**Violet Red Bile Broth**

**Intended Use:**
Recommended for detection and enumeration of coliforms from water and food.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>7.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Bile salts mixture</td>
<td>1.500</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.030</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.002</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

*Note: Formula adjusted, standardized to suit performance parameters*

**Directions**
Suspend 26.53 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 dispense into sterile tubes containing the inoculum.

**Principle And Interpretation**
Violet Red Bile Broth, a modification of MacConkey’s original formulation (5) is used for the enumeration of *coli-aerogens* 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. Lactose non-fermenters and late lactose fermenters produce pale coloured medium. Other related gram-negative bacteria can be suppressed by incubation at >42°C or by anaerobic incubation. Incubation may be carried out at >42°C for 18 hours, 32°C for 24-48 hours or 4°C for 10 days depending on the temperature characteristics of the organisms to be recovered (8). Violet red bile Broth is similar to VRBA (M049), except agar that is recommended by APHA (2,7).

Peptone and yeast extract 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 bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (6).

**Type of specimen**
Food samples; Water samples.

**Specimen Collection and Handling**
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (2,9). 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. Further biochemical tests are necessary for positive identification (5).
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 pinkish beige homogeneous free flowing powder

Colour and Clarity of prepared medium
Reddish purple coloured clear solution in tubes.

Reaction
Reaction of 2.65% 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>Colour of medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
<td>luxuriant</td>
<td>pink to pinkish red</td>
</tr>
<tr>
<td># Klebsiella aerogenes ATCC 13048</td>
<td>50-100</td>
<td>luxuriant</td>
<td>pink to pinkish red</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 13076</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>colourless to orangish yellow</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 25923</td>
<td>&gt;=10⁴</td>
<td>inhibited</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 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 (3,4).

Reference

Please refer disclaimer Overleaf.
**Disclaimer:**

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