Lauryl Tryptose Mannitol Broth w/ Tryptophan

**Intended Use:**
A single tube medium used for confirmation of *Escherichia coli* in drinking water.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>20.000</td>
</tr>
<tr>
<td>Mannitol</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>2.750</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>2.750</td>
</tr>
<tr>
<td>Sodium lauryl sulphate</td>
<td>0.100</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.200</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 35.8 grams in 1000 ml purified / distilled water. Warm gently to dissolve the medium completely. Dispense in tubes with inverted Durhams tubes. Sterilize by autoclaving at 115°C for 10 minutes. Cool to 45-50°C.

**Principle And Interpretation**

Identification of bacteria that constitute the coliform group sometimes is necessary to determine the nature of pollution. It is of particular importance in reference to distinguishing the presence of *Escherichia coli*, an indicator of fecal contamination. Lauryl Tryptose Mannitol Broth w/ Tryptophan is a single tube medium used for the confirmation of *E. coli* in drinking water as recommended by report 71 (2). This medium is also recommended by the ISO committee (3) and is also a suitable medium as per the requirements of the EC Directive (5) for the quality of drinking water. This medium may be used in parallel to Lauryl Tryptose Broth (M080) to detect non-lactose fermenting strains of *E. coli*.

Tryptose is the source of carbon, nitrogen, vitamins, amino acids and other essential growth requirements. Mannitol is the fermentable carbohydrate. Phosphates buffer the medium whereas sodium lauryl sulphate serves to inhibit accompanying non-coliform bacteria. L-tryptophan is a substrate of tryptophan deaminase enzyme.

*E. coli* is confirmed by gas and indole production when incubated at 44°C for 24 hours. If the indole test is negative even if in a single tube medium, repeat the test in Tryptone Water (M463). Each tube showing acid and gas in the multiple tube test is subcultured to a tube of Lauryl Tryptose Mannitol Broth with Tryptophan and incubated at 44°C.

**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. Incorrect inoculum concentration may produce inaccurate results.
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 3.58% 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 44°C for 24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Gas</th>
<th>Indole Production</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>positive reaction</td>
<td>positive reaction, red ring at the interface of the medium</td>
</tr>
</tbody>
</table>

# *Klebsiella aerogenes* ATCC 13048 (00175*)
>=10⁴ inhibited

*Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*)
>=10⁴ inhibited

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 (4,6).
Reference