Mannitol Salt Agar Plate

Intended Use:
Recommended for selective isolation of pathogenic Staphylococci.

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
Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate. Note: This product contains 7.5% Sodium chloride as one of its ingredients.

Note: 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).

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 mannnitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of S.aureus are usually mannnitol 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).

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

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. Read the label before opening the pack. 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).
4. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
5. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user’s unique requirement.
6. It is recommended to store the plates at 24-30°C to avoid minimum condensation.

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 90 mm disposable plates.

Colour of medium
Red coloured coloured medium

Quantity of medium
25 ml of medium in 90 mm disposable plates.

pH
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.

<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>Staphylococcus aureus</em> subsp.</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=50 %</td>
<td>yellow/white colonies surrounded by yellow zone</td>
</tr>
<tr>
<td><em>aureus</em> ATCC 6538 (00032*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8739</td>
<td>&gt;=10^4</td>
<td>inhibited</td>
<td>0 %</td>
<td></td>
</tr>
<tr>
<td>(00012*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp.</td>
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<td>yellow/white colonies surrounded by yellow zone</td>
</tr>
<tr>
<td><em>aureus</em> ATCC 25923 (00034*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>50 -100</td>
<td>good</td>
<td>40-50 %</td>
<td>red</td>
</tr>
<tr>
<td>ATCC 14990 (00132*)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
**Proteus mirabilis ATCC 12455**

50 -100 none-poor 0 -10 % yellow

**Escherichia coli ATCC 25922 (00013*)**

$>=10^4$ inhibited 0%

**Escherichia coli NCTC 9002 $>=10^4$ inhibited 0%

**# Klebsiella aerogenes $>=10^4$ inhibited 0%

**ATCC 13048 (00175*)**

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

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**Storage and Shelf Life**

On receipt store between 20-30°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 : 00/2020

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Please refer disclaimer Overleaf.
In vitro diagnostic medical device

CE Marking

Storage temperature

20°C–30°C

Do not use if package is damaged

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