>&gt;=18 hrs - 72 hrs</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis ATCC 12228 (00036</em>)*</td>
<td>50 -100</td>
<td>fair - good</td>
<td>15 -40</td>
<td>30 -40%</td>
<td>red</td>
<td>&gt;=18 hrs - 72 hrs</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis ATCC 14990 (00132</em>)*</td>
<td>50 -100</td>
<td>fair - good</td>
<td>15 -40</td>
<td>30 -40%</td>
<td>red</td>
<td>&gt;=18 hrs - 72 hrs</td>
</tr>
<tr>
<td>Proteus mirabilis ATCC 12453</td>
<td>50 -100</td>
<td>none-poor</td>
<td>0 -10</td>
<td>0 -10%</td>
<td>yellow</td>
<td>&gt;=18 hrs - 72 hrs</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
<td></td>
<td>&gt;=72 hrs</td>
</tr>
<tr>
<td>Escherichia coli NCTC 9002 &gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
<td></td>
<td></td>
<td>&gt;=72 hrs</td>
</tr>
<tr>
<td># Klebsiella aerogenes ATCC 13048</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
<td></td>
<td>&gt;=72 hrs</td>
</tr>
</tbody>
</table>

Key: (#) Formerly known as *Enterobacter aerogenes*, (*) Corresponding WDCM numbers

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,13).

Reference

5. European Pharmacopoeia, 2017, EDQM.
8. Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi

Revision : 03/2019

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.