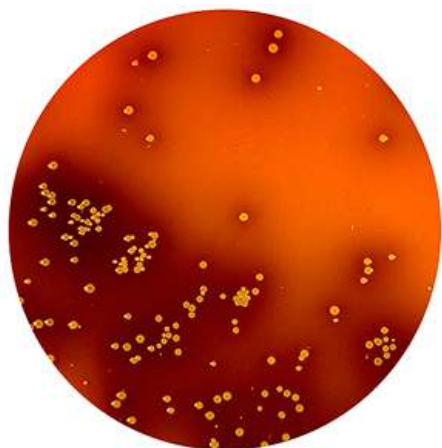


# LISTERIA PALCAM AGAR BASE LISTERIA PALCAM ANTIMICROBIC SUPPLEMENT LISTERIA SELECTIVE AGAR (PALCAM)

Dehydrated culture medium, selective supplement, ready-to use plates



## 1 - INTENDED USE

Selective and differential basal medium, selective supplement and ready to use plates for the isolation and enumeration of *Listeria* spp. from foodstuffs.

## 2 - COMPOSITIONS

### LISTERIA PALCAM AGAR BASE

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

Peptocomplex	10.00 g
Tryptose	10.00 g
Peptone	3.00 g
Yeast extract	3.00 g
Maize starch	1.00 g
Sodium chloride	5.00 g
Glucose	0.50 g
Mannitol	10.00 g
Aesculin	0.80 g
Ferric ammonium citrate	0.50 g
Lithium chloride	15.00 g
Phenol red	0.08 g
Agar	12.00 g

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

### LISTERIA SELECTIVE AGAR (PALCAM)

#### READY TO USE PLATES

Listeria PALCAM Agar Base	70.8 g
Polymyxin B sulphate	10.0 mg
Ceftazidime	20.0 mg
Acriflavine HCl	5.0 mg
Purified water	1000 mL

### LISTERIA PALCAM SELECTIVE SUPPLEMENT

#### (VIAL CONTENTS FOR 500 ML OF MEDIUM)

Polymyxin B sulphate	5 mg
Ceftazidime	10 mg
Acriflavine HCl	2.5 mg

## 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Identification of *Listeria monocytogenes* traditionally involves culture methods based on selective enrichment and plating on chromogenic media and aesculin containing media followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.<sup>1</sup>

Listeria PALCAM Agar Base is an aesculin based medium prepared without antibiotics and acriflavine; it is used with Listeria PALCAM Antimicrobial Supplement for the isolation and enumeration of *Listeria* spp. in foodstuffs.

The complete medium, prepared according to the formula developed by Van Netten et al.<sup>2</sup>, is recommended by FDA-BAM<sup>3</sup> as one of the aesculin based Listeria selective agars and may be used as second isolation medium as recommended by ISO 11290-1.<sup>4</sup>

PALCAM is the abbreviation for Polymyxin-Acriflavine-Lithium Chloride-Ceftazidime-Aesculin-Mannitol.

The complete PALCAM medium contains peptones which provide nitrogen, carbon and minerals for microbial growth. Selectivity is provided by the presence of lithium chloride, polymyxin B, ceftazidime and acriflavine. The medium uses two indicator systems: hydrolysis of aesculin and fermentation of mannitol. *Listeria* spp. hydrolyse aesculin, producing black zones around the colonies because of the formation of black iron phenolic compounds derived from the aglucon. *Listeria* spp. do not ferment mannitol while the most common contaminants, staphylococci and streptococci ferment it, causing the phenol red to change colour and the growth with colonies surrounded by a yellow halo.

## 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.4 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of Listeria PALCAM Antimicrobial Supplement reconstituted with 5 mL of sterile purified water under aseptic conditions. Mix well and pour into sterile Petri dishes.

## 5 - PHYSICAL CHARACTERISTICS

### Listeria PALCAM Agar

Dehydrated medium appearance	Light pink, fine, free-flowing powder
Solution and prepared plates appearance	Dark red, slightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2

### Listeria PALCAM Antimicrobial Supplement

Freeze-dried supplement appearance	short, dense, yellow-orange pellet
Reconstituted supplement appearance	yellow, limpid

## 6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Listeria PALCAM Agar Base	Dehydrated medium	4016042	500 g (7.1L)
Listeria PALCAM Agar Base	Dehydrated medium	4016044	5 kg (71 L)
Listeria PALCAM Antimicrobial Supplement	Freeze-dried supplement	4240042	10 vials, each for 500 mL of medium
Listeria Selective Agar (PALCAM)	Ready to use plates	541604	2 x 10 plates ø 90 mm



## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

## 8 - SPECIMENS

Food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.<sup>3,4</sup>

## 9 - TEST PROCEDURE

Perform selective enrichment of the sample with broths recommended by the chosen method of analysis.

The enrichment broths recommended by ISO 11290-1 are Half Fraser Broth and Fraser Broth, incubated at 30°C and 37°C for 24 hours, respectively, while FDA-BAM includes only one medium, Buffered Listeria Enrichment Broth without and with selective agents, with incubation at 30°C for 48 hours.

Streak a loopful of the incubated enriched broth on the surface of a PALCAM Medium plate and an ALOA plate to obtain well isolated colonies.

Examine the plates after incubation at 37°C for 24 ± 2 hours and after 48 ± 4 hours.

## 10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

After 24 h incubation at 35° C, typical *Listeria* spp. colonies are approximately 1 mm diameter, greyish green or olive-green colonies sometimes with black centres, surrounded by a black halo. Following 48 h incubation, typical *Listeria* species colonies are approximately 1.5-2 mm diameter, black with a black halo and sunken centre.

## 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
<i>L. monocytogenes</i> ATCC 19111	37°C / 48 H / A	grey-green colonies with black-brown halo
<i>E. faecalis</i> ATCC 19433	37°C / 48 H / A	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 medium and supplements (Test Batch:TB) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch and Tryptic Soy Agar.

Productivity is tested by a quantitative test with the target strains *L. monocytogenes* ATCC 19111 and *L. monocytogenes* ATCC 13932: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 48 hours. The colonies are enumerated on both batches and the productivity ratio (Pr: CFU<sub>TB</sub>/CFU<sub>TSA</sub>) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (grey-green colonies with black-brown halo) the results are considered acceptable and conform to the specifications. Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *L. innocua* ATCC 33090, and *L. ivanovii* ATCC 19119. The amount of growth and colonies characteristics are evaluated after incubation at 37°C for 48 hours: both *Listeria* strains exhibit a good growth with grey-green colonies with black-brown halo.

Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains +---. After incubation at 37°C for 48 hours, the growth of non-target strains is totally inhibited.

## 13 - LIMITATIONS OF THE METHOD

- As *L. grayi* ferments mannitol the colonies may have different characteristics from other species of *Listeria*.
- The PALCAM medium does not allow the differentiation of *L. monocytogenes* from other species of the genus *Listeria*.
- The complete identification of *L. monocytogenes* must be confirmed by suitable tests.

## 14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplement and the ready to use plates are for microbiological control only, 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 supplement shall be used in association according to the described directions.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Listeria PALCAM Antimicrobial Supplement is classified as hazardous. Before use, consult the 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](http://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.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- The selective supplement is sterilised by membrane filtration.
- Be careful when opening the metal ring of the supplement vial to avoid injury.
- 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 supplement and 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](http://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 products for the intended purpose.

### 15 - STORAGE CONDITIONS AND SHELF LIFE

#### Ready to use plates

Upon receipt, store plates in their original pack at +2 °C/ + 8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

#### 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).

#### Selective 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. 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
2. Van Netten. et al. Liquid and solid selective differential media for the detection and enumeration of *L. monocytogenes* and other *Listeria* spp. - *Int. J. Food Microbiol.*, 1989, vol. 8, p. 299-316.
3. ISO 11290:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods. Rev 10/2017

### TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 <i>In vitro</i> Diagnostic Medical Device	 Manufacturer	 This side up	 Store in a dry place
 Temperature limitation	 Content sufficient for <n> tests	 Consult instructions for use	 Use by	 Fragile	 Keep away from direct light

### REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/02
Revision 3	Reorganization and editing of sections 3, 9, 11, 16; inclusion of the section "Performances characteristics".	2023/01

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

