Iron Sulphite HiCynth™ Agar

Composition**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.3*</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium sulphite</td>
<td>0.500</td>
</tr>
<tr>
<td>Iron (III) citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.1±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

*Chemically defined peptone

Directions
Suspend 26 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation
Iron Sulphite Agar is a modification of Cameron Sulphite Agar developed by the National Canners Association of America (7). It was shown by Beerens (2) that 0.1% sulphite concentration in the original formula was inhibitory to some strains of Clostridium sporogenes. This observation was later confirmed by Mossel et al (5), who consequently showed that 0.05% sulphite concentration was not inhibitory to the organisms. Most clostridia have sulfite reductase in their cytoplasm but they are unable to expel them to the exterior. So when H₂S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate. Iron Sulphite HiCynth™ Agar is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones.

For the detection of organisms causing sulphide spoilage, two methods can be followed:

a) Deep-Shake Culture Method: Dispense the medium in 10 ml amounts in tubes. Inoculate the sample when the medium is at about 50°C. Allow to set and incubate at 55°C for 24-48 hours. Typical thermophilic species -Desulfotomaculum nigrificans, produces distinct black spherical colonies in the depth of the medium.

b) Attenborough and Scarr (1) Method: In this method, diluted samples of sugar or any other food are filtered through membrane filters.

Type of specimen
Food samples

Specimen Collection and Handling
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6). 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 species may show poor growth due to nutritional variations.
2. Further biochemical tests must be carried out for confirmation.

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 brownish yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Yellow coloured, slightly opalescent gel forms in Petri plates
Reaction
Reaction of 2.6% w/v aqueous solution at 25°C. pH : 7.1±0.2
pH
6.90-7.30
Cultural Response
Cultural characteristics observed under anaerobic conditions, after an incubation at 55-56°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium botulinum</em> ATCC 25763</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td><em>Clostridium butyricum</em> ATCC 13732</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td><em>Clostridium sporogenes</em> ATCC 19404 (00008*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td><em>Desulfotomaculum nigrificans</em> ATCC 19998</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good</td>
<td>40-50%</td>
<td>no blackening</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 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. 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 (3,4).

Please refer disclaimer Overleaf.
Reference


