Lysine Iron Agar

Intended use

Lysine Iron Agar is recommended for the differentiation of enteric organisms especially *Salmonella* Arizonae based on their ability to decarboxylate or deaminate lysine and to form hydrogen sulphide (H$_2$S).

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>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>1.000</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>10.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>0.040</td>
</tr>
<tr>
<td>Bromocresol purple</td>
<td>0.020</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.7±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.56 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes in slanted position to form slants with deep butts.

Principle And Interpretation

Lysine Iron Agar was developed by Edwards and Fife (1) to detect lactose fermenting Salmonellae. Salmonellae are known to decarboxylate lysine rapidly and produce large amounts of hydrogen sulphide (2, 3). This medium is a sensitive medium for the detection of lactose fermenting and lactose non-fermenting *Salmonella* species. Many strains of this group ferment lactose very rapidly thus suppressing H$_2$S production on Triple Sugar Iron Agar (M021). So there is a possibility that the organisms frequently found in food poisoning outbreaks could be overlooked. Thatcher and Clark (4) described the isolation of *Salmonella* species from foods from selective agar and to inoculate it on Lysine Iron Agar and Triple Sugar Iron (M021) together. Using these two media greater discrimination can be made between coliform organisms e.g. *Escherichia* and *Shigella* (5, 6).

Peptone and yeast extract provide essential nutrients. Dextrose is a source of fermentable carbohydrate. Ferric ammonium citrate and sodium thiosulphate are indicators of H$_2$S formation. Cultures that produce hydrogen sulphide cause blackening of the medium due to ferrous sulphide production. Lysine decarboxylation causes an alkaline reaction (purple colour) to give the amine cadaverine and the organisms which do not decarboxylate lysine, produce acid butt (yellow colour). Organisms that deaminate lysine, form alpha - ketocarboxylic acid, which reacts with iron salt near the surface of the medium under the influence of oxygen to form reddish-brown compound. The medium is stabbed to the base of the butt and streaked on slant.

Type of specimen

Pure isolate

Specimen Collection and Handling

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.
**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 greyish yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Purple coloured, clear to slightly opalescent gel forms in tubes as slants

**Reaction**
Reaction of 3.45% w/v aqueous solution at 25°C. pH : 6.7±0.2

**pH**
6.50-6.90

**Cultural Response**
M377: 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>Butt/Slant</th>
<th>H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrobacter freundii ATCC 8090</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>acidic reaction, yellowing of medium, alkaline reaction, purple reaction, or no colour change</td>
<td>positive blackening of medium</td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922 (00013</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>alkaline reaction, purple reaction, or no colour change</td>
<td>negative</td>
</tr>
<tr>
<td><em>Proteus mirabilis ATCC 25933</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>acidic reaction, yellowing of medium, deep red lysine deamination</td>
<td>positive blackening of medium</td>
</tr>
<tr>
<td><em>Salmonella Arizonae ATCC 13314</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>alkaline reaction, purple reaction, or no colour change</td>
<td>positive blackening of medium</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis ATCC 13076 (00030</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>alkaline reaction, purple reaction, or no colour change</td>
<td>positive blackening of medium</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium ATCC 14028 (00031</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>alkaline reaction, purple reaction, or no colour change</td>
<td>positive blackening of medium</td>
</tr>
<tr>
<td><em>Shigella flexneri ATCC 12022 (00126</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>acidic reaction, yellowing of medium, alkaline reaction, purple reaction, or no colour change</td>
<td>negative</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 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.

*Please refer disclaimer Overleaf.*
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 (7,8).

Reference