HiCrome™ MM Agar Modified

Intended use

HiCrome™ MM Agar Modified is recommended for identification and differentiation of *Salmonella* and non *Salmonella* like *Citrobacter* from water samples.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>6.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10.000</td>
</tr>
<tr>
<td>L-Lysine hydrochloride</td>
<td>5.000</td>
</tr>
<tr>
<td>D-Cellubiose</td>
<td>10.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.000</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>3.750</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.800</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>6.800</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>0.200</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.100</td>
</tr>
<tr>
<td>Agar</td>
<td>18.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.6±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.65 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ MM Agar was formulated by Miller and Mallison (1) for specific isolation and detection of Salmonellae. This medium is superior to XLT4 Agar in supporting growth of Salmonella due to the presence of appropriate proportion of four sugars. HiCrome™ MM Agar, Modified is a slight modification of HiCrome™ MM Agar and designed to differentiate *Enterobacteriaceae* especially *Salmonella* from *Proteus* and *Citrobacter* group. The utilization of sugars by organisms results in pH-changes. This is used as a means of distinguishing *Salmonella* from competing bacteria on the basis of colony colour.

*Salmonella* are gram negative, anaerobic, non sporulating rods in the family *Enterobacteriaceae* present in the stomach and intestinal tissues of human & animals and are found in their wastes. *Salmonella* usually are unable to ferment the sugars (2) that support growth of competing bacteria. Thus other bacteria tend to overgrow Salmonellae, masking their presence. Proteose peptone is a source of carbon, nitrogen and other essential amino acid and growth factor. Yeast extract which provides nitrogen and vitamin required for growth. To add to the differentiating ability of the formulation, an H$_2$S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. Bromothymol blue act as a pH indicator. The inclusion of sugars like lactose, sucrose, xylose and cellobiose provides source of fermentable carbohydrate which stimulate the better initial growth of *Salmonella* cells. Presence of lactose suppresses H$_2$S production by non-salmonellae like *Citrobacter freundii*. A chromogenic mixture, present in this medium helps to differentiate between lactose fermenters and nonfermenters. Lactose fermenters give bluish green coloured colonies, which would have been impossible to differentiate with an indicator based on pH change.

Type of specimen

Clinical samples - Blood, Faeces; Food samples; Water samples

Please refer disclaimer Overleaf.
Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(6)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :
In Vitro diagnostic Use only. 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.Due to nutritional variations, some strains show poor growth
2.Though most of the Salmonella produce H$_2$S certain non H$_2$S producing Salmonella species may appear as colourless colonies.
3.Certain Salmonella species which are lactose fermenters may show as bluish green coloured colonies
4. Further confirmation may be carried out on suspected colonies.

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
Firm, comparable with 1.8% Agar gel

Colour and Clarity of prepared medium
Bluish Green coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 8.07 % w/v aqueous solution at 25°C. pH : 7.6±0.2
pH
7.40-7.80

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>Recovery</th>
<th>Colour of Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrobacter freundii</em> ATCC 8090</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>Yellow coloured</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>Bluish green</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black centered</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 13076 (00030*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>black centered with yellow zone</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em> ATCC 6539</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>Black centered</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> ATCC 25933</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>Gray coloured</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC 13883</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>Yellowish green</td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

Please refer disclaimer Overleaf.
Storage and Shelf Life
Store between 2-8°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.

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 (3,4).

Reference

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

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