**Intended Use:**
Recommended for detection and enumeration of *Enterobacteriaceae* in raw food and clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.2*</td>
<td>7.000</td>
</tr>
<tr>
<td>HiCynth™ Peptone No.5*</td>
<td>3.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Synthetic detergent</td>
<td>1.500</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>10.000</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.030</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.002</td>
</tr>
<tr>
<td>Agar</td>
<td>12.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 38.53 grams in 1000 ml purified / 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**
Violet Red Bile Agar, a modification of MacConkey original formulation (7) is used for the enumeration of coliform bacterial group.

Violet Red Bile Glucose Agar w/o Lactose, a modification of VRBA (M049), was designed for the enumeration of *Enterobacteriaceae* (9). Violet Red Bile Glucose HiCynth™ Agar w/o Lactose is a modification of Violet Red Bile Glucose Agar wherein animal or vegetable peptones are replaced with chemically defined peptone to avoid BSE/TSE/GMO risks associated with animal peptones. It employs the selective inhibitory components crystals violet and bile salts and the indicator system glucose and neutral red. Sought bacteria will dissimilate glucose and produce purple zones around the colonies (3). ISO committee has also recommended this medium (4). Selectivity of VRBGA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C (10-12).

HiCynth™ Peptone No.2 and HiCynth™ Peptone No.5 serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Glucose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (8).

**Type of specimen**
Clinical samples; Food and dairy samples; Water samples

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,13,14). 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**
In Vitro diagnostic use. 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. Over incubation may result in reverting of reaction.
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 pinkish beige homogeneous free flowing powder

Gelling
Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium
Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 3.85% w/v of aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics was observed after an incubation at 35-37°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

<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 ATCC 25922 (00013</em>)*</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50 %</td>
<td>pink-red</td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 8739 (00012</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50 %</td>
<td>pink-red with</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bile precipitate</td>
</tr>
<tr>
<td><em>Escherichia coli NCTC 9002</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50 %</td>
<td>pink-red with</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bile precipitate</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa ATCC 9027 (00026</em>)*</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50 %</td>
<td>pink to red</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis ATCC 13076 (00030</em>)*</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50 %</td>
<td>light pink</td>
</tr>
<tr>
<td>#<em>Klebsiella aerogenes ATCC 13048 (00175</em>)*</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50 %</td>
<td>pink-red</td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp.aureus ATCC 25923 (00034</em>)*</td>
<td>&gt;=10^4</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp.aureus ATCC 6538 (00032</em>)*</td>
<td>&gt;=10^4</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.
# - Formerly known as *Enterobacter aerogenes*

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

Revision : 02/2019