Listeria Selective Broth Base

Intended Use:
Recommended for selective isolation and cultivation of *Listeria monocytogenes* from clinical specimens.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>17.000</td>
</tr>
<tr>
<td>Soya peptone</td>
<td>3.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>6.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>2.500</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>2.500</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.3±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 36 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 and aseptically add rehydrated contents of 1 vial of Listeria Selective Supplement II (FD063) or 2 vials of Listeria Selective Supplement II (FD063I) as desired. Mix well and dispense into sterile tubes or flasks or as desired.

**Principle And Interpretation**

*Listeria monocytogenes* is a short, gram-positive, non spore-forming rod shaped bacterium that appears coccoidal in older cultures. *Listeria* multiplies over a wide range of temperatures from 3°C to 45°C with optimum temperature range of 30°C to 37°C. *L.monocytogenes* has been isolated from numerous environmental sources such as silage, soil, decaying vegetation, sewage, damp earth, straw and faeces. Detection of *L.monocytogenes* in foods is not difficult. Low numbers of organisms are commonly isolated from raw milk, meat, vegetables, seafood and the food-processing environment. Enrichment procedures are used to isolate low numbers of *L.monocytogenes*. Injured *L. monocytogenes* are sublethally stressed as a result of exposure to heat, freezing or acidic conditions. Sublethally stressed *L. monocytogenes* require resuscitation in a non-selective medium at a temperature favouring repair of the sublethal injury.

Listeria Selective Broth is formulated as per Lovett et al (7) for the selective enrichment of *Listeria* species from milk and milk products and other foods. Listeria Selective Broth is recommended by ISO Committee (3) with a slight modification in the supplement (FD063I).

Tryptone, soya peptone and yeast extract provide carbon and nitrogen compounds, long chain amino acids, vitamins essential for bacterial metabolism. Dextrose is the energy source. The medium is rendered selective by addition of selective supplement. Cycloheximide inhibits the growth of saprophytic fungi. Nalidixic acid inhibits growth of gram-negative organisms and acriflavin suppresses gram-positive microorganisms. Acriflavin is an acridinic derivative with bacteriostatic properties towards many gram-positive bacteria and a weak fungicidal activity.

For enrichment, 25 grams or 25 ml sample is added to 225 ml medium in a stomacher bag. Homogenize the material if required. Incubation is carried out at 30°C for upto 7 days. Ajello et al (1) showed that incubation period of 7 days allows better recovery of environmentally stressed *Listeria* from milk and milk products. The enrichment broth is further subcultured on Listeria Selective Agar (M567) after 1, 2 and 7 days.

*Listeria monocytogenes* is a highly pathogenic organism and therefore proper precautions should be taken while handling them.

**Type of specimen**

Clinical samples - Body tissues or body fluids
Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:
In Vitro diagnostic Use only. 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 clinical specimens. Saftey guidelines may be referred in individual safety data sheets

Limitations:
1. Further biochemical tests are needed for a final identification of the isolated organisms.
2. *Listeria monocytogenes* is a highly pathogenic organism and therefore proper precautions should be taken while handling them.

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**
Fluorescent yellow coloured, clear solution in tubes

**Reaction**
Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.3±0.2

**pH**
7.10-7.50

**Cultural Response**
Cultural characteristics observed with added Listeria Selective Supplement II (FD063 / FD063I) after an incubation at 30-35°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans ATCC 10231</em></td>
<td>( \geq 10^4 )</td>
<td>inhibited</td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>( \geq 10^4 )</td>
<td>inhibited</td>
</tr>
<tr>
<td><em>Listeria monocytogenes ATCC 19111</em></td>
<td>50-100</td>
<td>luxuriant</td>
</tr>
<tr>
<td><em>Listeria monocytogenes ATCC 19112</em></td>
<td>50-100</td>
<td>luxuriant</td>
</tr>
<tr>
<td><em>Listeria monocytogenes ATCC 19118</em></td>
<td>50-100</td>
<td>luxuriant</td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp. aureus ATCC 25923</em></td>
<td>50-100</td>
<td>none-poor</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 2-8°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.

Please refer disclaimer Overleaf.
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 (4,5).

Reference


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