Fraser Broth Base

**Intended use**
Recommended, recommended as a primary as well as secondary enrichment medium, for the isolation and enumeration of *Listeria monocytogenes* from food and animal feeds. The composition and performance criteria of this media is as per the specification laid down in ISO 11290-1:2017 and ISO 11290-2:2017.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of animal tissues</td>
<td>5.000</td>
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<tr>
<td>Enzymatic digest of casein</td>
<td>5.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
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<tr>
<td>Meat extract</td>
<td>5.000</td>
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<tr>
<td>Sodium chloride</td>
<td>20.000</td>
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<tr>
<td>Disodium hydrogen phosphate dihydrate</td>
<td>12.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.350</td>
</tr>
<tr>
<td>Esculin</td>
<td>1.000</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>3.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**
Suspend 54.92 grams (the equivalent weight of dehydrated medium per litre) 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 Fraser Selective Supplement (FD125I) and 2 vials of Fraser Supplement (FD141) to 1000 ml medium for primary enrichment or 1 vial of each to 500 ml medium for secondary enrichment. Mix well and dispense in tubes or flasks as desired.

**Principle And Interpretation**
*L. monocytogenes* primarily causes meningitis, encephalitis or septicemia in humans (1,10). In pregnant women, *L. monocytogenes* often causes influenza like bacteremic illness that, if untreated, may lead to abortion, still birth or premature birth. Contaminated foods are the primary vehicles of transmission (9). Fraser Broth Base is based on the formulation of Fraser and Sperber (11) is used for the detection of *Listeria* species in food products. Fraser Broth Base is formulated so as to provide optimum conditions for the growth of *Listeria*. This medium is recommended by ISO for primary and secondary enrichment of *Listeria* species.
Peptone, Tryptone, yeast extract, and HM extract make the media highly nutritive by providing essential nutrients including carbonaceous and nitrogenous substances. Phosphates maintain the buffering capacity of the medium. All Listeria species exhibit beta-glucosidase activity which is evident by the blackening of the media. Listeria species hydrolyze esculin (substituted glucoside) to glucose and esculetin. The latter combines with ferric ions of ferric ammonium citrate (FD141), resulting in the formation of 6-7 dihydroxycoumarin, a black brown complex. Ferric ammonium citrate also enhances the growth of L.monocytogenes (8). The high salt tolerance (of sodium chloride) of Listeria is used as means to inhibit the growth of Enterococci. Lithium chloride is also used to inhibit Enterococci, which also possess the ability to hydrolyze esculin. Growth of accompanying bacteria is largely inhibited by the addition of Nalidixic acid and Acriflavin hydrochloride (FD125I).

**Type of specimen :**
Food samples

**Specimen Collection and Handling:**

1. **Initial suspension**
   This broth is used as a dilution fluid for the preparation of initial suspension
   25grams/25 ml of sample to 225 ml of the medium (M1327 + 1 vial of FD125I + 2 vials of FD141)

2. **Primary enrichment**
   The dilution prepared in Half Fraser broth is incubated at 30°C ± 1°C for 24-26 hours.
   The preenriched sample after incubation can be stored at 5°C for a maximum of 72 hours before transfer to Fraser Broth (secondary enrichment)
   A black colouration can develop during incubation.

3. **Secondary Enrichment**
   0.1 ml of culture from primary enrichment is added to 10 ml of Fraser Broth (secondary enrichment). It is incubated at 37°C ± 1°C for 24 ± 2 hours.
   Additional incubation of 24 hours for Listeria species other than L.monocytogenes is recommended to allow recovery of more species.
   The sample from primary enrichment and secondary enrichment is then subcultured on L.mono Differential Agar Base (M1540) and on Listeria Oxford Medium Base (M1145) or Listeria Identification Agar Base (PALCAM) (M1064I).
   Incubate at 37 ± 1 °C for 24 ± 2 hours. Additional incubation at 37 ± 1 °C for 24 ± 2 hours is recommended for Listeria spp. other than L.monocytogenes for recovery of more species. (6,7)

**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. Presence of L.monocytogenes is often masked by other Listeria species like L.inocua and L.ivanovii.
2. Further subculture of organisms on selective media is required.

**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**
Basal medium : Yellow coloured clear solution with slight precipitate. After addition : Fluorescent yellow coloured clear solution with slight precipitate forms in tubes.

**Reaction**
Reaction of 5.49% w/v aqueous solution at 25°C. pH : 7.2±0.2

**pH**
7.00-7.40
### Cultural Response
#### Half Fraser (Primary Enrichment)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Esculin Hydrolysis</th>
<th>Recovery on M1540*</th>
<th>Colour of colony on M1540*</th>
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<tr>
<td><em>Listeria monocytogenes</em> 1/2a ATCC 35152 (00109*) + <em>Escherichia coli</em> ATCC 25922 (00013*) + <em>Enterococcus faecalis</em> ATCC 29212 (00087*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction, blackening of medium</td>
<td>&gt;10 colonies</td>
<td>Blue green colonies w/ opaque halo</td>
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<tr>
<td><em>Listeria monocytogenes</em> 4b ATCC 13932 (00021*) + <em>Escherichia coli</em> ATCC 25922 (00013*) + <em>Enterococcus faecalis</em> ATCC 29212 (00087*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
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#### Selectivity

Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 37 ± 1°C for 24 ± 2 hours. Further subculture is carried out on M1540 at 37 ± 1°C for 48± 4 hours.

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<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>-</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8739 (00012*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>-</td>
<td>0</td>
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<tr>
<td><em>Enterococcus faecalis</em> ATCC &gt;=10⁴ 29212 (00087*)</td>
<td>none-poor</td>
<td>-</td>
<td>&lt;100 colonies</td>
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<td></td>
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<tr>
<td><em>Enterococcus faecalis</em> ATCC &gt;=10⁴ 19433 (00009*)</td>
<td>none-poor</td>
<td>-</td>
<td>&lt;100 colonies</td>
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### Fraser (Secondary Enrichment)

#### Productivity

Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 37 ± 1°C for 24 ± 2 hours. Further subculture is carried out on M1540 at 37 ± 1°C for 48± 4 hours.
Listeria monocytogenes
1/2a ATCC 35152
(00109*) +
Escherichia coli ATCC
25922 (00013*) +
Enterococcus faecalis
ATCC 29212 (00087*)

50-100 good-luxuriant positive reaction, blackening of medium >10 colonies Blue green colonies w/ opaque halo

>=10^4

Listeria monocytogenes
1/2a ATCC 35152
(00109*) +
Escherichia coli ATCC
8739 (00012*) +
Enterococcus faecalis
ATCC 19433 (00009*)

50-100 good-luxuriant positive reaction, blackening of medium >10 colonies Blue green colonies w/ opaque halo

>=10^4

Listeria monocytogenes
4b ATCC 13932
(00021*) +
Escherichia coli ATCC
25922 (00013*) +
Enterococcus faecalis
ATCC 29212 (00087*)

50-100 good-luxuriant positive reaction, blackening of medium >10 colonies Blue green colonies w/ opaque halo

>=10^4

Listeria monocytogenes
4b ATCC 13932
(00021*) +
Escherichia coli ATCC
8739 (00012*) +
Enterococcus faecalis
ATCC 19433 (00009*)

50-100 good-luxuriant positive reaction, blackening of medium >10 colonies Blue green colonies w/ opaque halo

>=10^4

Selectivity
Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 30 ± 1°C for 25 ± 1 hour. Further subculture is carried on Tryptone Soya Agar (M290) after an incubation at 37 ± 1°C for 48± 4 hours.

Escherichia coli ATCC
25922 (00013*)

>=10^4 inhibited - 0

Escherichia coli ATCC
8739 (00012*)

>=10^4 inhibited - 0

Enterococcus faecalis ATCC>=10^4
29212 (00087*)

none-poor - <100 colonies

Enterococcus faecalis ATCC>=10^4
19433 (00009*)

none-poor - <100 colonies

Storage and Shelf Life
Store between 10-30°C in a tightly closed container. 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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).
Reference

3. Fraser and Sperber, 1988, J. Food Prot., 51:762-76
6. 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
7. Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2, Detection method ; ISO 11290-2:2017

Disclaimer:

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