Coagulase Mannitol Agar Base

Intended Use:
Recommended for the primary isolation and differentiation of pathogenic Staphylococci from clinical specimens or for classifying pure cultures.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI infusion$</td>
<td>5.000</td>
</tr>
<tr>
<td>Tryptone</td>
<td>10.500</td>
</tr>
<tr>
<td>Soya peptone</td>
<td>3.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.500</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10.000</td>
</tr>
<tr>
<td>Bromo cresol purple</td>
<td>0.020</td>
</tr>
<tr>
<td>Agar</td>
<td>14.500</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 heart infusion (solids)

Directions
Suspend 47.02 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118 - 121°C (12-15 lbs pressure respectively) for 15 minutes. Cool to 45 - 50°C. Add 7 - 15% v/v sterile, pre-tested, rabbit plasma the basal medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The genus *Staphylococcus* comprises 28 accepted or proposed species, 14 of which may be encountered in human clinical specimens. Staphylococci are generally found on the skin and mucous membranes of humans and other animals. Some of the pathogenic staphylococci in both humans and animals produce an enzyme called coagulase and detection of this enzyme is used in the laboratory to identify these organisms (5).

These media are used for the isolation of *Staphylococcus aureus* from clinical specimens and for differentiation of *S.aureus* from other species on the basis of coagulase production and mannitol fermentation. Chapman for the first time introduced a medium for selective isolation and differentiation of Staphylococci (1). Tellurite-glycine media were designed by Zebovitz et al (8) and Marwin (6) for selectively isolating coagulase-positive Staphylococcal species. Present medium is based on Esber and Faulconer formulation (2). Mutant or old cultures of *S.aureus* may be weak coagulase producers. They should be freshly sub cultured and rechecked. *Escherichia coli* ferments mannitol and may be weakly coagulase positive. Coagulase production is dependent on the presence of a fermentable sugar like mannitol in this case. It is also dependent on the presence of a protein factor in the HI infusion and blood plasma (7). When mannitol is fermented, the pH of the medium surrounding the coagulase positive colonies drops. This drop in pH is indicated by the change in colour of the bromocresol purple indicator, which turns yellow and exhibits yellow zones around the colonies.

An opaque area of coagulated plasma forms around the colonies of coagulase positive organisms. *Staphylococcus epidermidis* is coagulase negative and mannitol non-fermenting species, which does not change the colour of the medium. Coagulase negative species may ferment mannitol and produce a yellow zone around the colonies but an opaque zone will not be formed.

Type of specimen
Clinical samples: skin and mucous membranes

Specimen Collection and Handling

Please refer disclaimer Overleaf.
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). 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. Some mutant strains of *S.aureus* may show coagulase weak or negative reaction. The culture should be retested in case of doubt.
2. Some strains of *E.coli* may show mannitol fermentation and weak coagulase positive reaction.
3. Gram strain may help in distinguishing between species

**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 light grey homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.45% Agar gel

**Colour and Clarity of prepared medium**
Purple coloured, slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 4.7% w/v aqueous solution at 25°C. pH : 7.4±0.2

**pH**
7.20-7.60

**Cultural Response**
Cultural characteristics observed with added 7-15% v/v sterile pretested, rabbit plasma at 35-37°C for 18-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Mannitol fermentation</th>
<th>Coagulate production</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em> ATCC 12228 (00036*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>(\geq70)%</td>
<td>negative reaction, purple colour</td>
<td>negative reaction, no opaque zone formation positive reaction, colonies surrounded by opaque zone</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus</em> ATCC 25923 (00034*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>(\geq70)%</td>
<td>positive reaction, yellow colour</td>
<td></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**

Please refer disclaimer Overleaf.
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 (3,4).

Reference