ChromArt

ALOA®
AGAR LISTERIA ACC. TO OTTAVIANI & AGOSTI
ALOA® ENRICHMENT-SELECTIVE SUPPLEMENTS

Dehydrated culture medium, selective supplement and enrichment, ready to use media in plates and flasks

ALOA:
colonies of *L.monocytogenes* and *L.innocua*

1 - INTENDED USE

For the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. in samples of the food chain and in environmental samples.

2 - COMPOSITION *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

AGAR LISTERIA ACC. TO OTTAVIANI & AGOSTI (ALOA®)

DEHYDRATED MEDIUM AND READY TO USE MEDIUM IN FLASKS: TYPICAL FORMULA FOR 1 L OF WATER

Meat peptone	18.00 g
Tryptone	6.00 g
Yeast extract	10.00 g
Sodium pyruvate	2.00 g
Glucose	2.00 g
Magnesium glycerophosphate	1.00 g
Magnesium sulphate	0.50 g
Sodium chloride	5.00 g
Lithium chloride	10.00 g
Disodium hydrogen phosphate anhydrous	2.50 g
5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.05 g
Agar	13.50 g

ALOA® SELECTIVE SUPPLEMENT	(vial contents for 500 mL of medium)	(vial contents for 200 mL of medium)
Nalidixic acid. sodium slat	0.010 g	0.004 g
Ceftazidime	0.010 g	0.004 g
Cicloheximide	0.025 g	0.01 g
Polymyxin B sulphate	38,350 UI	15,340 UI

ALOA® ENRICHMENT SUPPLEMENT	(vial contents for 500 mL of medium)	(vial contents for 200 mL of medium)
L-α-fosphatidylinositol	1.0 g	0.4 g

ALOA®-AGAR LISTERIA ACC. TO OTTAVIANI & AGOSTI, READY-TO-USE PLATES

Meat peptone	18.000 g
Tryptone	6.000 g
Yeast extract	10.000 g
Sodium pyruvate	2.000 g
Glucose	2.000 g
Magnesium glycerophosphate	1.000 g
Magnesium sulphate	0.500 g
Sodium chloride	5.000 g
Lithium chloride	10.000 g
Disodium hydrogen phosphate anhydrous	2.500 g
5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.050 g
Agar	13.500 g
Nalidixic acid, sodium salt	0.020 g
Ceftazidime	0.020 g
Cicloheximide	0.050 g
L-α-fosphatidylinositol	2.00 g
Polymyxin B sulphate	76,700 IU
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Agar Listeria acc. to Ottaviani and Agosti (ALOA) is a chromogenic and selective medium for the detection and enumeration of *L. monocytogenes* and *Listeria* spp. in samples of the food chain and in environmental samples due to its ability to differentiate *L. monocytogenes* from other *Listeria* species, even in the presence of a mixed flora.
ALOA medium is recommended by ISO 11290-1[1] and ISO 11290-2[2] for detection and enumeration of *L. monocytogenes* and *Listeria* spp. and it is also cited by FDA-BAM[3], APHA[4] and other regulatory agencies[5,6].
ALOA medium was conceived by Franco Ottaviani and Marco Agosti[7] and industrialised by Biolife in the mid-1990s.
ALOA has been compared by several authors with PALCAM and Oxford media and some other chromogenic media: all results confirm the superiority of ALOA medium over conventional media and other chromogenic media.[8-13]

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Lequerq[14] reported that ALOA was the best medium of the four tested and that its introduction into the laboratory methods to replace Oxford and PALCAM increases the isolation and counting of atypical *L. monocytogenes* strains.
Gracieux et al.[15] reported a higher recovery rate of virulent, hypovirulent and avirulent strains of *L. monocytogenes* with ALOA than with PALCAM medium and other chromogenic media.
Sacchetti et al.[16] reported that in an experiment with 132 food samples, ALOA and a second chromogenic medium allowed faster detection of *L. monocytogenes* with greater sensitivity and specificity than PALCAM medium.
According to Jadhav et al.[17], identification with MALDI-TOF was optimal with colonies grown on ALOA medium.
Peptones and yeast extract provide nitrogen, carbon, vitamins particularly of the B-group and trace elements for microbial growth. Glucose is a source of carbon and energy, sodium chloride maintains the osmotic balance, and sodium phosphate bibasic is included as a buffer system. Sodium pyruvate aids in resuscitation of stressed cells and magnesium salts stimulate the growth of *Listeria* spp. The selective action is due to the presence of lithium chloride in the basal medium and the addition of the antimicrobial mixture of the selective supplement containing ceftazidime, polymyxin B, nalidixic acid and cycloheximide. The medium markedly reduces the growth of the majority of concomitant Gram-positive and Gram-negative bacteria, as well as of yeasts and fungi.
The differential property of ALOA is due to the presence of the chromogenic compound 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside, a substrate for the detection of the enzyme ß-glucosidase, which is common to all *Listeria* species. Specific differential action is achieved with a substrate for phospholipase C (PI-PLC: phosphatidyl inositol phospholipase C). *Listeria monocytogenes* cleaves this specific substrate added to the medium base producing an opaque halo around the colonies. Most *Listeria ivanovii* also produce an opaque halo around the colonies after 48 h incubation. With the combined action of the two substrates, it is possible to differentiate the following colonies: *L. monocytogenes*: blue-green colonies surrounded by an opaque halo, *Listeria* other than-*monocytogenes* and *ivanovii*: blue-green colonies without the opaque halo.

4A- Directions for medium preparation (dehydrated medium)

Suspend 35.3 g in 500 mL of cold purified water, heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C add the contents of one vial of ALOA Enrichment Supplement pre-warmed to 48-50°C, and the contents of one vial of ALOA Selective Supplement, reconstituted with 5 mL of ethanol/sterile distilled water (1:1). Mix well and distribute into sterile Petri dishes.

4B- Directions for medium preparation (REF 511605K3 medium in flasks and supplements)

Dissolve the contents (200 mL) of one flask of ALOA medium in a water bath at 100°C. Cool to 47-50°C add the contents of one vial of ALOA Enrichment Supplement pre-warmed to 48-50°C, and the contents of one vial of ALOA Selective Supplement, reconstituted with 5 mL of ethanol/sterile distilled water (1:1). Mix well and distribute into sterile Petri dishes.

5 - Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	yellowish, opalescent
Prepared flasks appearance	yellowish, opalescent
Freeze-dried selective supplement	white, high, compact pellet; colourless and clear solution after reconstitution
Enrichment supplement appearance	cloudy, yellow suspension with a slight precipitate
Final pH of complete medium (at 20-25°C)	7.2 ± 0.2

6 - Materials provided – packaging

Product	Type	REF	Pack
Agar Listeria acc. to Ottaviani & Agosti (ALOA®)	Dehydrated medium	4016052	500 g (7,1 L)
Agar Listeria acc. to Ottaviani & Agosti (ALOA®)	Dehydrated medium	4016054	5 kg (71 L)
ALOA® Enrichment Selective Supplements	Freeze-dried and liquid supplements	423501	4+4 vials, each for 500 mL of medium
ALOA® Enrichment Selective Supplements	Freeze-dried and liquid supplements	423505	5+5 vials, each for 200 mL of medium
ALOA® -Agar Listeria acc. to Ottaviani & Agosti	Ready-to-use plates	541605	2 x 10 plates ø 90 mm
ALOA® -Agar Listeria acc. to Ottaviani & Agosti	Ready-to-use plates	501605P	5 plates ø 150 mm
ALOA® Flasks Kit	Ready-to-use flasks and supplements	511605K3	4x200mL ALOA flasks + 4 vials of ALOA Enrichment Supplement and 4 vials of ALOA Selective Supplement, each for 200 mL of medium base

7 - Materials required but not provided

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

8 - Specimens

Foods, animal deeding stuffs, food chain and environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[1-6]

9 - test procedure

Detection of *Listeria monocytogenes* and *Listeria* spp (ISO 11290-1)[1]

1. In general, to prepare the initial suspension, add a test portion of 25 g or 25 mL to 225 g or 225 mL of Fraser Broth Half Concentration, to obtain a tenfold dilution, and homogenize.
2. Incubate the primary enrichment medium at 30 °C for 25 h ± 1 h.
3. Transfer 0.1 mL of the culture to a tube or bottle containing 10 mL of secondary enrichment medium (Fraser Broth) and incubate for 24 h ± 2 h at 37 °C. In the case of *Listeria* spp. other than *Listeria monocytogenes* detection, additional 24 h incubation can allow for recovery of more species.

4. From the primary enrichment culture inoculate, by means of a loop, the surface of the first selective plating medium, Agar Listeria according to Ottaviani and Agosti (ALOA), to obtain well-separated colonies. Proceed in the same way with the second selective plating-out medium of choice (e.g., PALCAM or Oxford Agar).
5. From the secondary enrichment medium, repeat the procedure with the two selective plating-out media.
6. Incubate ALOA plates at 37°C ± 1°C for 24 ± 2 hours; if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
7. Incubate the second plating out medium according to the Instructions for Use
8. Examine the dishes for the presence of presumptive colonies of *L. monocytogenes* or *Listeria* spp.

Notes
It is possible to store at 5 °C the pre-enriched sample after incubation before transfer to Fraser broth for a maximum of 72 h.
Half-Fraser broth and Fraser broth can be refrigerated at 5 °C before isolation on selective agar for a maximum of 72.
After incubation, ALOA plates can be refrigerated at 5 °C for a maximum of 48 h before reading.

Enumeration of *Listeria monocytogenes* and of *Listeria* spp (ISO 11290-2)[2]

1. Prepare a sample suspension in Buffered Peptone Water or other suitable enrichment broth according to ISO 6887 (all parts); in case both determination and counting are performed according to parts 1 and 2 of ISO 11290, the sample suspension may be made in half-Fraser broth (with or without the addition of the selective supplement).
2. Inoculate 0.1 mL of the sample suspension and 0.1 mL of further decimal dilutions onto 90 mm plates of ALOA medium.
3. For samples with suspected low number of target-strains, inoculate 1 mL of the sample suspension and 1 mL of further decimal dilutions onto 140 mm plates of ALOA medium.
4. Examine after incubation at 37°C for 24 ± 2 hours and, if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
5. Count *L. monocytogenes* colonies and *Listeria* spp. colonies in plates with less than 150 colonies (90 mm diameter plates) or 360 colonies (140 mm plates) according to the section "reading and interpretation".

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies on plating out media.
Consider as presumptive *L. monocytogenes* the blue-green colonies surrounded by an opaque halo.
Consider as presumptive *Listeria* spp. the blue-green colonies with or without opaque halo.
Second plating-out medium: after incubation at the temperature described by the Instructions for Use, examine for the presence of typical colonies according to the characteristics of the chosen medium.

Confirm typical colonies by the methods and tests indicated in ISO 11290-1 or ISO 11290-2, after purification of the colonies in Tryptic Glucose Yeast Agar.
The mandatory confirmatory tests for *L.monocytogenes*, according to ISO 11290 and using ALOA medium, are the following: β-hemolysis (+), carbohydrate utilization (L-rhamnose +; D-xylose -). Optional confirmatory tests for *L.monocytogenes* are:, catalase (+), mobility at 25°C (+). The mandatory confirmatory tests for *Listeria* spp. are: microscopic examination, catalase (+); optional tests are: VP (+), mobility at 25°C (+).
Miniaturized galleries for the biochemical identification of *Listeria monocytogenes* may be used (Listeria Monoconfirm Test REF 193000)

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 13932	37°C /24-48h A	green-blue colonies surrounded by an opaque halo
L. monocytogenes NCTC 7973	37°C /24-48h A	green-blue colonies surrounded by an opaque halo
L. innocua ATCC 33090	37°C /24-48h A	green-blue colonies without opaque halo
L. ivanovii ATCC 19119	37°C /24-48h A	green-blue colonies with opaque halo
E. coli ATCC 25922	37°C / 48h A	inhibited
E. faecalis ATCC 19433	37°C / 48h 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 ALOA dehydrated and ready-to-use medium and supplements are tested for productivity and selectivity by comparing the results with Tryptic Soy Agar.
Productivity is tested by a quantitative test with the target strains *L.monocytogenes* ATCC 13932 and NCTC 7973: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 35-37°C for 24-48 hours. The colonies are enumerated on both batches and the productivity ratio (*Pr*) is calculated. If *Pr* is ≥ 0.5 and if the colonies morphology and colour are typical (green-blue colonies with opaque 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 24-48 hours: *L.innocua* grows with green-blue colonies without opaque halo while *L.ivanovii* exhibits a good growth after 48 hour of incubation with green-blue colonies and an opaque 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, *S.aureus* ATCC 25923, *S.sciuri* wild strain CB 16.1, and *C.albicans* ATCC 10231. After incubation at 35-37°C for 48 hours, the growth of non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- The reading of plates with abundant growth can be facilitated by comparing the opacity of the medium at the edges where there may be no growth with that in the centre of the plate or by comparing with an uninoculated plate. Plates with a confluent and intense growth of *L. monocytogenes* will still appear intensely opaque; in the case of intense growth of *Listeria* sp. other than *monocytogenes* the plates will not be opaque. If there is any doubt, the colonies should be re-isolated.
- *L.ivanovii* at 24 hours and especially after 48 hours of incubation, presents blue-green colonies with an opaque halo. In these cases, confirmatory tests will allow correct identification.
- Some strains of *Bacillus cereus*, which are resistant to the selective agents, may produce flat, wrinkled, non-homogenous white to blue colonies with a large, intense halo.
- It has been reported[18] that some species of 6 genera of Gram-positive bacteria can grow on ALOA and sometimes generate blue or bluish colonies: *Bacillus* spp. (*B. circulans, B. clausii, B. licheniformis, B. oleronius*, *Cellulosimicrobium funkei*), *Enterococcus* spp. (*E. faecalis, E. faecium/durans*), *Kocuria kristinae, Marinilactibacillus psychrotolerans, Rothia terrae, Staphylococcus* spp. (*S.sciuri, S. saprophyticus subsp. saprophyticus/xylosus*), *Streptococcus*.
- Some strains of *L. monocytogenes* exposed to stress conditions, particularly acid stress, can show a very weak halo (or even no halo).[1,2]
- Some rare *L. monocytogenes* are characterized by a slow PIPLC activity. Such bacteria are detected when the total duration of incubation is more than, for example, four days. Some of these strains could be pathogenic. No *L. monocytogenes* strains have been described as PIPLC negative.[1,2]
- Rare strains of *L.monocytogenes* may not exhibit β-haemolysis.[1,2] If typical colonies on ALOA are β-haemolysis negative, additional confirmatory tests (Gram, catalase, mobility, CAMP test, PCR) are recommended.

14 - PRECAUTIONS AND WARNINGS

- ALOA medium and supplements 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. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Material 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, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- Be careful when opening the metal ring of the supplements to avoid injury.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving.
- The selective supplement is sterilized by membrane filtration, the enrichment supplement is sterilized by autocalving.
- 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.
- 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 supplements and the inoculated plates 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 product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +2°C /+8°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).

Freeze-dried 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).

Liquid supplement

Store until the expiry date stated on the label, at +2°C /+8°C. Do not use beyond this date. Open the liquid enrichment bottle with aseptic precautions and store at 2-8°C until the expiry date if the contents are not fully used.

Ready to use flasks

Upon receipt, store flasks in their original pack at +2°C /+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).

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°C /+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).

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 (plates/flasks) and the applied storage conditions (temperature and packaging). According to Corry *et al.*[19] the self-prepared plates can be stored at +2°C /+8°C in the dark and protected against evaporation for up to four weeks.

16 - REFERENCES

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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	For single use only	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 7	Updated layout and content	2022/06

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