ALOA® FLASKS KIT

Ready to use flasks and supplements for the preparation of ALOA medium for the detection and enumeration of *L. monocytogenes* in foods and animal feeding stuffs.

![Image of L. innocua and L. monocytogenes colonies](image)

**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 200 ml of medium)**

- Nalidixic Acid: 4 mg
- Ceftazidime: 4 mg
- Cycloheximide: 10 mg
- Polymyxin B: 15340 IU

**ALOA Enrichment Supplement (vial contents for 200 ml of medium)**

- L-α-fosphatidylinositol: 0.4 g

**DIRECTIONS FOR PREPARATION FROM DEHYDRATED MEDIUM**

Dissolve the contents of the bottle in a temperature controlled water batch (100°C). Cool to 45-50°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 2 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-ß-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 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). 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. monocytogenes 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**

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

**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.

**REFERENCES**


**PACKAGING**

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