

Specification

Chromogenic medium for the isolation of *Listeria spp* and the presumptive identification of *L. monocytogenes*.

Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared Plates 90 mm with: 21 ± 2 ml	1 box with 2 cellophane bags with 10 plates/bag.	3 months	2-14 °C

Composition

Composition (g/l):	
Meat Peptone.....	18.00
Casein Digest.....	6.000
Yeast Extract.....	10.00
Sodium pyruvate.....	2.000
Glucose.....	2.000
Magnesium glycerophosphate.....	1.000
Magnesium sulfate.....	0.500
Sodium chloride.....	5.000
Lithium chloride.....	10.00
Disodium hydrogen phosphate (anhydr.)...	2.500
BCI glucopyranoside.....	0.050
Nalidixate.....	0.020
Ceftazidime.....	0.020
Polymyxine B.....	76.700 UI
Cycloheximide.....	0.050
Phosphatidyl-Inositol.....	2.000
Agar.....	12.000

Description /Technique

Description

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 that is present only in *Listeria monocytogenes* and some strains of *Listeria ivanovii*.

The combination of both substrates allows the differentiation *L. monocytogenes* that produces colonies blue-green in colour but surrounded by an opaque zone from the other *Listeria* species that growth with blue-green colonies without any halo. This differentiation is evident after incubate the plates for 24±2 hours at 37 °C.

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

There are a lot of standardized methodology (ISO, FDA-BAM, AOAC, AFNOR, etc.). The technician must follow the protocol validated in his laboratory.

Incubate the plates right side up aerobically at 37°C for 24- 48 h.

After incubation, the colonies that have appeared onto the surface of the agar with the following appearances:

L.monocytogenes - Blue-green Colonies surrounded by opaque halo

L.innocua - Blue-green Colonies without opaque halo

Other bacteria - Blue, Colourless or completely inhibited, without halo.

Quality control

Physical/Chemical control

Color : Yellowish

pH: 7.2 ± 0.2 at 25°C

Microbiological control

Spiral Spreading: Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10⁴-10⁶ CFU (selectivity).

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

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 37 °C ± 1, reading after 44 ± 4h

Microorganism

L. monocytogenes ATCC® 13932, WDCM 00021

Listeria innocua ATCC® 33090, WDCM 00017

Enterococcus faecalis ATCC® 29212, WDCM 00087

Escherichia coli ATCC® 25922, WDCM 00013

L. monocytogenes ATCC® 35152, WDCM 00109

Growth

Good (≥ 50 %) - Blue-green colonies with opaque halo

Good - Blue colonies without white halo

Inhibited

Inhibited

Good (≥ 50 %) - Blue-green colonies with opaque halo

Sterility Control

Incubation 48 h at 30-35 °C and 48 h 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. Environ. 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. Gaithersburg. 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 selettivo 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. Environ. 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.

Storage

Storage conditions: 2-14°C

Avoid direct contact with surfaces that can freeze product.