Mannitol Salt Agar

**Intended Use:**
Recommended for the selection and subculture of *Staphylococcus aureus* in accordance with the harmonized method of USP/EP/BP/JP/IP.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>10.000</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>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

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

# - Equivalent to Beef extract

**Directions**

Mannitol Salt Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized, hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium in tubes as butts/slants or in plates as desired and allow to solidify. If on plate, either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically.

**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 (10). Staphylococci have the unique ability of growing on a high salt containing media (8). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5%NaCl was studied by Chapman (2). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (10, 5, 3, 12, 11). 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 (4).

HM peptone B and proteose peptone supply essential growth factors and trace nutrients to the growing bacteria. Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. 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] (10). Lipase activity of *S.aureus* can be detected by supplementing the medium with egg yolk emulsion.

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) (9). 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 (9).

**Type of specimen**
Clinical samples: pus; Food and dairy samples, water samples.

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (12,1). 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. A possible *S.aureus* must be confirmed by the coagulase test.
2. 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) (9).  
3. 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 (9).

**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**
Sterile Mannitol Salt Agar in glass bottle.

**Colour of medium**
Red coloured coloured medium

**Quantity of medium**
100ml of medium in glass bottle.

**Reaction**
7.20-7.60

**Sterility Test**
Passes release criteria

**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

**Cultural Response**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultural Response</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=50 %</td>
<td>yellow/white colonies surrounded by yellow zone</td>
<td>18-72 hrs</td>
</tr>
<tr>
<td>subsp. <em>aureus</em> ATCC 6538 (00032*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8739</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0 %</td>
<td></td>
<td>&gt;=72 hrs</td>
</tr>
<tr>
<td>(00012*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>18-72 hrs</td>
</tr>
<tr>
<td>subsp. <em>aureus</em> ATCC 25923 (00034*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**HiMedia Laboratories**

### Technical Data

<table>
<thead>
<tr>
<th>Strain</th>
<th>Concentration</th>
<th>Inhibition</th>
<th>Color</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis ATCC 14990</em></td>
<td>50 - 100</td>
<td>red</td>
<td>18 - 72 hrs</td>
<td></td>
</tr>
<tr>
<td><em>Proteus mirabilis ATCC 12453</em></td>
<td>50 - 100</td>
<td>none-poor</td>
<td>18 - 72 hrs</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922 (00013</em>)*</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0%</td>
<td>&gt;=72 hrs</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes ATCC 13048 (00175</em>)*</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0%</td>
<td>&gt;=72 hrs</td>
</tr>
<tr>
<td><strong>Key:</strong> (*) Corresponding WDCM numbers.</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

### Storage and Shelf Life

On receipt store between 15-25°C 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


Revision : 01/ 2019

Please refer disclaimer Overleaf.
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