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>

**Directions**

Suspend 57.02 grams in 1000 ml purified / 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-sporulating 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 (5) and suspected foodstuffs (1,6,8,9) and for microbial limit test (7). 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 H\textsubscript{2}S gas. This reductive enzymatic process is attributed to thiosulphate reductase. Production of H\textsubscript{2}S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of H\textsubscript{2}S 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 H\textsubscript{2}S production. *Shigella* species form colourless colonies, which do not produce H\textsubscript{2}S. 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 (2,3).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,8,9).

**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 (4).

**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.

**Cultural Response**

<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>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>fair</td>
<td>20-30%</td>
<td>pink with bile precipitate cream pink</td>
</tr>
<tr>
<td># Klebsiella aerogenes ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>fair</td>
<td>20-30%</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 50-100 (00087*)</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td></td>
<td>colourless, may have black centre</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> ATCC 25933</td>
<td>50-100</td>
<td>fair-good</td>
<td>30-40%</td>
<td>colourless with black centre</td>
</tr>
<tr>
<td><em>Salmonella Choleraesuis</em> ATCC 12011</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</em> ATCC 6539</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless with black centre</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless with black centre</td>
</tr>
</tbody>
</table>
HiMedia Laboratories
Technical Data

Salmonella Enteritidis ATCC 50-100
13076 (00030*)

Shigella flexneri ATCC 12022 (00126*)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth Characteristics</th>
<th>Percentage</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Enteritidis ATCC 50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless with black centre</td>
</tr>
<tr>
<td>Shigella flexneri ATCC 12022 (00126*)</td>
<td>good</td>
<td>40-50%</td>
<td>colourless</td>
</tr>
</tbody>
</table>

* 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 (2,3).

Reference

In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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