**Saline Nutrient Agar**

**Intended Use:**
Recommended for isolation and cultivation of *Vibrio parahaemolyticus* from food products or animal feeding products.

### 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>HM extract#</td>
<td>3.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>30.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>pH after sterilization (at 25°C)</td>
<td>8.50±0.2</td>
</tr>
</tbody>
</table>

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

# - Equivalent to Meat extract

### Directions

Suspend 53.0 grams in 1000 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. Mix well and pour in sterile Petri plates.

### 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 Nutrient Agar is recommended by ISO 8914:1990 (1) for isolating and enumerating *Vibrio parahaemolyticus* from food and animal feed.

Peptone and HM extract provide nitrogen compounds, growth factors and vitamins for the growth of *Vibrio parahaemolyticus*. High sodium chloride content and alkaline pH of the medium provides conditions that facilitate easy growth of *Vibrio parahaemolyticus*, while restricting the growth of most gram-negative microorganisms.

For isolation and confirmation of *Vibrio parahaemolyticus*, five typical colonies from Thiosulfate Citrate Bile Sucrose Agar (TCBS) or Triphenyltetrazolium Chloride Soya Tryptone Agar (TSAT) are subculture onto Saline Nutrient Agar followed by biochemical confirmation.

### 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. This medium is general purpose medium and may not support the growth of fastidious organisms.
2. Salt tolerant bacteria may grow.
3. Further isolation and biochemical tests must be carried out for confirmation.

### 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

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.3% w/v aqueous solution after sterilization at 25°C. pH : 8.5±0.2
pH
8.30 - 8.70

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>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio parahaemolyticus ATCC 17802 (00037*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Vibrio chlorae ATCC 15748</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td># Klebsiella aerogenes ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</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 20-30°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

Disclaimer :
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.