SS Agar, Modified

Intended Use:
SS Agar (Salmonella Shigella Agar) Modified is used for the selective isolation and differentiation of *Salmonella* and *Shigella* species from pathological specimens, suspected foodstuffs etc.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>HM Peptone B #</td>
<td>5.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Bile salts mixture</td>
<td>5.500</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>8.500</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.00033</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>12.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

# Equivalent to Beef extract

Directions

Suspend 57.02 grams in 1000 ml distilled water. Heat to boiling with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Overheating may destroy the selectivity of the medium. Cool to about 45-50°C. Mix and pour into sterile Petri plates.

Principle And Interpretation

*Salmonella* and *Shigella* are gram-negative, facultatively anaerobic, non-spore-forming rods in the family *Enterobacteriaceae*. They are widely distributed in animals, infecting mainly the stomach and the intestinal tissues. SS Agar is recommended as differential and selective medium for the isolation of *Salmonella* and *Shigella* species from pathological specimens (1) and suspected foodstuffs (2, 3, 4, 5) and for microbial limit test (6). SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

Peptone and HM Peptone B provide essential growth nutrients. Lactose is the fermentable carbohydrate. Brilliant green, bile salts and thiosulphate selectively inhibit gram-positive and coliform organisms. Sodium thiosulphate is reduced by certain species of enteric organisms to sulphite and H2S gas. This reductive enzymatic process is attributed to thiosulphate reductase. Production of H2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of H2S with ferric ions or ferric citrate, indicated by black centered colonies.

The high selectivity of Salmonella Shigella Agar allows the use of large inocula directly from faeces, rectal swabs or other materials suspected of containing pathogenic enteric bacilli. On fermentation of lactose by few lactose-fermenting normal intestinal flora, acid is produced which is indicated by change of colour from yellow to red by the pH indicator neutral red. Thus these organisms grow as red-pigmented colonies. Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centers. *Salmonella* species exhibit colourless colonies with black centers resulting from H2S production. *Shigella* species form colourless colonies, which do not produce H2S. While using samples suspected of being exposed to treatments that might have damaged the viability of microorganisms due to processing of food materials or samples from patients under antibiotic treatment etc., previous enrichment in Selenite cystine Broth Base (M025) or Tetrathionate Broth Base (M032) is necessary. Inoculate SS Agar plates with the enriched culture. After incubation the suspicious colonies should be subcultured on differential media to be identified biochemically or serologically.
Type of specimen
Clinical: faeces, blood, rectal swabs; Suspected foodstuffs

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,3,4,5).

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. The medium is highly selective and may be toxic to certain Salmonella or Shigella species. Hence it is recommended to use to inoculate plates of less inhibitory media parallel to SS Agar, such as Hektoen Enteric Agar (M467) or Deoxycholate Citrate Agar (M065) for easier isolation of Shigella species (7).

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 1.2% Agar gel

Colour and Clarity of Prepared Medium
Reddish orange coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.7% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

<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>Cultural Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>50-100</td>
<td>fair</td>
<td>20-30%</td>
<td>pink with bile precipitate</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes ATCC 13048</em></td>
<td>50-100</td>
<td>fair</td>
<td>20-30%</td>
<td>cream pink</td>
</tr>
<tr>
<td><em>Enterococcus faecalis ATCC 29212</em></td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Proteus mirabilis ATCC 25933</em></td>
<td>50-100</td>
<td>fair-good</td>
<td>30-40%</td>
<td>colourless, may have black centre</td>
</tr>
<tr>
<td><em>Salmonella Choleraeuis ATCC 12011</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless with black centre</td>
</tr>
<tr>
<td><em>Salmonella Typhi ATCC 6539</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless with black centre</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
Salmonella Typhimurium  ATCC 14028
- 50-100
- good-luxuriant
- >=50%
- colourless with black centre

Salmonella Enteritidis ATCC 50-100
- 13076
- good-luxuriant
- >=50%
- colourless with black centre

Shigella flexneri ATCC 12022
- 50-100
- good
- 40-50%
- colourless

Storage and Shelf Life
Store below 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 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 (8,9).

Reference
Disclaimer:

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