L. mono Differential HiVeg™ Agar Base

Intended use

L. mono Differential HiVeg™ Agar Base has been recommended for the selective and differential isolation of *Listeria monocytogenes*.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ peptone No. 1</td>
<td>18.000</td>
</tr>
<tr>
<td>HiVeg™ hydrolysate</td>
<td>6.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>2.000</td>
</tr>
<tr>
<td>Glucose (Dextrose)</td>
<td>2.000</td>
</tr>
<tr>
<td>Magnesium glycerophosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>10.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>2.500</td>
</tr>
<tr>
<td>Chromogenic substrate</td>
<td>0.050</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td><strong>Formula adjusted, standardized to suit performance parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

Directions

Suspend 36.02 grams in 460 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. Aseptically add sterile contents of 1 vial of L mono Enrichment Supplement I (FD214) and sterile rehydrated contents of L mono Selective Supplement I (FD212), L mono Selective Supplement II (FD213). Mix well and pour into sterile Petri plates.

Principle And Interpretation

*Listeria monocytogenes* is a gram-positive foodborne human pathogen responsible for serious infections in pregnant women that may ultimately result in abortion, stillbirth, birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles. The pathogenicity of *Listeria ivanovii* for humans is uncertain. Since *L. monocytogenes* and *L. innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford).

L mono Differential HiVeg™ Agar Base is a slight modification of L mono Differential Agar Base which is based on the formulation of Ottoviani and Agosti (4, 5) for the selective and differential isolation of *Listeria monocytogenes* from food and animal feeds. It is prepared by replacing animal based peptones with veg peptones which are free from BSE/TSE risks. HiVeg™ Peptone No.1, HiVeg™ Hydrolysate, yeast extract and sodium pyruvate supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Glucose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplements (FD212 and FD213) inhibit accompanying microflora and allow the growth of *Listeria* species. *Listeria* species hydrolyse the chromogenic substrate which produces green coloured colonies. Differentiation of *Listeria monocytogenes* from other *Listeria* species is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies.

Type of specimen

Clinical samples- blood ; Food and animal feeds, environmental samples in the area of food manufacturing and handling.

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For food and animal feeds, environmental samples follow appropriate techniques for handling specimens as per established guidelines (3). 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. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Further biochemical tests must be carried out to differentiate between *L. monocytogenes* and *L. ivanovii*.

**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 amber coloured, opalescent gel forms in Petri plates

**Reaction**
Reaction of 7.2% w/v aqueous solution at 25°C. pH : 7.2±0.2

**pH**
7.00-7.40

**Cultural Response**
Cultural characteristics observed with added sterile *L. mono* Selective Supplement I (FD212), *L. mono* Selective Supplement II (FD213) and *L. mono* Enrichment supplement I (FD214) 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>Colour of Colony</th>
<th>PIPLC activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans ATCC 10231</em></td>
<td>&gt;10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis ATCC 29212</em></td>
<td>&gt;10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis ATCC 19433</em></td>
<td>&gt;10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>&gt;10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 8739</em></td>
<td>&gt;10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa ATCC 27853</em></td>
<td>&gt;10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Listeria innocua ATCC 33090</em></td>
<td>&gt;10⁴</td>
<td>luxuriant</td>
<td>&gt;50%</td>
<td>greenish-blue</td>
<td>negative</td>
</tr>
<tr>
<td><em>Listeria grayi ATCC 19120</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;50%</td>
<td>greenish-blue</td>
<td>negative</td>
</tr>
</tbody>
</table>
**Listeria ivanovii ATCC 19119**  
50-100 luxuriant >=50% greenish-blue positive, opaque halo around the colony exhibiting phosphatidyl-inositol specific phospholipase activity

**Listeria monocytogenes ATCC 35152 (00109*)**  
50-100 luxuriant >=50% greenish-blue positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity

**Listeria monocytogenes ATCC 13932 (00021*)**  
50-100 luxuriant >=50% greenish-blue positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity

**Listeria monocytogenes ATCC 19112**  
50-100 luxuriant >=50% greenish-blue positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity

**Listeria seeligeri ATCC 35967**  
50-100 luxuriant >=50% greenish-blue negative

**Listeria welshimeri ATCC 43549**  
50-100 luxuriant >=50% greenish-blue negative

Key : ( *) Corresponding WDCM numbers.

**Storage and Shelf Life**

Store dehydrated powder and the prepared medium at 2-8°C in tightly closed container. 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 (1,2).

**Reference**

3. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1 , Detection method ; ISO 11290-1:2017
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.