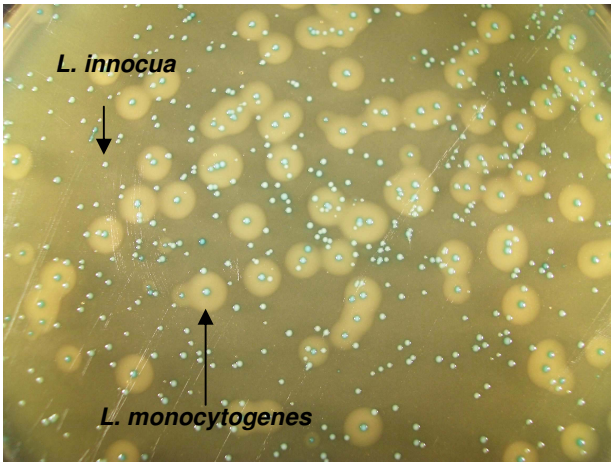
ALOA®

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

Powder medium, selective and enrichment supplements and ready to use plates for the detection and enumeration of *L. monocytogenes* in foods and animal feeding stuffs.



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

TYPICAL FORMULAS

Agar Listeria acc. to Ottaviani & Agosti (ALOA) (g/l)

Meat peptone	18.00
Tryptone	6.00
Yeast extract	10.00
Sodium pyruvate	2.00
Glucose	2.00
Magnesium glycerophosphate	1.00
Magnesium sulphate	0.50
Sodium chloride	5.0
Lithium chloride	10.0
Disodium hydrogen phosphate anhydrous	2.5
5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.05
Agar	13.5

ALOA Selective Supplement (vial contents for 500 ml of medium)

Nalidixic Acid,	10 mg
Ceftazidime	10 mg
Cycloheximide	25 mg
Polymyxin B	38350 IU

ALOA Enrichment Supplement (vial contents for 500 ml of medium)

L-α-fosphatidylinositol	1.0 g

Agar Listeria acc. to Ottaviani & Agosti (ALOA) - ready to use plates

ALOA	1000 ml
ALOA Selective Supplement	10 ml
ALOA Enrichment Supplement	40 ml

DIRECTIONS FOR PREPARATION FROM DEHYDRATED MEDIUM

Suspend 35.3 g in 500 ml of cold distilled water, heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 48-50°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 in sterile Petri dishes. Aspect of the medium: homogeneously turbid.

Final pH 7.2 ± 0.2

DESCRIPTION

Agar Listeria acc. to Ottaviani & Agosti (ALOA), complete with selective and enrichment supplements, is a selective and differential medium for the isolation of *Listeria* spp. from foodstuffs and other samples and for the identification of *L. monocytogenes*. The selectivity of the medium is due to lithium chloride and to the addition of antimicrobial selective mixture containing ceftazidime, polymyxin B, nalidixic acid and cycloheximide. The differential activity is due to the presence in the medium of the chromogenic compound X-glucoside as a substrate for the detection of ß-glucosidase enzyme, common to all *Listeria* species. The specific differential activity is obtained by means a substrate (L-α-phosphatidylinositol) for a phospholipase C enzyme that is present in *L. monocytogenes* and in some strains of *L.ivanovii.* Thanks to the combination of both substrates, it is possible to differentiate the colonies of *Listeria* spp., which grow with a green-blue colour, from the colonies of *L. monocytogenes,* which grow with a green-blue colour surrounded by an opaque halo.
Agar Listeria acc. to Ottaviani & Agosti (ALOA) allows to differentiate *L. monocytogenes* even in presence of a mixed flora, after incubation of 24 +/- 2 hours in an easy and reliable way, as well as direct streaking, or after enrichment in the usual selective liquid media.
ALOA has been testes by several authors in comparison with PALCAM and Oxford media (1,8,10,11,13) and with other chromogenic media (3,9). All these studies shown this medium to be superior to PALCAM and Oxford Media and to other available chromogenic media. ALOA medium has been validated as ready to use plates (1) and it is recommended by ISO 11290-1 Amd.1:2004 and by ISO 11290-2 Amd.1:2004 (5a, 5b) for the detection and enumeration of *L.monocytogenes* in foods and animal feeding stuffs.

TECHNIQUE

Agar Listeria acc. to Ottaviani & Agosti (ALOA) can be used according to the usual methods for the detection of *L. monocytogenes* after 2 steps or 1 step enrichment and for the enumeration of *L.monocytogenes*.

1 step enrichment method (rapid method)

If 1 step enrichment procedure is chosen the following "rapid" method, validated by AFNOR, may be followed:
Inoculate the sample in Fraser Broth Half Concentration in a ratio of 1:10 (e.g. 25 g sample + 225 ml of enrichment broth). Incubate at 30°C for 24+/-2 hours. Transfer a loopful of enrichment broth to the surface of ALOA plates. Examine the plates after incubation at 37°C for 24 +/- 2 hours.
Consider as *L. monocytogenes* the green-blue colonies surrounded by an opaque halo (typical colonies). Confirm the presumptive *L.monocytogenes* colonies with ALOA Confirmation Agar (REF 401606) or other suitable confirmation tests.
Consider as *Listeria* sp. non-*monocytogenes* the green- blue colonies without the opaque halo.
If no typical colonies are present after 24 h of incubation or if no growth occurs, re-incubate the plates for further 18-24 hours. If no typical colonies develop, the sample can be considered *L.monocytogens* free. If typical colonies grow in the second period of incubation confirm these colonies as described above.

2 steps enrichment method (ISO 11290-1)

The procedure recommended by ISO 11290 part 1 (detection) is the follow:
Make a 1:9 dilution of the sample in Fraser Broth Half Concentration (eg 25 g of sample + 225 ml of liquid medium). Incubate at 30°C for 24 hours.
Streak 0.1 ml aliquots of Fraser Broth Half Concentration onto a plate of ALOA medium and onto a second selective plating medium of choice. Incubate ALOA plates at 37°C for 24 ± 2 hours. Reincubate negative plates for a further 24 ± 2 hours.
Subculture 0.1 ml Fraser Broth Half Concentration into 10 ml of Fraser Broth and incubate at 37°C for 24 hours. If no growth occurs incubate a further 24 hours.

Streak 0.1 ml aliquots of Fraser Broth onto a plate of ALOA medium and onto a second selective plating medium of choice. Incubate ALOA plates at 37°C for 24 ± 2 hours. Re-incubate negative plates for a further 24 ± 2 hours.
Confirm the typical colonies as described into ISO standard.

Enumeration method (ISO 11290-2)
The procedure recommended by ISO 11290 part 2 (enumeration) is the follow:
Make a 1:9 dilution of the sample in Fraser Broth Half Concentration or in Buffered Peptone Water (e.g. 25 g of sample + 225 ml of liquid medium). Incubate at 20°C for 1 hour.
Streak or spread 0.1 ml of resuscitated suspension onto a plate of ALOA medium and incubate at 37°C for 24 ± 2 hours. Reincubate negative plates for a further 24 ± 2 hours.
Confirm the typical colonies as described into ISO standard.

USER QUALITY ASSURANCE (37°C –24 h)
Productivity control
L.monocytogenes ATCC 19111: growth, green-blue colonies surrounded by an opaque halo
L.monocytogenes ATCC 13932*:* growth, green- blue colonies surrounded by an opaque halo
Specificity control: *L.innocua ATCC 33090:* growth*,* green-blue colonies without opaque halo
Selectivity control: *E.coli* ATCC 25922, *E.faecalis* ATCC 19433*, C.albicans* ATCC 10231: inhibited

STORAGE
Dehydrated medium and selective/enrichment supplement: 2-8°C
Ready to use plates: 2-8°C
User prepared plates: up to 7 days at 2-8°C

WARNING
ALOA Selective Supplement contains cycloheximide, which is toxic and causes severe irritation to the skin and mucous membranes. The product is classified as T+; see the precautions to be taken on the product label

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5a-ISO 11290-1:1996 Amd.1:2004. Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method- Amendment 1: Modification of the isolation media and the haemolysis test and inclusion of precision data.
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PACKAGING

Dehydrated medium

4016052	**Agar Listeria acc. to Ottaviani & Agosti (ALOA),**	500 g (7,1 l)
4016054	**Agar Listeria acc. to Ottaviani & Agosti (ALOA),**	5 kg (71 l)

Selective supplements

423501	**ALOA Enrichment Selective Supplements,**	4+4 vials each for 500 ml of medium
423505	**ALOA Enrichment Selective Supplements,**	5+5 vials each for 200 ml of medium

Ready to use plates

541605	**Agar Listeria acc. to Ottaviani & Agosti (ALOA),**	20 ready to use plates, ø 90 mm
501605P	**Agar Listeria acc. to Ottaviani & Agosti (ALOA),**	5 ready to use plates, ø 140 mm

Kit

511605K3 **ALOA Flasks Kit** 4x200ml ALOA flasks + 4 vials of ALOA Enrichment Supplement and 4 vials of ALOA Selective Supplement, each for 200ml of medium base

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