**Listeria Oxford HiCynth™ Medium Base**

**Intended use**
Recommended for isolation of *Listeria* species from pathological specimens.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.1*</td>
<td>23.000</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>15.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Corn starch</td>
<td>1.000</td>
</tr>
<tr>
<td>Esulin</td>
<td>1.000</td>
</tr>
<tr>
<td>Ammonium ferric citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Agar</td>
<td>10.000</td>
</tr>
<tr>
<td><strong>Final pH ( at 25°C)</strong></td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 27.75 grams in 500 ml purified / distilled water. Heat to boiling 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 the rehydrated contents of 1 vial of Oxford Listeria Supplement (FD071) or 1 vial of Listeria Moxalactam Supplement (FD126). Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

*Listeria monocytogenes* is the only species of the genus *Listeria* that is important as a human pathogen. *Listeria seeligeri*, *Listeria welshimeri* and *Listeria ivanovi* have been related with animal diseases. In any case, all the species are pathogenic between the ovine and bovine cattle. Positive diagnosis of listeriosis can be obtained only by the isolation and cultivation of the responsible bacteria from blood or CSF samples of the affected organisms. Listeria Oxford Medium Base is based on the formulation described by Curtis et al (2) for isolation of *L. monocytogenes* from clinical and food specimens. Listeria Oxford HiCynth™ Medium Base is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. HiCynth™ Peptone No.1 serves as the source of carbon, nitrogen, vitamins and other essential nutrients to the organisms. Corn starch serves to neutralize the toxic metabolites formed. Lithium chloride and the antibiotics inhibit gram-negative bacteria and most gram-positive organisms but certain strains of Staphylococci may grow as esculin negative colonies. Cycloheximide is used to reduce fungal contamination; cefotetan and phosphomycin are inhibitors of bacterial overgrowth. Acriflavin, colistin sulphate and lithium chloride inhibit bacteria other than *Listeria* species. Alternatively moxalactam (FD126) can be added which inhibits both gram-positive and gram-negative bacteria.

* Listeria monocytogenes* hydrolyzes esculin to esculetin and dextrose. Esculetin reacts with ferric ions and produces black zones around the colonies. Although the selectivity of the medium is enough to allow the isolation and differentiation by direct surface inoculation, a previous dilution of the inoculum is advisable or even more when the sample is highly polluted. The techniques for isolation vary with the material under examination (8). For all specimens selective and cold enrichment is recommended (3,4). For faecal and biological specimens, the sample is homogenized in 0.1% Peptone Water (M028) and 0.1 ml amount is either directly plated on Listeria Selective Medium or inoculated into the Selective Enrichment Broth and incubated at 30°C for 7 days and then further inoculated on Listeria Selective Medium. For food and environmental samples selective enrichment is generally used. For isolation of *Listeria* from food (milk and milk products), add 25 ml or 25 grams of sample to 225 ml of Listeria Enrichment Broth, UVM (M890A). Homogenize and mix carefully. Incubate for 48 hours at 30°C. Streak the enriched cultures onto Listeria Oxford medium Base and incubate aerobically for 48 hours at 37°C. Take 5 typical colonies (esculin positive) and inoculate onto Soyabean Casein Digest HiCynth™ Medium (MCD290). Incubate for 24 hours and then use these colonies for biochemical confirmation.

**Type of specimen**

Clinical samples - Body tissues or body fluids, Food and dairy samples

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Please refer disclaimer Overleaf.
Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:
In Vitro diagnostic Use. 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. Safety guidelines may be referred in individual safety data sheets.

Limitations:

1. Further biochemical tests are needed for a final identification of the isolated organisms.

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
Light yellow to dark yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium
Dark amber coloured clear to slightly opalescent gel with a blue cast forms in Petri plates

Reaction
Reaction of 5.55% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added Oxford Listeria Supplement (FD071) or Listeria Moxalactam supplement(FD126), after an incubation at 35-37°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Esculin Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis ATCC 6633 (00003*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212 (00087*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Enterococcus hirae ATCC 10541</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes serovar 1 ATCC 19111 (00020*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>Positive reaction, blackening of medium around the colony</td>
</tr>
<tr>
<td>Listeria monocytogenes ATCC 19112</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>Positive reaction, blackening of medium around the colony</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
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 inorder 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 (5,6).

References

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<thead>
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<th>Listeria monocytogenes</th>
<th>50-100</th>
<th>luxuriant</th>
<th>&gt;=50%</th>
<th>positive reaction, blackening of medium around the colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 19117</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)</th>
<th>50-100</th>
<th>good</th>
<th>40-50%</th>
<th>negative reaction</th>
</tr>
</thead>
</table>

Key : *Corresponding WDCM numbers.
In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
23 Vadhami Industrial Estate,
LBS Marg, Mumbai-86, MS, India

CE Partner 4U, Esdoornlaan 13, 3951 DB Maarn The Netherlands,
www.cepartner4u.eu