M-Lauryl Sulphate HiVeg™ Broth

Intended Use

M-Lauryl Sulphate HiVeg™ Broth is used for enumeration of *Escherichia coli* and coliforms in water, using membrane filter technique.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ special peptone</td>
<td>39.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>6.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>30.000</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.200</td>
</tr>
<tr>
<td>Sodium lauryl sulphate (SLS)</td>
<td>1.000</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**

**Directions**

Suspend 76.2 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

**Principle And Interpretation**

M-Lauryl Sulphate HiVeg™ Broth is a modification of M-Lauryl Sulphate Broth. It is prepared by replacing animal based peptones with veg peptones and it is free from BSE/TSE risk.

The membrane filter technique is used to test relatively large volumes of samples. It is extremely useful in monitoring drinking water and a variety of natural waters (1). The earlier medium used to detect coliforms in water employed bile salts as the selective agent. This was replaced with Teepol by Burman (2). The effectiveness of teepol was demonstrated earlier (6, 7).

M-Lauryl Sulphate Broth is similar to this medium, the only difference being the use of sodium lauryl sulphate as the inhibitory agent instead of teepol. It is recommended for enumeration of *Escherichia coli* and coliforms using membrane filtration technique (8, 4). M-Lauryl Sulphate HiVeg Broth is prepared by replacing animal based peptones by veg peptones in M-Lauryl Sulphate Broth.

An absorbent pad is saturated with M-Lauryl Sulphate HiVeg™ Broth. The filter, through which the water sample is passed, is aseptically placed on this saturated absorbent pad, with face upwards. Burman (3) recommended the following incubation temperatures and durations:

Unchlorinated waters:
- Coliform organisms: 4 hours at 30°C followed by 14 hours at 35°C
- *Escherichia coli*: 4 hours at 30°C followed by 14 hours at 44°C

Non-chlorinated organisms benefit from 4 hours incubation at 30°C but chlorinated organisms require 6 hours incubation at 25°C. After incubation, yellow colonies are formed which should be confirmed further.

HiVeg™ special peptone and yeast extract act as a source of nitrogen, carbon and amino acids. Lactose is the source of fermentable carbohydrate. Phenol red serves as an indicator. Sodium lauryl sulphate inhibits gram-positive bacteria.

**Type of specimen**

Water samples

**Specimen Collection and Handling**

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
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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations
1. Some strains 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 coloured homogeneous free flowing powder

Colour and Clarity of prepared medium
Red coloured clear solution without any precipitate

Reaction
Reaction of 7.62% w/v aqueous soluti at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed on membrane filter after an incubation at 44°C for 18 - 24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Colour of Colony on Membrane</th>
<th>Growth on membrane (at 35-37°C)</th>
<th>Growth on membrane (at 43-45°C)</th>
<th>Colour of Colony on Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em> subsp. <em>spizizenii ATCC 6633</em> (00003*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922</em> (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>luxuriant</td>
<td>yellow</td>
</tr>
<tr>
<td># Klebsiella aerogenes ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>inhibited</td>
<td>yellow</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium ATCC 14028</em> (00031*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>inhibited</td>
<td>pink</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 15-25°C. se 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. mproper 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. se 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 (5,9).
Reference


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