Intended Use:
Recommended for the selective enumeration of presumptive *Escherichia coli* by MPN technique from water samples and from clinical samples.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ hydrolysate</td>
<td>20.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.000</td>
</tr>
<tr>
<td>Synthetic detergent no. 1</td>
<td>1.500</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>4.000</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>Final pH (at 25°C)</td>
<td>6.9±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 37.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in test tubes containing inverted Durhams tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the concentration of medium in accordance with sample size.

Principle And Interpretation
EC Medium is used for detection of coliforms during bacteriological examination of water, milk and foods. It was originally described by Hajna and Perry (3). This medium was later used by Fishbein and Surkiewicz to carry out *Escherichia coli* confirmatory tests (2). It is also used in MPN methods (1) and is often used for confirmation of coliforms. EC HiVeg™ Broth is same as EC Broth except that the animal based peptones are completely replaced with vegetable peptones to avoid the BSE/TSE risks associated with animal peptones.

The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or non-fecal) when used as a confirmatory test (6). EC HiVeg™ Broth should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of faecal coliforms is required. HiVeg™ hydrolysate provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Lactose is the fermentable sugar. Synthetic detergent no. 1 inhibit gram-positive bacteria especially bacilli and faecal Streptococci. Phosphates control the pH during fermentation of lactose. Gas production in a fermentation tube within 24 hour or less is a presumptive evidence of the presence of coliform bacteria. This medium can be used at 37°C for the detection of coliform organisms or at 44.5°C for the isolation of *Escherichia coli* from water and shellfish or 45.5°C for foods.

When using sample more than 10 ml, the medium must be reconstituted at a concentration equivalent to that specified on the directions, once the sample is added, the working procedure is as follows:
Transfer a loopful of culture from all the tubes of Lauryl Sulphate HiVeg™ Broth (MV080) showing gas formation within 24 hours and from all the tubes showing bacterial growth within 48 hours to EC HiVeg™ Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water bath and incubate at 44°C for 24 hours. Consider the growth showing gas production as positive.

Calculate the density of the faecal coliform organisms by using MPN tables. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (8). Gas formation at 44.5°C or 45.5°C (and 37°C) *Escherichia coli*, possibly also other coliforms. Gas formation at 37°C Coliform bacteria without *Escherichia coli*.
Type of specimen
Clinical - faeces; Food samples; Water sample.

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,9,10).
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.

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. For identification, organisms must be in pure culture.
2. Morphological, biochemical and/or serological tests should be performed for final identification.

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
Yellow coloured, clear solution without any precipitate

Reaction
Reaction of 3.7% w/v aqueous solution at 25°C. pH : 6.9±0.2
pH
6.70-7.10

Cultural Response
Cultural characteristics observed after an incubation at 44.5°C ± 0.2 for 24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumonia</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction</td>
</tr>
<tr>
<td>ATCC 13883 (00097*)</td>
<td></td>
<td></td>
<td>negative reaction</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>50-100</td>
<td>fair to good</td>
<td>negative reaction</td>
</tr>
<tr>
<td>ATCC 27853 (00025*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td>ATCC 29212 (00087*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis subsp.</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td>spizizenii ATCC 6633</td>
<td>(00003*)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction</td>
</tr>
<tr>
<td>ATCC 25922 (00013*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># Klebsiella aerogenes</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td>ATCC 13048 (00175*)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key *
- Corresponding WDCM Numbers ;
# - Formerly known as Enterobacter aerogenes

Please refer disclaimer Overleaf.
Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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 (4,5).

Reference
In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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