Gelatin Iron Agar

Intended use
Gelatin Iron Agar is used for detecting gelatin liquefaction and hydrogen sulphide production.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>25.000</td>
</tr>
<tr>
<td>HM extract #</td>
<td>7.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Gelatin</td>
<td>120.000</td>
</tr>
<tr>
<td>Ferrous chloride</td>
<td>0.500</td>
</tr>
<tr>
<td>Agar</td>
<td>1.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

# - Equivalent to Meat extract

Directions
Suspend 15.9 grams in 100 ml warm purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense in test tubes as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation Composition**
Gelatin liquefaction along with the production of hydrogen sulphide is one of the characteristics used in the classification of bacteria. Hydrogen sulphide can be produced in small amounts from sulphur containing amino acids by a large number of bacteria. Methods to detect hydrogen sulphide production by suspending strips of paper impregnated with lead acetate above cultures are of variable sensitivity and are of limited value. The hydrogen sulphide production test combined with gelatin liquefaction test is useful for group differentiation within the Enterobacteriaceae species (3). Few Clostridia exhibit gelatinase activity as well as H2S production. Escherichia coli grow well on this medium but show neither gelatinase activity nor H2S production.

The medium consists of peptone, HM extract and gelatin, which provide nitrogen compounds and also the carbon compounds for the growing organisms. Gelatin acts as solidifying agent and is the substrate for the organisms producing gelatinase enzyme. Ferrous chloride aids in the detection of hydrogen sulphide indicated by black precipitate. Gelatin is usually liquefied by Clostridium perfringens within 24 to 48 hours.

Type of specimen
Isolated Microorganism

Specimen Collection and Handling
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
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. Due to nutritional variations, some strains may show poor growth.
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
Cream to yellow homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.1% Agar gel and 12.0% Gelatin gel.

Colour and Clarity of prepared medium
Light yellow coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 15.9% w/v aqueous solution at 25°C. pH: 7.0±0.2
pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Gelatinase reaction</th>
<th>H₂S production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)</td>
<td>50-100 luxuriant</td>
<td>positive reaction</td>
<td>negative, no blackening of medium</td>
<td></td>
</tr>
<tr>
<td>Clostridium perfringens ATCC 12924</td>
<td>50-100 luxuriant</td>
<td>positive reaction</td>
<td>positive, blackening of medium</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100 luxuriant</td>
<td>negative reaction</td>
<td>negative, no blackening of medium</td>
<td></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 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. 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.
Disclaimer:

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