Lipovitellin Salt Mannitol Agar Base

**Intended use**
Recommeneded for selective isolation and identification of pathogenic *Staphylococcus aureus* by detecting lipase production and mannitol fermentation from clinical and non-clinical specimens.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM Peptone B#</td>
<td>1.000</td>
</tr>
<tr>
<td>Peptone, special</td>
<td>10.000</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><strong>Final pH ( at 25°C)</strong></td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 11.1 grams in 93 ml purified/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 and aseptically add 7 ml of sterile Egg Yolk Emulsion (FD045) to get a final concentration of 2%. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**
The coagulase-positive species of *Staphylococcus* i.e. *Staphylococcus aureus* is well-documented as a human opportunistic pathogen (7). *S.aureus* is also isolated from recreational water like swimming pools, and are thus indicators of health risk (2,3,6,9). *S.aureus* is relatively resistant to the effect of disinfectant like chlorine and sodium chloride. Lipovitellin Salt Mannitol Agar Base, recommended by APHA (1) is used for the selective isolation and identification of pathogenic *S.aureus* by detecting lipase production and mannitol fermentation.

HM Peptone B and peptone special serve as source of nitrogenous and carbonaceous compounds, long chain amino acids vitamins and other essential nutrients required for bacterial growth. Sodium chloride in higher concentration makes the medium selective for *Staphylococcus* by inhibiting accompanying flora. D-Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by the pH indicator dye, namely phenol red. Lipovitellin is a lipo phosphoprotein, which is combined with lecithin in the yolk of eggs. It is also known as vitellin or ovovitellin and is inhibitory to majority of bacteria except *Staphylococcus*. Egg yolk emulsion serves as a source of lipids for lipase activity. Inoculate tubes of M-Staphylococcus Broth (M1120). Incubate at 35-37°C for 24 hours. Streak plates of Lipovitellin Salt Mannitol Agar Base with a loopful of culture from positive (turbid) tubes. Incubate at 35-37°C for 24-48 hours. Opaque, yellow zones around the colonies are positive evidence of Lipovitellin + lipase activity (opaque) and mannitol fermentation (yellow)(1).

**Type of specimen**
Clinical samples - Blood, skin exudates, pus samples; Food samples; Water samples

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (8).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (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 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. This medium is selective medium and may suppress the growth of certain Staphylococcal species.
2. Due to high sodium chloride concentration, the medium has a tendency to form lumps on exposure to air.

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
Basal medium: Red coloured clear to slightly opalescent gel After addition of 2% Egg Yolk Emulsion :Pink coloured opaque 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 with added egg yolk emulsion, after an incubation at 35-37°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
<th>Lipase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)</td>
<td>50-100</td>
<td>good to luxuriant</td>
<td>&gt;=70%</td>
<td>yellow colonies positive, with yellow opaque zone around the colonies</td>
<td>iridescent sheen on the colony surface and medium</td>
</tr>
</tbody>
</table>

Key: *Corresponding WDCM numbers.

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. 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 (4,5).
Reference


Disclaimer:

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