Salt Polymyxin Broth Base

**Intended use**

Recommended for detection and enumeration of *Vibrio* species. The composition and performance criteria of this medium are as per the specifications laid down in ISO 8914:1990.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone #</td>
<td>9.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.700</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>18.000</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**

* # Peptic digest of animal tissue

**Directions**

Suspend 14.85 grams in 500 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 - 50°C and aseptically add rehydrated contents of 1 vial of Polymyxin B Selective Supplement (FD003). Mix well and dispense into sterile tubes or flasks as desired.

**Principle and Interpretation**

*Vibrios* are fairly easy to isolate from both clinical and environmental material, though some species may require growth factors and/or vitamins. *Vibrio parahaemolyticus* is the leading cause of bacterial diarrhoea associated with the consumption of contaminated food products. Salt Polymyxin Broth (M821) is formulated as per the recommendation of APHA (4). ISO Committee (2) has recommended a modification (M821I) of this medium for detection and enumeration of salt tolerant *Vibrio parahaemolyticus*.

Peptone and yeast extract provide nitrogenous compounds, carbon, sulphur, trace elements, long chain amino acids and vitamin B complex, essential for the growth. Polymyxin B sulphate inhibits gram-positive organisms.

Weigh 50 grams of sample into a blender. Add 450 ml phosphate buffer saline dilution water and blend for 1 minute at 8000 rpm. This constitutes 1:10 dilution. Prepare 1:100, 1:1000, 1:10000 dilutions or higher, if necessary, in PCB. Inoculate 3 x 10 ml portion of the 1 : 10 dilutions into 3 tubes containing 10 ml of enrichment broth i. e. Salt Polymyxin Broth Base-2x concentration. This represents the 1 gram portion. Similarly inoculate 3 x 1 ml of dilutions into 10 ml of single strength Salt Polymyxin Broth Base. Incubate tubes at 35 ± 2°C for 24 hours.

After incubation a loopful is subcultured on solid medium such as TCBS Agar (M189) for further studies. *V. parahaemolyticus* appears as round, green or bluish colonies, 2-3 mm in diameter while *V. cholerae* forms yellow coloured colonies.

**Type of specimen**

Food samples and animal feeding stuffs

**Specimen Collection and Handling**

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (1,4). 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.
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 without any precipitate

Reaction
Reaction of 2.97% 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 24-48 hours with added Polymyxin B Selective Supplement (FD003).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio cholerae ATCC 14035</td>
<td>50-100</td>
<td>luxuriant</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus ATCC 17802</td>
<td>50-100</td>
<td>luxuriant</td>
</tr>
</tbody>
</table>

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. 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 (2,3).

Reference