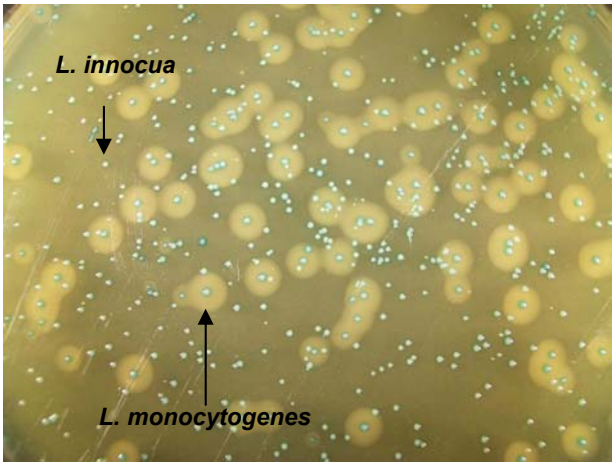
ALOA®

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*



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

Typical Formula

TYPICAL FORMULA (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	15.0
Nalidixic Acid	20 mg
Ceftazidime	20 mg
Cycloheximide	50 mg
Polymyxin B	76700 IU
L-α-fosphatidylinositol	2.0 g

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-fosphatidylinositol) 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 isolation of *L. monocytogenes* after 2 steps or 1 step enrichment. 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 20°C for 18-24 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) confirmed with the rapid test Monocytogens ID Discs (cat. n° 193005) 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.
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. Reincubate negative plates for a further 24 ± 2 hours.
Confirm the typical colonies as described into ISO standard.
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.

Storage
Store at 2-8° - When stored as directed the plates remain stable until the expiry date shown on the label. Do not use beyond stated expiry date.

References
1-Artault, S., Bind,J.L., Delaval,Y., Dureuil, N., Gaillard, N. (2000) AFNOR Validation of the ALOA method for the detection *of Listeria monocytogenes* in foodstuffs. Colloque de la Societé Francaise de Microbiologie, Paris, 19-20 Octobre, 2000.
2-Bauwens L. Vercammen F. Hertsens A. (2003) Detection of Listeria spp. In zoo animal faeces: use of immunomagnetic separation and a chromogenic medium Vet. Microbiol. 91, 115-123
3-Beumer, L.L. (2001) Horizontal method for the detection of *Listeria monocytogenes* ISO 11290-1. Change of Isolation Media. Wageningen University, The Nederlands.
4-Flamini, L., Rossi, I. Pondini, F. (1999) Conteggio rapido di *Listeria monocytogens* per inclusione in terreno selettivo e differenziale (ALOA). Industrie Alimentari, XXXVIII, febbraio, 127.

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.
5b-ISO 11290-2:1998 Amd.1:2004. Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method- Amendment 1: Modification of the enumeration medium. Mioni, R., Grimaldi,
6-Gracieux P., Roche S.M., Pardon P., Velge P. (3003) Hypovirulent L.monocytogenes strains are less frequently recovered than virulent strains on PALCAM and Rapid'L.mono media. Int. J. Food Microbiol. 83, 133-145.
7-Leclerq A. (2004) Atypical colonial morphology and low recovery *of L. monocytogenes* strains on Oxford, PALCAM, Rapid'L.mon and ALOA solid media. J. of Microbiological Methods, 57, 252-258.
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9-Moroder L (2002) Comparison of alternative methods for the enumeration of *Listeria monocytogenes* in food. FEMS-Symposium on the Versatility of Listeria species. Izmir, October 10-11, 2002
10-Ottaviani, F., Ottaviani, M., Agosti, M. (1997) Esperienze su un agar selettivo e differenziale per *Listeria monocytogens*. Industrie Alimentari, XXXVI, luglio-agosto, 888.
11-Ottaviani, F., Ottaviani, M., Agosti, M. (1997) Differential agar medium for *Listeria monocytogenes*. Quinper Froid Symposium Proceedings, P6 A.D.R.I.A. Quinper (F) 16-18 June, 1997
12-Sacchetti R., Bianucci F., Ambrogiani E. (2003) Detection of L.monocytogenes in foodstuffs using chromogenic isolation media. New Microbiol. 26, 269-274.
13-Vlaemynck, G., Lafarge, V., Scotter, S. (2000) Improvement of the detection of *Listeria monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium. J.Appl. Microbiol., 88, 430.

Packaging

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