Mannitol Salt Agar

M118

Intended Use:
Mannitol Salt Agar is used as a selective media for the isolation of pathogenic Staphylococci from clinical and non-clinical samples.

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
Suspend 111.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, add 5% v/v Egg Yolk Emulsion (FD045). 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 (1). Staphylococci have the unique ability of growing on a high salt containing media (2). 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 (1, 4-7). 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 (8). 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. Coagulate-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](1). 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 (10,11).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,12).
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
NA

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

Reaction
Reaction of 11.1% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

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>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcus aureus</strong> subsp. aureus ATCC 6538 (00032*)</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>Escherichia coli ATCC 8739 &gt;=10^3 (00012*)</td>
<td>inhibited</td>
<td>0 %</td>
<td></td>
<td></td>
<td>&gt;=72 hrs</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong> subsp. aureus ATCC 25923 (00034*)</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><strong>Staphylococcus epidermidis</strong> ATCC 12228 (00036*)</td>
<td>50-100</td>
<td>fair-good</td>
<td>30-40 %</td>
<td>red</td>
<td>18-72 hrs</td>
</tr>
<tr>
<td><strong>Staphylococcus epidermidis</strong> ATCC 14990 (00132*)</td>
<td>50-100</td>
<td>fair-good</td>
<td>30-40 %</td>
<td>red</td>
<td>18-72 hrs</td>
</tr>
<tr>
<td><strong>Proteus mirabilis</strong> ATCC 12453</td>
<td>50-100</td>
<td>none-poor</td>
<td>0-10 %</td>
<td>yellow</td>
<td>18-72 hrs</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>&gt;=10^3</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
<td>&gt;=72 hrs</td>
</tr>
</tbody>
</table>
Escherichia coli NCTC 9002 >=10³ 
inhibited 0% 
>=72 hrs

Enterobacter aerogenes 
ATCC 13046 (00175*) >=10³ 
inhibited 0% 
>=72 hrs

Key: *Corresponding WDCM numbers.

**Storage and Shelf Life**
Store below 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 inorder 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 (10,11).

**Reference**

Revision : 03 / 2018
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.