Crystal Violet Lactose Broth

Intended Use:
Recommended for detection of coliforms in water filtration control works.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>5.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.00143</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 16 grams in 1000 ml purified/distilled water. Distribute in 10 ml quantities in tubes with inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If the inoculum is more than 1 ml double or multiple strength media must be used.

Principle And Interpretation

The extent to which total coliforms are present in the source water can indicate the general quality of that water and the likelihood that the water is faecally contaminated. Total coliforms are currently controlled in drinking water regulations (i.e. Total Coliform Rule) because their presence above the standard indicates inefficacy in treatment or in the distribution system. Environmental Protection Agency (EPA) requires all public water systems to monitor for total coliforms in distribution systems. If coliforms are found, then the public water system must further analyze coliform-positive sample for specific types of coliforms (i.e., faecal coliforms or E. coli). EPA is increasing protection from pathogens in surface water systems as part of the Interim Enhanced Surface Water Treatment Rule. Crystal Violet Lactose Broth is used for the detection of coliforms in such water filtration control works.

The media contains proteose peptone and peptone as sources of carbon, nitrogen, vitamins and minerals. Dipotassium hydrogen phosphate and Potassium dihydrogen phosphate provide buffering to the medium. Lactose is the carbon and energy source. Crystal violet inhibits most of the gram positive organisms.

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. 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. This medium is general purpose medium and may not support the growth of fastidious organisms.

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 light tan homogeneous free flowing powder

Colour and Clarity of prepared medium
Light purple coloured, clear solution in tubes

Reaction
Reaction of 1.6% 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 40-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Colour of medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>purple</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>50-100</td>
<td>none</td>
<td>light yellow</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em> ATCC 12228</td>
<td>50-100</td>
<td>fair</td>
<td>purple/very</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> ATCC 19615</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>slightly yellow</td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

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 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

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