HiCrome™ Coliform HiCynth™ Agar w/ SLS

Intended use

Recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water, food and clinical samples.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ peptone No.3#</td>
<td>3.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>3.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.700</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>1.000</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium lauryl sulphate</td>
<td>0.100</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>0.200</td>
</tr>
<tr>
<td>Agar</td>
<td>12.000</td>
</tr>
</tbody>
</table>

Final pH (at 25°C) 6.8±0.2

**Formula adjusted, standardized to suit performance parameters

# - Chemically defined peptones

Directions

Suspend 27 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. Add 5mg/l novobiocin before autoclaving the medium, when a high number of gram positive accompanying bacteria are expected. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ Coliform Agar w/ SLS is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (7). HiCrome™ Coliform HiCynth™ Agar w/ SLS is prepared by completely replacing animal based peptones with chemically defined peptones to avoid BSE/TSE/GMO risks associated with animal peptones.

HiCynth™ peptone No.3 and sodium pyruvate provide essential growth nutrients to the organisms. The phosphates buffer the medium well. The medium composition helps even the sub lethally injured coliforms to grow rapidly. Sodium lauryl sulphate inhibits gram-positive organisms. The chromogenic mixture contains two chromogenic substrates. The enzyme β-galactosidase produced by coliforms cleaves one chromogen, resulting in the salmon red colouration of coliform colonies. The enzyme β-glucuronidase produced by *E. coli*, cleaves X-glucuronide. *E.coli* forms dark blue to violet coloured colonies due to cleavage of both the chromogens (2,5,6). The addition of L-Tryptophan improves the indole reaction, thereby increasing detection reliability in combination with the two chromogens. To confirm *E.coli*, add a drop of Kovacs reagent (R008) on the dark-blue to violet colony. Formation of cherry-red colour indicates positive reaction.

Type of specimen

Clinical samples - faeces, urine, food samples; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (8).

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 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.

Please refer disclaimer Overleaf.
**Limitations:**
1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Certain species of *Shigella* and *Salmonella* are ß-glucuronidase positive.

**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 beige homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.2% Agar gel.

**Colour and Clarity of prepared medium**
Light yellow, clear to slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 2.7% w/v aqueous solution at 25°C. pH : 6.8±0.2

**pH**
6.60-7.00

**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 24 hours (48 hours if necessary).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of Colony</th>
<th>Indole production</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrobacter freundii ATCC 8090</em></td>
<td>50-100</td>
<td>good-luxuriant &gt;=50%</td>
<td>salmon to red</td>
<td>negative reaction</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 8739 (00012)</em></td>
<td>50-100</td>
<td>good-luxuriant &gt;=50%</td>
<td>dark blue/violet</td>
<td>positive, confirmation of red colour around the colony by addition of Kovacs reagent (R008)</td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter cloacae ATCC 23355 (00082)</em></td>
<td>&gt;=10³</td>
<td>inhibited 0%</td>
<td>salmon to red</td>
<td>negative reaction</td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis ATCC 29212 (00087)</em></td>
<td>&gt;10³</td>
<td>good-luxuriant &gt;=50%</td>
<td>light pink</td>
<td>negative reaction</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae ATCC 13883 (00097)</em></td>
<td>50-100</td>
<td>good-luxuriant &gt;=50%</td>
<td>colourless</td>
<td>negative reaction</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Enteritidis ATCC 13076 (00030)</em></td>
<td>50-100</td>
<td>good 40-50%</td>
<td>colourless</td>
<td>negative reaction</td>
<td></td>
</tr>
<tr>
<td><em>Shigella flexneri ATCC 12022 (00126)</em></td>
<td>50-100</td>
<td>good 40-50%</td>
<td>colourless</td>
<td>negative reaction</td>
<td></td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**
Store dehydrated powder and prepared medium on receipt at 2-8°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.

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 (3,4).

Reference


Revision : 02/2019