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

Dehydrated culture medium and supplement

LISTERIA FRASER BROTH

Ready to use medium in flasks

1 - INTENDED USE

Listeria Fraser Broth Base, with the addition of ferric ammonium citrate, is used for secondary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples of the food chain (ISO 11290-1).

2 - COMPOSITION

LISTERIA FRASER BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Enzymatic digest of animal tissue	5.00 g
Enzymatic digest of casein	5.00 g
Meat extract	5.00 g
Yeast extract	5.00 g
Sodium chloride	20.00 g
Disodium hydrogen phosphate anhydrous°	9.50 g
Potassium dihydrogen phosphate	1.35 g
Aesculin	1.00 g
Lithium chloride	3.00 g
Acriflavine HCl	25.00 mg
Nalidixic Acid	20.00 mg

*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, TERRENO PRONTO IN FLACONE E PROVETTA
FORMULA TIPICA PER LITRO

Enzymatic digest of animal tissue	5.00 g
Enzymatic digest of casein	5.00 g
Meat extract	5.00 g
Yeast extract	5.00 g
Sodium chloride	20.00 g
Disodium hydrogen phosphate anhydrous°	9.50 g
Potassium dihydrogen phosphate	1.35 g
Aesculin	1.00 g
Lithium chloride	3.00 g
Acriflavine HCl	25.00 mg
Nalidixic Acid	20.00 mg
Ferric Ammonium Citrate	0,500 g

LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)

	VIAL CONTENTS FOR 500 mL OF MEDIUM REF 4240056	VIAL CONTENTS FOR 5 L OF MEDIUM REF 42185056 and 42185056A
Ferric Ammonium Citrate	0.25 g	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-FSIS[6] 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. Sperberby[7] 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 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 Fraser Broth to be prepared. Acriflavine and nalidixic acid, being thermostable, are included in the medium base.
Fraser Broth is used for secondary 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]
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. Fraser Broth contains double the concentrations of acriflavine and nalidixic acid compared to Half-Fraser Broth. Aesculin is hydrolysed. to glucose and aesculetin (6-7-dihydroxycoumarin): aesculetin reacts with the iron salts in the medium, giving it a brown-black colour.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 27.5 g in 500 mL (or 275 g in 5 litres) of cold purified water. Heat to boiling with frequent agitation to completely dissolve the powder. Autoclave at 121°C for 15 minutes and cool to 25-50°C. 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 litres of 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 pellet; brown opalescent solutions after reconstitution
Final pH of complete media (at 20-25°C)	7.2 ± 0.2

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6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Listeria Fraser Broth Base	Dehydrated medium	4015962 4015964	500 g (9.1 L) 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	Ready to use flasks	5115962 5115963 5115964	6 x 90 mL 6 x 225 mL 6 x 200 mL
Listeria Fraser Broth	Ready to use vials	551596 551596N	20 x 10 mL 20 x 9 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]

1. 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.
2. Incubate the primary enrichment medium at 30 °C ± 1°C for 25 h ± 1 h.
3. 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.
4. 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 401605), 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).
5. From the secondary enrichment medium, repeat the procedure with the two selective plating-out media.
6. 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.
7. Incubate the second plating out medium according to the instructions for use
8. 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.

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-haemolysis (+), 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 *L. 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°/ T / ATM	EXPECTED RESULTS
L.monocytogenes ATCC 13932+ *E. faecalis* ATCC 29212+ *E. coli* ATCC 25922	37°C / 24h / A	> 10 typical colonies after subculture on ALOA
L.monocytogenes NCTC 7973+ *E. faecalis* ATCC 29212+ *E. coli* ATCC 25922	37°C / 24h / A	> 10 typical colonies after subculture on ALOA
E. faecalis ATCC 29212 *E. coli* ATCC 25922	37°C / 24h / A 37°C / 24h / A	< 100 colonies after subculture on TSA 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 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 37°C for 24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 13932. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1 and the tubes shall exhibit blackening.

Productivity and selectivity are tested together with mixtures of ≤100 CFU of target organisms and ≥1000 CFU of non- target organisms per test tubes, incubating at 37°C for 24 hours. Mixtures of target and non-target strains: *L. monocytogenes* ATCC 13932+*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 ≥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 and tubes are subject to terminal sterilization by autoclaving.
- The supplements are 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 supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets 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 tubes and bottles

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

Dehydrated medium

Upon receipt, store at +10°C/+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°C/+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