**Intended Use:**
Recommended for identification of members of *Enterobacteriaceae* especially *Salmonella* species. The composition and performance criteria of this medium are as per the specifications laid down in ISO 1993, Draft ISO DIS 6579-1:2017.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>20.000</td>
</tr>
<tr>
<td>HM extract #</td>
<td>3.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.000</td>
</tr>
<tr>
<td>Glucose (Dextrose)</td>
<td>1.000</td>
</tr>
<tr>
<td>Iron(III) citrate</td>
<td>0.300</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>0.300</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.024</td>
</tr>
<tr>
<td>Agar</td>
<td>12.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 64.62 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in sloped form with a butt of depth about 2.5cm-5cm.

Note: For better results, the medium can be sterilized by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

**Principle And Interpretation**
Triple Sugar Iron Agar was originally proposed by Sulkin and Willett (9) and modified by Hajna (3) for identifying *Enterobacteriaceae*. This medium complies with the recommendation of APHA, for the examination of meat and food products (8), for the examination of milk and dairy products (10) and for microbial limit test for confirming the presence of *Salmonella* (1,2) and in the identification of gram-negative bacilli (1, 7). ISO Committee (4) has recommended a slight modification in the original medium for the identification of *Salmonella*.

Peptone, yeast extract and HM extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose(glucose) are the fermentable carbohydrates. Sodium thiosulphate and ferrous ions make H₂S indicator system. Phenol red is the pH indicator. Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More amount of acids are liberated in butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amounts of acid present in the butt. Thus the appearance of an alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and/or sucrose. Bacteria that ferment lactose or sucrose (or both), in addition to glucose, produce large amounts of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (CO₂) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. Thiosulphate is reduced to hydrogen sulphide by several species of bacteria and H₂S combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. Reduction of thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube. Triple Sugar Iron Agar should be used in parallel with Urea Agar/Broth (M112/M111) to distinguish between *Salmonella* and *Proteus* species. The reactions can be summarized as follows:
Alkaline slant / acid butt-only glucose fermented
Acid slant / acid butt-glucose and sucrose fermented or glucose and lactose fermented or all the three sugars, glucose, lactose and sucrose fermented.
Bubbles or cracks present—gas production
Black precipitate present—H₂S gas production

**Type of specimen**
Food and Dairy products.

**Specimen Collection and Handling:**
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,10). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**
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 specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations :**
1. Some members of the *Enterobacteriaceae* and H₂S producing *Salmonella* may not be H₂S positive on TSI Agar. Some bacteria may show H₂S production on Kligler Iron Agar but not on TSI Agar. This can happen because utilization of sucrose in TSI Agar suppresses the enzymic pathway that result in H₂S production.

**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**
Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants.

**Reaction**
Reaction of 6.45% 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 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Slant</th>
<th>Butt</th>
<th>Gas</th>
<th>H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrobacter freundii</em> ATCC 8090</td>
<td>50-100</td>
<td>luxuriant</td>
<td>acidic reaction, yellowing of the medium</td>
<td>acidic reaction, yellowing of the medium</td>
<td>positive reaction</td>
<td>positive, blackening of medium</td>
</tr>
<tr>
<td># <em>Klebsiella aerogenes</em> ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>acidic reaction, yellowing of the medium</td>
<td>acidic reaction, yellowing of the medium</td>
<td>positive reaction</td>
<td>negative, no blackening of medium</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>acidic reaction, yellowing of the medium</td>
<td>acidic reaction, yellowing of the medium</td>
<td>positive reaction</td>
<td>negative, no blackening of medium</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
Salmonella Typhimurium ATCC 14028

<table>
<thead>
<tr>
<th>Phenotypic Properties</th>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic Reaction</td>
<td>Positive</td>
<td>Blackening of the medium</td>
</tr>
<tr>
<td>Yellowing of the Medium</td>
<td>Positive</td>
<td>Blackening of the medium</td>
</tr>
</tbody>
</table>

Storage and Shelf Life

Store between 10-30°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 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.

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Please refer disclaimer Overleaf.
Reference


Reference


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