



LISTERIA FRASER BROTH BASE HALF CONCENTRATION LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)

Dehydrated culture medium and supplement LISTERIA FRASER BROTH Ready to use medium in flask

1 - INTENDED USE

Listeria Fraser Broth Base Half Concentration is used, with the addition of ferric ammonium citrate, for primary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples of the food chain (ISO 11290-1) and as diluent for sample preparation in the enumeration procedure (ISO 11290-2).

2 - COMPOSITION**LISTERIA FRASER BROTH BASE HALF CONCENTRATION, DEHYDRATED MEDIUM****TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER***

Proteose peptone	5.000 g
Tryptone	5.000 g
Beef extract	5.000 g
Yeast extract	5.000 g
Sodium chloride	20.000 g
Disodium hydrogen phosphate anhydrous°	9.500 g
Potassium dihydrogen phosphate	1.350 g
Aesculin	1.00 g
Lithium chloride	3.000 g
Acriflavin HCl	0.0125 g
Nalidixic Acid	0.010 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

° Equivalent to 12 g of disodium hydrogen phosphate dihydrate

LISTERIA FRASER BROTH HALF CONCENTRATION ,**READY TO USE MEDIUM IN FLASKS****FORMULA TIPICA PER LITRO**

Proteose peptone	5.000 g
Tryptone	5.000 g
Beef extract	5.000 g
Yeast extract	5.000 g
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Disodium hydrogen phosphate anhydrous°	9.500 g
Potassium dihydrogen phosphate	1.350 g
Aesculin	1.00 g
Lithium chloride	3.000 g
Acriflavin HCl	0.0125 g
Nalidixic Acid	0.010 g
Fe Ammonium Citrate	0,500 g

LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)**VIAL CONTENTS FOR 500 ML OF MEDIUM**

REF 4240056

Ferric Ammonium Citrate

0.25 g

VIAL CONTENTS FOR 5 L OF MEDIUM

REF 42185056 AND 42185056S

2.5 g

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Although improved control measures since the 1990s have significantly reduced the prevalence of *L.monocytogenes* in many food categories, particularly in meat and meat products, it remains a significant cause of foodborne illness.¹

Identification traditionally involves culture methods based on selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.²

ISO,^{3,4} FDA,⁵ USDA-FSIS⁶ protocols differ in the recommended culture media but they all involve one or more enrichment steps followed by plating into one or two selective isolation media. Fraser Broth was developed by Judy A. Fraser and William H. Sperberby⁷ by a modification of the USDA secondary enrichment broth through the addition of lithium chloride and ferric ammonium citrate. The efficacy of Fraser Broth was documented by testing a wide range of food and environmental samples from food processing facilities.

Listeria Fraser Broth Base Half Concentration contains all the basic ingredients with the exception of ferric ammonium citrate which is contained in the freeze-dried supplement that enable the complete medium Half-Fraser Broth to be prepared.

Half-Fraser Broth is used for primary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples from the food chain according to ISO 11290-1.³ Half-Fraser Broth can be used for sample preparation in the enumeration procedure according to ISO 11290-2.⁴

Peptones and yeast extract provide nitrogen, carbon, vitamins particularly of the B-group and trace elements for microbial growth; phosphates are used as buffering agents to control the pH in the medium. Selectivity is provided by the presence of nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine which inhibits many Gram-positive bacteria; lithium chloride and the high salt (NaCl) tolerance of *Listeria* are used to inhibit growth of enterococci. Half-Fraser Broth contains half the concentrations of acriflavine and nalidixic acid compared to Fraser Broth. Esculin is hydrolysed. to glucose and aesculetin (6-7-dihydroxycoumarin): aesculetin reacts with the iron salts in the medium, giving it a brown-black colour. Since all *Listeria* spp. hydrolyse esculin, cultures which do not blacken can be considered to be *Listeria*-free.⁷

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 27.4 g in 500 mL of cold purified water. Heat to boiling to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Cool to room temperature. To 500 mL of medium add the contents of one vial of Listeria Fraser Supplement (Fe Ammonium Citrate) (REF 4240056) reconstituted with 5 mL of sterile purified water. To 5 liters of culture medium add the contents of one vial of Listeria Fraser Supplement (Fe Ammonium Citrate) (REF 42185056 or 42185056A) reconstituted with 20 mL of sterile purified water. Mix well and pour into sterile flasks under aseptic conditions.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance

beige, fine, homogeneous, free-flowing powder

Prepared flasks appearance

yellow-brown, limpid

Freeze-dried selective supplements

low, fragile brown tablets; brown opalescent solutions after reconstitution

Final pH of complete media (at 20-25°C)

7.2 ± 0.2



**6 - MATERIALS PROVIDED – PACKAGING**

Product	Type	REF	Pack
Listeria Fraser Broth Base Half Concentration	Dehydrated medium	4015942	500 g (9.1 L)
		4015944	5 kg (91 L)
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	4240056	10 vials, each for 500 mL of medium
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	42185056	1 vial for 5 L of medium
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	42185056A	9 vials, each for 5 L of medium
Listeria Fraser Broth Half Concentration	Ready to use flasks	5115942	6 x 90 mL
Listeria Fraser Broth Half Concentration	Ready to use flasks	5115943	6 x 225 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, sterile flasks and tubes, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS

Foods, animal deeding stuffs, food chain and environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.^{3,4}

9 - TEST PROCEDURE**Detection of *Listeria monocytogenes* and *Listeria* spp (ISO 11290-1)³**

- In general, to prepare the initial suspension, add a test portion of 25 g or 25 mL to 225 g or 225 mL of Half-Fraser Broth, to obtain a tenfold dilution, and homogenize.
- Incubate the primary enrichment medium at 30 °C ± 1°C for 25 h ± 1 h.
- Transfer 0.1 mL of the culture to a tube or bottle containing 10 mL of secondary enrichment medium (Fraser Broth) and incubate for 24 h ± 2 h at 37 °C ± 1°C. In the case of *Listeria* spp. other than *Listeria monocytogenes* detection, additional 24 h incubation can allow for recovery of more species.
- From the primary enrichment culture inoculate, by means of a loop, the surface of the first selective plating medium, Agar *Listeria* according to Ottaviani and Agosti (ALOA) (REF 401505), to obtain well-separated colonies. Proceed in the same way with the second selective plating-out medium of choice (e.g., PALCAM or Oxford Agar, REF 401604 or 401600).
- From the secondary enrichment medium, repeat the procedure with the two selective plating-out media.
- Incubate ALOA plates at 37°C ± 1°C for 24 ± 2 hours; if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
- Incubate the second plating out medium according to the Instructions for Use
- Examine the dishes for the presence of presumptive colonies of *L. monocytogenes* or *Listeria* spp.

Notes

It is possible to store at +5 °C the pre-enriched sample after incubation before transfer to Fraser broth for a maximum of 72 h.

Half-Fraser broth and Fraser broth can be refrigerated at +5 °C before isolation on selective agar for a maximum of 72.

After incubation, ALOA plates can be refrigerated at +5 °C for a maximum of 48 h before reading.

Enumeration of *Listeria monocytogenes* and of *Listeria* spp (ISO 11290-2)⁴

- Prepare a sample suspension in Buffered Peptone Water or other suitable enrichment broth according to ISO 6887 (all parts); in case both detection and enumeration are performed according to parts 1 and 2 of ISO 11290, the sample suspension may be made in half-Fraser broth (with or without the addition of the selective supplement).
- Inoculate 0.1 mL of the sample suspension and 0.1 mL of further decimal dilutions onto 90 mm plates of ALOA medium.
- For samples with suspected low number of target-strains, inoculate 1 mL of the sample suspension and 1 mL of further decimal dilutions onto 140 mm plates of ALOA medium.
- Examine after incubation at 37°C for 24 ± 2 hours and, if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
- Count *L. monocytogenes* colonies and *Listeria* spp. colonies in plates with less than 150 colonies (90 mm diameter plates) or 360 colonies (140 mm plates) according to the section "reading and interpretation".

10 - READING AND INTERPRETATION

After incubation, typically *Listeria* spp. produce a blackening of the two enrichment broths.

After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies on plating out media.

With ALOA plates, consider as presumptive *L. monocytogenes* the blue-green colonies surrounded by an opaque halo; consider as presumptive *Listeria* spp. the blue-green colonies with or without opaque halo.

Second plating-out medium: examine for the presence of typical colonies according to the characteristics of the chosen medium.

Confirm typical colonies by the methods and tests indicated in ISO 11290-1 or ISO 11290-2, after purification of the colonies in Tryptic Soy Yeast Extract Agar (REF 402166).

The mandatory confirmatory tests for *L.monocytogenes*, according to ISO 11290 and using ALOA medium, are the following: β-hemolysis (+), carbohydrate utilization (L-rhamnose +; D-xylose -). Optional confirmatory tests for *L.monocytogenes* are: catalase (+), mobility at 25°C (+), CAMP Test (+). The mandatory confirmatory tests for *Listeria* spp. are: microscopic examination, catalase (+); optional tests are: VP (+), mobility at 25°C (+).

Miniaturized galleries for the biochemical identification of *Listeria monocytogenes* may be used (*Listeria* Monoconfirm Test REF 193000)

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.³

CONTROL STRAINS	INCUBATION T° / τ / ATM	EXPECTED RESULTS
<i>L.monocytogenes</i> +	ATCC 13932 30°C / 24h / A	> 10 typical colonies after subculture on ALOA
<i>E. faecalis</i> +	ATCC 29212	
<i>E. coli</i>	ATCC 25922	





<i>L. monocytogenes</i> +	NCTC 7973	30°C / 24h / A	> 10 typical colonies after subculture on ALOA
<i>E. faecalis</i> +	ATCC 29212		
<i>E. coli</i>	ATCC 25922		
<i>E. faecalis</i>	ATCC 29212	30°C / 24h / A	< 100 colonies after subculture on TSA
<i>E. coli</i>	ATCC 25922	30°C / 24h / A	totally inhibited after subculture on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC:National Collection of Type Cultures

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated *Listeria* Fraser Broth Base Half Concentration, supplemented with *Listeria* Fraser Supplement-Fe Ammonium Citrate (REF 4240056), is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 30°C for 24 hours and recording the highest dilution showing growth in Reference Batch (G_{RB}) and in Test Batch (G_{TB}). Productivity is tested with the following target strains: *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 13932. The productivity index $G_{RB}-G_{TB}$ for each test strain shall be ≤ 1 and the tubes shall exhibit blackening.

Productivity and selectivity are tested together with mixtures of approximately 100 CFU of target organisms and 1000 CFU of non-target organisms per test tubes, incubating at 30°C for 24 hours. Mixtures of target and non-target strains: *L. monocytogenes* ATCC 19111+*E. coli* ATCC 25922+*E. faecalis* ATCC 29212 and *L. monocytogenes* NCTC 7973+*E. coli* ATCC 25922+*E. faecalis* ATCC 29212. After incubation of inoculated tubes and sub-culture on ALOA plates, the target strains will show more than 10 colonies per plate.

Moreover, selectivity is tested by inoculating approximately 1000 CFU per tube of the following non-target strains: *E. faecalis* ATCC 29212, and *E. coli* ATCC 25922. After incubation *E. faecalis* exhibits a growth with less than 100 CFU after subculture on Tryptic Soy Agar while *E. coli* is totally inhibited. Selectivity is tested also with the non-target strain *C. albicans* ATCC 18804 by dilution to extinction method: the strain is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.
- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.

14 - PRECAUTIONS AND WARNINGS

- The products here described are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass. Be careful when opening the metal ring of the supplements to avoid injury.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving.
- The supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplements and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready-to-use medium in flasks

Upon receipt, store flasks in their original pack at 2-8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

Dehydrated medium

Upon receipt, store at +10/+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the





container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Freeze-dried supplement

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Buchanana RL *et al.* A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments Food Control Volume 75, May 2017, Pages 1-13
2. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. FEMS Microbiol Rev. 2005 Nov;29(5):851-75
3. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1: Detection method.
4. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 2: Enumeration method.
5. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods, April 2022.
6. USDA-FSIS. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Ready-To-Eat, Siluriformes (Fish) and Egg Products, and Environmental Samples. MLG 8.13, 10/01/2021
7. Fraser JA, Sperber WH. Rapid Detection of *Listeria* spp. in Food and Environmental Samples by Esculin Hydrolysis. J Food Prot 1988 Oct;51(10):762-765.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/07
Revision 2	Updated content	2024/06

Note: minor typographical, grammatical, and formatting changes are not included in the revision history

