HiCrome™ ECC Agar

**Intended Use:**
Recommended as a differential medium for presumptive identification of *Escherichia coli* and other coliforms in food, environmental and clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone, special</td>
<td>5.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>2.500</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>3.500</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>20.300</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.030</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 55.83 grams in 1000 ml 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**

*Escherichia coli*, a member of the family *Enterobacteriaceae* is a part of normal flora of the intestinal tract of humans and a variety of animals. Although most of *E. coli* does not cause gastrointestinal illnesses, certain groups of *E. coli* can cause life-threatening diarrhoea and sever sequelae or disability (1). HiCrome™ ECC Agar is a differential medium recommended for the presumptive identification of *E. coli* and other coliforms in food and environmental samples (2). The medium contains two chromogens. One of the chromogen is cleaved by the enzyme glucuronidase produced by *E. coli* to give blue to purple coloured colonies whereas the other chromogen is cleaved by the enzyme galactosidase, produced by majority of coliforms, resulting in the formation of rose-pink coloured colonies (5,6).

Peptone special, yeast extract provide nitrogenous, carbonaceous substances, long chain amino acids, vitamin B complex and other essential growth nutrients. Lactose is the fermentable carbohydrate, which aids in detecting lactose fermenters with neutral red as an indicator. Disodium hydrogen phosphate and potassium dihydrogen phosphate buffers the medium well. Sodium chloride maintains the osmotic equilibrium. Dry the surface of plate medium.

Dilute the food sample by 1:5 or 1:10 with 0.1% sterile Peptone Water (M028) and homogenize in a blender or a stomacher. Spread 0.5 ml or 1.0 ml of the homogenate over the agar surface with a sterile glass spreader and incubate the plates at 37°C for 18-24 hours. Count the blue/purple colonies and multiply with the dilution factor. The number of *E. coli* is reported per gram of food. The medium should be used only for in-vitro diagnostic purpose. Wear mask while handling the dehydrated product and avoid contact with eyes.

**Type of specimen**
Clinical samples, Food and environmental 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 (7).
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. β-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Some species may show poor growth due to nutritional variations.

**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 pink homogeneous free flowing powder

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

**Colour and Clarity of prepared medium**
Reddish pink coloured, opaque gel forms in Petri plates

**Reaction**
Reaction of 5.58% 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 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>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>blue/purple</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853 (00025*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>straw</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC 13883 (00097*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>rose/pink</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 13076 (00030*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>pink</td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

**Storage and Shelf Life**
Store dehydrated powder and prepared medium on receipt at 2-8°C. Use before expiry period 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).
Reference


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