Triple Sugar Iron Agar

**Intended Use:**
Recommended for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Tryptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>HM Peptone B*</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>Dextrose (Glucose)</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.200</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>
</tbody>
</table>

**Final pH (at 25°C)** 7.4±0.2

*Formula adjusted, standardized to suit performance parameters

*Equivalent to Beef extract

**Directions**

Triple Sugar Iron Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized, hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium in tubes as butts/slants or in plates as desired and allow to solidify. If on plate, either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically.

**Principle And Interpretation**

Triple Sugar Iron Agar was originally proposed by Sulkin and Willett (8) and modified by Hajna (3) for identifying *Enterobacteriaceae*. This medium complies with the recommendation of APHA, for the examination of meat and food products (7), for the examination of milk and dairy products (9) and for microbial limit test for confirming the presence of *Salmonellae* (1,2) and in the identification of gram-negative bacilli (1, 6).

Tryptone, peptone, yeast extract and HM peptone B provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose 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:

*Equivalent to Beef extract*
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**
Isolated microorganism from water, food, or clinical sample.

**Specimen Collection and Handling:**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,9).
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. 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**
Sterile Triple Sugar Iron Agar in glass bottle.

**Colour medium**
Pinkish red coloured

**Quantity of medium**
100ml of medium in glass bottle.

**Reaction**
7.20-7.60

**Sterility test**
Passes release criteria

**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># Klebsiella aerogenes 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.*
<table>
<thead>
<tr>
<th><strong>HiMedia Laboratories</strong></th>
<th><strong>Technical Data</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>Klebsiella pneumoniae ATCC 13883 (00097</em>)</em>*</td>
<td><strong>50-100 luxuriant</strong></td>
</tr>
<tr>
<td><strong>Salmonella Paratyphi A ATCC 9150</strong></td>
<td><strong>50-100 luxuriant</strong></td>
</tr>
<tr>
<td><strong>Salmonella Typhi ATCC 6539</strong></td>
<td><strong>50-100 luxuriant</strong></td>
</tr>
<tr>
<td><em><em>Salmonella Typhimurium ATCC 14028 (00031</em>)</em>*</td>
<td><strong>50-100 luxuriant</strong></td>
</tr>
<tr>
<td><em><em>Shigella flexneri ATCC 12022 (00126</em>)</em>*</td>
<td><strong>50-100 luxuriant</strong></td>
</tr>
<tr>
<td><em><em>Escherichia coli ATCC 8739 50-100 (00012</em>)</em>*</td>
<td><strong>luxuriant</strong></td>
</tr>
<tr>
<td><strong>Escherichia coli NCTC 9002 50-100</strong></td>
<td><strong>luxuriant</strong></td>
</tr>
<tr>
<td><strong>Klebsiella pneumoniae ATCC 10031</strong></td>
<td><strong>50-100 luxuriant</strong></td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

# Formerly known as Enterobacter aerogenes

**Storage and Shelf Life**
On receipt store between 15-25°C. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

**Reference**

Please refer disclaimer Overleaf.

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In vitro diagnostic medical device
CE Marking
Storage temperature
Do not use if package is damaged

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