LISTERIA BUFFERED ENRICHMENT BROTH

Dehydrated culture medium

1 - INTENDED USE

Buffered enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in food and environmental samples.

2 - COMPOSITION

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

Pancreatic digest of casein	17.00 g
Soy peptone	3.00 g
Yeast extract	6.00 g
Sodium chloride	5.00 g
Glucose	2.50 g
Dipotassium hydrogen phosphate	9.60 g
Potassium dihydrogen phosphate	3.85 g
Acriflavine HCl	15.00 mg
Nalidixic acid	40.00 mg
Cycloheximide	50.00 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

Listeria Buffered Enrichment Broth is a modification of the formulation devised by Lovett et al.[1] with added buffering strength and is used for the detection of *L. monocytogenes* in food and environmental samples. The enrichment and selective properties have been improved by increasing the buffering capacity of the original medium.
Casein peptone, yeast extract and soy peptone provide essential nitrogen and carbon-based nutrients, vitamins and trace elements for microbial growth; glucose is a carbohydrate that increases the growth rate of *Listeria*; phosphates act as a buffer system; sodium chloride maintains osmotic balance. Selectivity is provided by cycloheximide, an antifungal compound, 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. Because all these antimicrobials are thermostable, they are included in the powdered medium and can be sterilised by autoclaving.[2,3]

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 47 g in 1000 mL of cold purified water. Mix thoroughly and warm if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Listeria Buffered Enrichment Broth	Dehydrated medium	401601B2	500 g (10.5 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

1. Add 25 g of sample to 225 mL of Listeria Buffered Enrichment Broth. Blend until the test portion is thoroughly dispersed.
2. Incubate the enrichment medium for 24 and 48 hours at 30°C.
3. After 24- and 48-hours incubation, streak a loopful of the enrichment culture onto ALOA Agar plate (code 401605) and PALCAM Agar plate (code 401604) or Oxford Agar plate (code 401600).
4. Incubate at 37°C and examine for the presence of typical colonies at both 24 h and 48 h.

10 - READING AND INTERPRETATION

After incubation, *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.

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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes ATCC 19111	30°C / 48h / A	growth
S. aureus ATCC 25923	30°C / 48h / A	inhibited

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

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12- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Listeria Buffered Enrichment Broth 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 48 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (GrTB). 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 48 hours. Mixture 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 ALOA plates, the target strains will show more than 10 colonies per plate.
Moreover, selectivity is tested by inoculating approximately 10000 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 UFC after subculture on Tryptic Soy Agar whereas *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 material under examination and local laws. Refer to various compendia or to national regulations for the complete procedures.

14 - PRECAUTIONS AND WARNINGS

- This product 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.
- Listeria Buffered Enrichment Broth 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium 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 Sheet of the product 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

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).
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. Lovett J, Francis DW, Hunt JM. Listeria monocytogenes in Raw Milk: Detection, Incidence, and Pathogenicity. J Food Prot 1987; 50:188-19
2. Martindale The Extra Pharmacopoeia (1982) Twenty-eighth Edition. The Pharmaceutical Press, London.
3. Haley, L.D., Trandel, J.B., Coyle, M.B. (1980) Practical methods for culture and identification of fungi in the clinical microbiological laboratory. Cumitech n. 11, ASM, Washington, D.C.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

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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