Intended use

Violet Red Bile HiCynth™ Agar is selective medium used for the isolation, detection and enumeration of coli-aerogenes bacteria in water, milk, other dairy food products and from clinical samples.

### Ingredients

<table>
<thead>
<tr>
<th>Component</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>Lactose</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>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters
*- Chemically defined peptones

### Directions

Suspend 41.53 grams in 1000 ml purified / distilled water. Heat with stirring to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and pour into sterile Petri plates containing the inoculum. If desired, the medium can be sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

### Principle And Interpretation

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The historical definition of this group has been based on the method used for detection i.e. lactose fermentation. This group is defined as all aerobic and facultative anaerobic, gram-negative, non-spore-forming rod shaped bacteria that ferment lactose with gas and acid formation within 48 hour at 35°C (7,12). Examination of foods, ingredients and raw materials, for the presence of marker groups such as coliforms is the one of the common tests.

Violet Red Bile Agar, a modification of MacConkey's original formulation (7) is used for the enumeration of coli-aerogenes bacterial group. It relies on the use of the selective inhibitory components crystals violet and bile salts and the indicator system lactose, and neutral red. Thus, the growth of many unwanted organisms is suppressed, while tentative identification of sought bacteria can be made. Organisms, which rapidly attack lactose, produce purple colonies surrounded by purple halos. Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones (3). VRBA is recommended by APHA (1,11). Selectivity of VRBA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C (8-10). It is also recommended by ISO (4). Violet Red Bile HiCynth™ Agar is a modification wherein animal based peptones are replaced with chemically defined peptones to avoid BSE/TSE/GMO risks associated with animal peptones.

HiCynth™ Peptone No.2 and HiCynth™ Peptone No.5 serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Lactose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and synthetic detergent help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Violet Red Bile HiCynth™ Agar is not completely specific for enteric; other accompanying bacteria may give the same reaction. Further biochemical tests are necessary for positive identification (11).

### Type of specimen

Clinical samples - Blood; 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 (1,11,12).
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.

Limitations:
1. 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 pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel.

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

Reaction
Reaction of 4.15% w/v aqueous solution at 25°C, pH : 7.4±0.2

pH
7.20-7.60

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># Klebsiella aerogenes</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>pink to pinkish red</td>
</tr>
<tr>
<td>ATCC 13048 (00175*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>pinkish red with bile precipitate</td>
</tr>
<tr>
<td>25922 (00013*)</td>
<td></td>
<td></td>
<td></td>
<td>Colourless to orangish yellow</td>
</tr>
<tr>
<td>Salmonella Enteritidis</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td></td>
</tr>
<tr>
<td>ATCC 13076 (00030*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>subsp.aureus ATCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25923 (00034*)</td>
<td></td>
<td></td>
<td></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).

Please refer disclaimer Overleaf.
References


Disclaimer:
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In vitro diagnostic medical device
CE Marking
Storage temperature

Do not use if package is damaged

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