Saline Tryptone / Tryptophan Medium

**Intended Use:**
Recommended for detection of indole production by *Vibrio parahaemolyticus*. The composition and performance criteria are in accordance with ISO 8914:1990.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10.000</td>
</tr>
<tr>
<td>DL-tryptophan</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>30.000</td>
</tr>
<tr>
<td>Final pH (after sterilization) at 25°C</td>
<td>7.50</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 41 grams in 1000 ml purified / distilled water. Heat, if necessary to dissolve the medium completely. Mix well and dispense in quantities of 5ml into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Principle And Interpretation**

*Vibrio parahaemolyticus* is a halophilic estuarine organism. This organism can be isolated from a variety of sea food product and marine environments. The organism, when isolated from fresh sea food, is usually found in low number and is sensitive to refrigeration and heat. Saline Tryptone/ Tryptophane Medium is in accordance with ISO 8914:1990 (2) recommended for detection of *Vibrio parahaemolyticus* on the basis of indole production.

Tryptone provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. High concentration of sodium chloride and alkaline pH of the medium provides condition that facilitates easy recovery of *V.parahemolyticus* and restrict the growth of other bacteria. *Vibrio parahaemolyticus* break down tryptophane into indole and alpha-aminopropionic acid. The presence of indole in the medium can be detected by Kovac’s reagent (R008).

Inoculate Saline Tryptone/ Tryptophan Medium with the suspected colony and incubate at 35-37°C for 24hrs. After incubation add 1ml of Kovac’s reagent (R008). The formation of red ring indicates a positive reaction, while yellow-brown ring indicates a negative reaction.

**Type of specimen**
Food and dairy samples.

**Specimen Collection and Handling:**
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,5,6). 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 and serological tests must be carried out for further 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
Light yellow coloured clear solution

Reaction
Reaction of 4.1% w/v aqueous solution at 25°C. pH : 7.50

pH
7.50

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>Indole test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>50-100</td>
<td>luxuriant</td>
<td>positive reaction, red ring at the interface of the medium on addition of Kovac's reagent (R008)</td>
</tr>
<tr>
<td>ATCC 17802 (00037*)</td>
<td></td>
<td></td>
<td></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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference