# LISTERIA UVM1 ENRICHMENT BROTH

**Dehydrated and ready-to-use culture medium**

## 1 - INTENDED USE

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

## 2 - COMPOSITION

**TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)**

| | |
|---|---|
| Proteose peptone | 5.00 g |
| Tryptone | 5.00 g |
| Beef extract | 5.00 g |
| Yeast extract | 5.00 g |
| Sodium chloride | 20.00 g |
| Sodium phosphate bibasic | 12.00 g |
| Potassium dihydrogen phosphate | 1.35 g |
| Aesculin | 1.00 g |
| Acriflavine | 12.00 mg |
| Nalidixic acid | 20.00 mg |

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

## 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, FDA, USDA-FSIS 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.
Originally described by Donnelly and Baigent[3], the University of Vermont Modified (UVM) medium was adjusted by MacCalin and Lee[4] decreasing the nalidixic acid concentration and increasing the acriflavine concentration. Listeria UVM1 Enrichment Broth corresponds to the formulation modified by MacCalin and Lee and meets the requirements of USDA-FSIS.[5]
Listeria UVM1 Enrichment Broth is recommended as primary enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in food according to USDA-FSIS MLG 8.11,[6,7] followed by a secondary enrichment in MOPS-BLEB.
Essential growth factors for microbial growth are provided by peptones which are sources of nitrogen, carbon and minerals and by yeast extract which is a source of vitamins, particularly of the B-group. 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, an acridine derivative with bacteriostatic properties towards many Gram-positive bacteria and weak antifungal activity. The high salt (NaCl) tolerance of *Listeria* is used to inhibit growth of enterococci. Esculin is hydrolysed by all *Listeria* species.

## 4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 54.4 g in 1000 mL of cold purified water. Mix thoroughly and warm to completely dissolve the powder. Distribute a suitable volume of broth in flasks and autoclave at 121°C for 15 minutes.

## 5 - PHYSICAL CHARACTERISTICS

| | |
|---|---|
| Dehydrated medium appearance | beige, fine, homogeneous, free-flowing powder |
| Prepared flasks appearance | yellow, limpid |
| Final pH of complete media (at 20-25°C) | 7.2 ± 0.2 |

## 6 - MATERIALS PROVIDED – PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| Listeria UVM1 Enrichment Broth | Dehydrated medium | 4015982<br>4015984 | 500 g (9.2 L)<br>5 kg (92L) |
| Listeria UVM1 Broth | Ready-to-use flasks | 5115983 | 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 or tubes, 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.[6]

## 9 - TEST PROCEDURE

1. Perform the primary enrichment by adding 225 ml of Listeria UVM1 Enrichment Broth to 25 g or 25 ml of sample. Homogenise for 2 minutes and incubate at 30° ± 2°C for 20-26 hours.
2. Transfer 0.1 mL from the UVM broth into 10 mL of MOPS-BLEB (REF 401601M) and incubate at 35° ± 2°C for 18-24 hours
3. 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.
4. Use the culture in MOPS-BLEB for inoculating a second MOX medium plate (culture procedure only) or for molecular detection of *Listeria monocytogenes.*

## 10 - READING AND INTERPRETATION

After incubation, *Listeria* spp. produce a turbidity into the enrichment broth.

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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[6] for the identification of colonies.

## 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 19117 | 30°C / 24h / A | good growth |
| *E. coli* ATCC 25922 | 30°C / 24h / A | inhibited |
| *E. faecalis* ATCC 29212 | 30°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 Listeria UVM1 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 30°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.
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. After incubation of inoculated tubes and sub-culture on PALCAM 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

- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.
- Techniques for the detection of *Listeria* in foods vary, depending on the material under examination and local laws. Refer to various compendia or national regulations for the complete procedures.

## 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. 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 flasks to prevent injury due to breakage of glass.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving
- Each ready-to-use flasks 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 of the products 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°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).

## 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. Donnelly CW, Baigent GJ. Method for flow cytometric detection of Listeria monocytogenes in milk. Appl Environ Microbiol1986; 52:689.
4. McClain D, Lee WH. Development of USDA-FSIS method for isolation of Listeria monocytogenes from raw meat and poultry J Ass Off Assol Chem. 1988; 71: 660
5. Laboratory Guidebook, Notice of Change: Media and Reagents. USDA-FSIS, Chapter MLG Appendix 1.09, 12/29/2017.
6. 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.
7. 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<br>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 |

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

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