MOPS-BUFFERED LISTERIA ENRICHEMENT BROTH (MOPS-BLEB)

Dehydrated and ready-to-use culture medium

MOPS-BLEB- From left: uninoculated tube, growth of *L. monocytogenes*

1 - INTENDED USE

Secondary enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in foods according to USDA-FSIS.

2 - COMPOSITION

DEHYDRATED MEDIUM AND READY TO USE TUBES

TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Pancreatic digest of casein	17.000 g
Soy peptone	3.000 g
Yeast extract	6.000 g
Sodium chloride	5.000 g
Glucose	2.500 g
Dipotassium hydrogen phosphate	2.500 g
MOPS free acid	6.700 g
MOPS sodium salt	10.500 g
Cycloheximide	0.05 g
Acriflavine HCl	0.015 g
Nalidixic Acid	0.040 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

MOPS Buffered Listeria Enrichment Broth (MOPS-BLEB) is prepared according to a modification described by USDA-FSIS[1] of the original formulation of J. Lovett[2], with the introduction of a strong buffer system that improves the enrichment properties. The medium is recommended by USDA-FSIS[3,4] as the second enrichment broth to be used for subculture of the primary enrichment in UVM 1 medium.
Casein peptone, yeast extract and soy peptone provide essential nitrogen and carbon-based nutrients and trace elements for microbial growth; glucose is a carbohydrate that increases the growth rate of *Listeria*; MOPS compounds and dipotassium hydrogen phosphate act as a buffer system; sodium chloride maintains osmotic balance. Selectivity is provided by the presence of the antifungal compound cycloheximide, nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine, an acridine derivative with bacteriostatic properties towards many Gram-positive bacteria and weak antifungal activity.

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 53.3 g in 1000 mL of cold purified water. Mix thoroughly and warm to completely dissolve the powder. Distribute 10 mL in tubes and autoclave at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, limpid
Final pH of complete medium (at 20-25°C)	7.3 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
MOPS Buffered Listeria Enrichment Broth (MOPS-BLEB)	Dehydrated medium	401601M2 401601M4	500 g (9.4 L) 5 kg (94 L)
MOPS Buffered Listeria Enrichment Broth (MOPS-BLEB)	Ready-to-use tubes	551601M	20 x 10 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

Red meat, poultry, ready-to-eat siluriformes (fish) and egg products, and environmental samples. When collecting, storing, transporting and preparing food samples, follow the rules of good laboratory practice and refer to USDA-FSIS document MLG 8.11.[3]

9 - TEST PROCEDURE

1. Perform the primary enrichment by adding 225 ml of Listeria UVM1 Enrichment Broth (REF 4015982) to 25 g or 25 ml of sample.
2. Homogenise for 2 minutes and incubate at 30° ± 2°C for 20-26 hours.
3. Transfer 0.1 mL from the UVM broth into 10 mL of MOPS-BLEB and incubate at 35° ± 2°C for 18-24 hours
4. At the same time, from the primary enrichment broth, streak 0.1 mL onto a MOX medium plate (401601 Listeria Oxford Agar Base + 4240039 MOX COL Selective Supplement) and incubate at 35° ± 2°C for 24-28 hours.
5. Use the culture in MOPS-BLEB for inoculating a second MOX medium plate and incubate at 35° ± 2°C for 24-28 hours (culture procedure only) or for molecular detection of *Listeria monocytogenes.*

10 - READING AND INTERPRETATION

After incubation, typically *Listeria* spp. produce a turbidity into the enrichment broth.
After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Follow the procedure described by USDA-FSIS MLG method 8.11[3] for the identification of colonies.

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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 19111	35°C / 24h / A	good growth
E. coli ATCC 25922	35°C / 24h / A	inhibited
E. faecalis ATCC 29212	35°C / 24h / A	partially inhibited

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

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready to use MOPS Buffered Listeria Enrichment Broth are 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 35°C for 18-24 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 13932, *L. monocytogenes* NCTC 7973, *L. innocua* ATCC 33090 and *L. gray* ATCC 25401. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is assessed by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of non-target organisms in test tubes, incubating at 35°C for 24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}) 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.

13 - LIMITATIONS OF THE METHOD

- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Dehydrated MOPS-BLEB is classified as hazardous. Before use, consult the Safety Data Sheet.
- 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 this 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving.
- Each ready-to-use tube is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material 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

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).
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).

Ready-to-use medium in tubes

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

16 - REFERENCES

1. Laboratory Guidebook, Notice of Change: Media and Reagents. USDA-FSIS, Chapter MLG Appendix 1.09, 12/29/2017.
2. Lovett, J., Francis D.W. and Hunt J.M. (1987) Listeria monocytogenes in raw milk: detection, incidence and pathogenicity. J. Food Prot. 50:188-192

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3. Laboratory Guidebook, Notice of Change: Isolation and Identification of Listeria monocytogenes from Meat, Poultry, Ready to Eat Siluriformes (Fish) and Egg Products, and Environmental Sponges. USDA-FSIS, Chapter MLG 8.11, 1/02/2019.
4. Laboratory Guidebook, Notice of Change: Flow Chart Specific for FISI Isolation and Identification of Listeria monocytogenes - Isolation and Identification of Listeria monocytogenes (Culture Method only). USDA-FSIS, Chapter MLG 8 Appendix 1.4, 1/02/2019.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
emperature nitation	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 0	First issue	2022/07

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

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