

Specification

Selective supplement for isolation and confirmation of *Listeria monocytogenes*. formulated according to ISO 11290-1 and 2:1996 Amd 2004

Presentation

10 Freeze dried vials

with: 3 ± 0.1 g

Packaging Details

22 \pm 0.25 x 55 \pm 0.5 mm glass vials, tag labelled, plastic cap - 10 vials per box.

Shelf Life

49 months

Storage

2-25 °C

Composition

Compositon (g/vial)

Polymyxin B.....	38350 IU
Cycloheximide.....	0.025
Ceftazidime.....	0.010
Nalidixic acid.....	0.010

Note: Each vial is sufficient to supplement 470 ml of Listeria Agar Base according to Ottaviani and Agosti

Reconstitute the original freeze-dried vial by adding 1 vial with Sterile distilled water..... 6 ml

Description /Technique

Description:

Completed with all its supplements the Agar Listeria Ottaviani & Agosti is a selective and differential medium for the detection of *Listeria* species and the presumptive identification of *Listeria monocytogenes*.

The selectivity is achieved by the high concentration of lithium chloride and the mixture of antimicrobics. The differential activity is due to the chromogenic substrate to detect the β -glucosidase enzyme that is present in all *Listeria* species.

The specific identification is obtained by the L- α -phosphatidylinositol, that acts as substrate for a phospholipase C present only in *Listeria monocytogenes* and some strains of *Listeria ivanovii*.

The combination of both substrates allows the differentiation *L. monocytogenes*, which grow in produces colonies blue-green in colour and surrounded by an opaque zone, from the other *Listeria* species, which blue-green colonies but without any halo. This differentiation is evident after incubating the plates for 24 \pm 2 hours at 37 °C.

Sometimes, especially with highly contaminated samples, it is possible that some colonies, white in colour, are not *Listeria* growth. In this case an enrichment step is recommended prior to plate inoculation.

Observations: Most *Listeria ivanovii* also produce an opaque halo around the colonies after 48 h of incubation. This presumptive evidence must be confirmed by performing the biochemical or serological identification tests (Rhamnose / Xylose sugar fermentation, hemolysis tests, CAMP test, etc.) or any test confirming the species without hesitation.

Technique:

Add 1 bottle supplement Ottaviani & Agosti (L-alpha-phosphatidylinositol) and 1 vial supplemet Ottaviani & Agosti for complete 500 ml medium.

Homogenize by mixing and distribute in Petri dishes. The solidified cool medium appears homogeneously turbid.

There are many standardised methodologies (ISO, FDA-BAM, AOAC, AFNOR, etc.). The technician must follow the protocol validated in his laboratory.

Quality control

Physical/Chemical control

Color : White

Microbiological control

Spiral Spreading: Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / $10^4\text{-}10^6$ CFU (selectivity).

Microbiological control according to ISO 11133:2014/A1:2018.

Aerobiosis. Incubation at 35 ± 2 °C, reading after 18-24 hours.

Microorganism

Escherichia coli ATCC® 25922, WDCM 00013

Growth

Enterococcus faecalis ATCC® 29212, WDCM 00087

Inhibited

L. monocytogenes ATCC® 13932, WDCM 00021

Inhibited

Listeria innocua ATCC® 33090, WDCM 00017

Good - Blue colonies with white halo

L. monocytogenes ATCC® 35152, WDCM 00109

Blue colonies without white halo

L. monocytogenes ATCC® 35152, WDCM 00109

Good - Blue colonies with white halo

Sterility control

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

Bibliography

- Artault, S., J.L. Bind, Y. Delaval, N. Dureuil, N. Gallart (2000) AFNOR validation of the ALOA method for the detection of Listeria monocytogenes in foodstuffs. Coll. Soc. Fran. Microbiol. 19-20 Oct. Paris.
- Bannerman, E.S. & J. Bille (1988) A new selective medium for isolating Listeria from heavily contaminated material. Appl.m Environm. Microbiol. 54:1:165-167.
- Greenwood, M., C. Willis, P. Dosweell, G. Allen & K. Pathak (2005) Evaluation of chromogenic media for the detection of Listeria species in food.
- Hitchins, A.D. & K. Jinneman (1998) Listeria monocytogenes in FDA-BAM 8th edition Revision A. Updater January 2003. AOAC Intl. Gathersburg. MD. USA.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 11290-1:2017 Standard. Microbiology of the food chain. Horizontal method for the detection and enumeration of Listeria monocytogenes and for Listeria spp.- Part 1: Detection Method
- ISO 11290-2:2017 Standard. Microbiology of the food chain. Horizontal method for the detection and enumeration of Listeria monocytogenes and for Listeria spp.- Part 2: Enumeration Method
- Jantzen, M.M., J. Navas, M. de Paz, B. Rodriguez, W.P. da Silva & M. Nuñez (2006) Evaluation of ALOA plating medium for its suitability to recover high pressure-injured Listeria monocytogenes from ground chicken meat. Letters Appl. Microbiol 43:313-317
- Manafi, M. W. Kneifel & S. Bascomb (1991) Fluorogenic and chromogenic substrates used in bacterial diagnostics. Microbiol Rev. 55:3:335-348
- Ottaviani, F., M. Ottaviani & M. Agosti (1997) Esperienza su un agar salettivo e differenziale per Listeria monocytogenes. Industrie Alimentari 36:1-3
- Victor Lachica, R. (1990) Selective plating medium for quantitative recovery of food-borne Listeria monocytogenes. Appl. Environm. Microbiol. 56:1:167-169
- Watkins, J. & K.P. Sleath (1981) Isolation and enumeration of Listeria monocytogenes from sewage, sewage sludge and river water. J. Appl. Bacteriol. 50:1-9
- UNE-EN ISO 11133 (2014). Microbiología de los alimentos para consumo humano, alimentación animal y agua.-Preparación, producción, conservación y ensayos de rendimiento de los medios de cultivo.