Rapid HiColiform™ HiVeg™ Broth

Intended use
Recommended for detection and confirmation of *Escherichia coli* and total coliforms from water and clinical samples, using a combination of chromogenic and fluorogenic substrates.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ special peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>1.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>2.700</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium lauryl sulphate</td>
<td>0.100</td>
</tr>
<tr>
<td>Chromogenic substrate</td>
<td>0.080</td>
</tr>
<tr>
<td>Fluorogenic substrate</td>
<td>0.050</td>
</tr>
<tr>
<td>IPTG</td>
<td>0.100</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 16.03 grams in 1000 ml purified/distilled water. For double strength broth use 32.06 grams of M1453A in 1000 ml distilled water. Heat if necessary 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 dispense as desired.

Principle And Interpretation
Rapid HiColiform Broth is a modification of LMX Broth described by Manafi and Kneifel (2). This medium is useful for the detection and confirmation of *Escherichia coli* and total coliforms in water samples on the basis of chromogenic and fluorogenic substrates (2-9). Rapid HiColiform HiVeg™ Broth is prepared by completely replacing animal based peptones with vegetable peptones to avoid BSE/TSE risks associated with animal peptones.

The fluorogenic substrate is split by enzyme β-D-glucuronidase specifically found in *Escherichia coli*. The reaction is indicated by the development of a blue fluorescence under UV light. The presence of total coliforms is indicated by blue-green colourations due to the cleavage of the chromogenic substrate. IPTG amplifies enzyme synthesis and increases the activity of β-D-galactosidase. To confirm presence of *E.coli* overlay the medium with Kovacs reagent. The layer turns red within 2 minutes in case of positive reaction.

HiVeg™ special peptone serves as a source of carbon and nitrogen compounds, long chain amino acids, vitamins and other essential growth nutrients. Sorbitol is the fermentable carbohydrate. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium lauryl sulphate makes the medium selective by inhibiting accompanying microflora, especially the gram-positive organisms.

Type of specimen
Clinical samples- 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 (10).

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.

Please refer disclaimer Overleaf.
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. 97% of the E.coli strains are β-D-glucuronidase positive, however few strains may show negative fluorescence.

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

Colour and Clarity of prepared medium
Light yellow coloured, clear solution having slight precipitate in tubes

Reaction
Reaction of 1.60% 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>Colour of Medium</th>
<th>Fluorescence (under uv)</th>
<th>Indole reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td># Klebsiella aerogenes</td>
<td>50-100</td>
<td>luxuriant</td>
<td>blue-green</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>ATCC 13048 (00175*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># Formerly known as Enterobacter aerogenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC</td>
<td>50-100</td>
<td>luxuriant</td>
<td>blue-green</td>
<td>positive</td>
<td>positive reaction</td>
</tr>
<tr>
<td>25922 (00012*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: *Corresponding WDCM numbers.

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 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

Please refer disclaimer Overleaf.

Disclaimer:
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