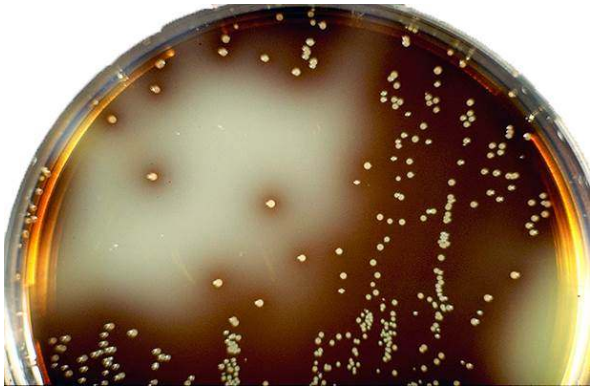
LISTERIA OXFORD AGAR BASE
LISTERIA OXFORD ANTIMICROBIC SUPPLEMENT
LISTERIA MOX-COL ANTIMICROBIC SUPPLEMENT
LISTERIA SELECTIVE AGAR (OXFORD)

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



Oxford Medium: colonies of *Listeria monocytogenes*

1 - INTENDED USE

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

2 - COMPOSITIONS

LISTERIA OXFORD AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptocomplex	10.00 g
Tryptose	10.00 g
Peptone	3.00 g
Maize starch	1.00 g
Sodium chloride	5.00 g
Aesculin	1.00 g
Ferric ammonium citrate	0.50 g
Lithium chloride	15.00 g
Agar	12.00 g

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

LISTERIA MOX-COL ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Moxalactam	10.0 mg
Colistin sulphate	5.0 mg

LISTERIA SELECTIVE AGAR (OXFORD)
(READY -TO-USE PLATES)

Listeria Oxford Agar Base	1000 mL
Cycloheximide	400 mg
Colistin sulphate	20 mg
Cefotetan	2 mg
Fosfomycin	10 mg
Acriflavine	5 mg

LISTERIA OXFORD ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cycloheximide	200.0 mg
Colistin sulphate	10.0 mg
Cefotetan	1.0 mg
Fosfomycin	5.0 mg
Acriflavine	2.5 mg

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 on chromogenic and aesculin containing media followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.[2]
Listeria Oxford Agar Base is an aesculin based medium prepared without antibiotics and acriflavine; it can be used with Listeria Oxford Antimicrobic Supplement or with Listeria MOX-COL Antimicrobic Supplement for the isolation and enumeration of *Listeria* spp. in foodstuffs.
The complete medium known as "Oxford Medium" is prepared according to the formula developed by Curtis et al.[3] and is recommended by FDA-BAM[4] as one of the aesculin based Listeria selective agars and may be used as second isolation medium as recommended by ISO 11290-1.[5]
The complete Oxford medium contains peptones which provide nitrogen, carbon and minerals for microbial growth. Selectivity is provided by the presence of lithium chloride, active against streptococci, cycloheximide active against yeasts and moulds, cefotetan and fosfomycin active on Gram-positive and Gram-negative bacteria. Aesculin and ferric iron act as indicator system: *Listeria* spp. hydrolyse aesculin, producing black zones around the colonies because of the formation of black iron phenolic compounds derived from the aglucon.
The "MOX" medium is a modification of the formulation described by McClain and Lee[6], with a reduced concentration of moxalactam in order to obtain a better growth of *Listeria* spp. It is recommended by USDA-FSIS[7,8] and FDA-BAM[2] for the detection of *L.monocytogenes*. MOX formulation with moxalactam, colistin and lithium chloride is considered superior for the inhibition of methicillin resistant staphylococci and *Proteus* spp.

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 28.7 g of dehydrated medium 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.

Oxford medium

Add the content of one vial of Listeria Oxford Antimicrobic Supplement (REF 4240038) reconstituted with 5 mL of a solution of 1:1 ethanol/sterile purified water, under aseptic conditions. Mix well and pour into sterile Petri dishes.

MOX-COL medium

Add the content of one vial of Listeria MOX-COL Antimicrobic Supplement (REF 4240039) reconstituted with 5 mL of sterile purified water, under aseptic conditions. Mix well and pour into sterile Petri dishes.

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5 - PHYSICAL CHARACTERISTICS

Listeria OXFORD Medium

Dehydrated medium appearance	beige, fine, free-flowing powder
Solution and prepared plates appearance	amber, slightly opalescent with a blue ring at the surface of the liquid
Final pH at 20-25 °C	7.0 ± 0.2
Listeria OXFORD Antimicrobic Supplement	
Freeze-dried supplement appearance	short, fragile, yellow-orange pellet
Reconstituted supplement appearance	yellow-orange, limpid
Listeria MOX-COL Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless, limpid

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Listeria Oxford Agar Base	Dehydrated medium	4016002	500 g (8.7 L)
Listeria Oxford Agar Base	Dehydrated medium	4016004	5 kg (87 L)
Listeria Oxford Antimicrobic Supplement	Freeze-dried supplement	4240038	10 vials, each for 500 mL of medium
Listeria MOX-COL Antimicrobic Supplement	Freeze-dried supplement	4240039	10 vials, each for 500 mL of medium
Listeria Oxford Selective Agar	Ready to use plates	541600	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.[4,5,7]

9 - TEST PROCEDURE

Perform selective enrichment of the sample with the broths recommended by the chosen method of analysis.
Generally, the primary enrichment broth is incubated at 30°C and the secondary enrichment broth is incubated at 37°C for 24 hours.
The enrichment broths recommended by ISO 11290-1 are Half Fraser Broth and Fraser Broth; the selective broths indicated by USDA-FSIS are UVM1 and MOPS-BLEB, 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 onto the surface of an Oxford Medium plate or MOX-COL Medium plate and of 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 37° C typical *Listeria* species colonies are approximately 1 mm diameter, grey-brown with brown or black halo.
Following 48 h incubation typical *Listeria* species colonies are approximately 2-3 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
L.monocytogenes	ATCC 19111	37°C / 48 H / A	grey colonies with black-brown halo
E.faecalis	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 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 13932 and *L.monocytogenes* ATCC 19111: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 35-37°C for 48 hours. The colonies are enumerated on both batches and the productivity ratio (Pr: CFU_{TB}/CFU_{TSA}) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (grey 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 35-37°C for 48 hours: *L.innocua* and *L.ivanovii* exhibits a good growth after 48 hour of incubation with grey colonies with black-brown halo.
The 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 *E.faecalis* ATCC 19433, *E.coli* ATCC 25922, and *C.albicans* ATCC 10231. After incubation at 37°C for 48 hours, the growth of non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- The Oxford medium does not allow the differentiation of *L.monocytogenes* from other species of the genus *Listeria*.
- The identification of *L.monocytogenes* must be confirmed by suitable tests.

14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplements and the ready to use plates 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.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Safety Data Sheets.

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- 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.
- 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 supplements are sterilised by membrane filtration.
- Be careful when opening the metal ring of the supplements vials 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 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 Sheet 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 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 supplements

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).

15 – 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. Curtis GDW, Mitchell RG, King AF, Emma J. A selective differential medium for the isolation of Listeria monocytogenes. Lett Appl Microbiol1989; 8:95-98.
4. 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.
5. 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.
6. Mc Clain 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
7. 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
8. Laboratory Guidebook, Notice of Change: Media and Reagents. USDA-FSIS, Chapter MLG Appendix 1.09, 12/29/201
9. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II). Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:222-4.

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 2	Updated layout and content	2022/02
Revision 3	Reorganization and editing of sections 2, 3, 9, 11, 16; inclusion of the section "Performances characteristics".	2022/08

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

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