Fraser Broth Base

M1327

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, ISO 11290-2:2017 and ISO 11133:2014 (E) /Amd.: 2020.

Composition**

ISO 11290 Specification - Half Fraser & Fraser

Ingredients	g / L
Enzymatic digest of animal tissues	5.000
Enzymatic digest of casein	5.000
Yeast extract	5.000
Meat extract	5.000
Sodium chloride	20.000
Disodium hydrogen phosphate dihydrate	12.000
Potassium dihydrogen phosphate	1.350
Esculin	1.000
Lithium chloride	3.000
Final pH (at 25°C)	7.2±0.2

Fraser Broth : Half Fraser & Fraser broth

Ingredients	g / L
Peptone #	5.000
Tryptone $	5.000
Yeast extract	5.000
HM extract ##	5.000
Sodium chloride	20.000
Disodium hydrogen phosphate dihydrate	12.000
Potassium dihydrogen phosphate	1.350
Esculin	1.000
Lithium chloride	3.000
Final pH (at 25°C)	7.2±0.2

Supplements to be added after autoclaving

	Half fraser g / L	Fraser g / L
Acriflavin hydrochloride	0.0125	0.025
Nalidixic acid, sodium salt	0.01	0.02
Ammonium Iron citrate	0.5	0.50

	Half fraser g / L	Fraser g / L
FD125I	**1 vial**	**2 vials**
Acriflavin hydrochloride	0.0125	0.025
Nalidixic acid, sodium salt	0.01	0.02
FD141	**2 vials**	**2 vials**
Ammonium Iron citrate	0.5	0.50

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Enzymatic digest of animal tissues

$ - Equivalent to Enzymatic digest of casein

- Equivalent to Meat extract

Directions

Suspend 54.92 gram (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

Listeria species are widely distributed in the environment. They have been isolated from soil, decaying vegetable matter, silage, sewage, water, animal feed, fresh and frozen poultry, meats, raw milk, cheese and asymptomatic human and animal carriers (1). *L.monocytogenes* primarily causes meningitis, encephalitis or septicemia in humans (2,3). In pregnant women, *L.*monocytogenes often causes influenza like bacteremic illness that, if untreated, may leaded to ammionitis and infection of the fetus, resulting in abortion, still birth or premature birth. Contaminated foods are the primary vehicles of transmission (4). Fraser Broth Base is based on the formulation of Fraser and Sperber (5) is used for the detection of *Listeria* species in food products (6). 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 (7,8,9).

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* (10). 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 an 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 HiCrome™ Listeria Ottaviani-Agosti Agar Base (M1540I) 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. (7,8)

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Presence of *L.monocytogenes* is often masked by other *Listeria* species like *L.inocua* and *L.ivanovii.*
4. 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 theexpiry 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)

Organism	Inoculum (CFU)	Growth	Esculin Hydrolysis	Recovery on M1540I*	Colour of colony on M1540I*
Productivity					
Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 30 ± 1°C for 25 ± 1 hour. Further subculture is carried out on M1540I at 37 ± 1°C for 48± 4 hours.					
Listeria monocytogenes 1/2a ATCC 35152 (00109*) +	50-100	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Escherichia coli ATCC 25922 (00013*) +	>=10^4				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4				
Listeria monocytogenes 1/2a ATCC 35152 (00109*) +	50-100	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Escherichia coli ATCC 8739 (00012*) +	>=10^4				
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4				
Listeria monocytogenes 4b ATCC 13932 (00021*) +	50-100	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Escherichia coli ATCC 25922 (00013*) +	>=10^4				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4				
Listeria monocytogenes 4b ATCC 13932 (00021*)+	50-100	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Escherichia coli ATCC 8739 (00012*) +	>=10^4				
Enterococcus faecalis ATCC 19433 (00009*)	>=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.

Organism	Inoculum (CFU)	Growth	Recovery on M290
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	none-poor	<100 colonies
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	none-poor	<100 colonies

Fraser (Secondary Enrichment)

Organism	Inoculum (CFU)	Growth	Esculin Hydrolysis	Recovery on M1540I*	Colour of colony on M1540I*
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 M1540I 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 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 1/2a ATCC 35152 (00109*) + *Escherichia coli* ATCC 8739 (00012*) + *Enterococcus faecalis* ATCC 19433 (00009*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 4b ATCC 13932 (00021*) + *Escherichia coli* ATCC 25922 (00013*) + *Enterococcus faecalis* ATCC 29212 (00087*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 4b ATCC 13932 (00021*) + *Escherichia coli* ATCC 8739 (00012*) + *Enterococcus faecalis* ATCC 19433 (00009*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo

Selectivity

Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 37 ± 1°C for 24 ± 2 hour. Further subculture is carried on Tryptone Soya Agar (M290) after an incubation at 37 ± 1°C for 48± 4 hours.

Organism	Inoculum (CFU)	Growth	Recovery on M290
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	none-poor	<100 colonies
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	none-poor	<100 colonies

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and 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.

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 (1,4).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Nieman R. E., and Lorber B., 1980, Rev. Infect. Dis. 2 : 207-2
3. Schuchat A. B., Swaminathan and C. V. Broome, Clin. Microbiol., Rev. 4 : 169-1
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Fraser and Sperber, 1988, J. Food Prot., 51:762-76
6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. 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
8. 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
9. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014 (E) /Amd.: 2020
10. Cowart R. E. and Foster BG., 1985, J. Infect. Dis.; 151:17

Revision : 07/ 2024