


Formula * in g/L

Meat peptone.....	18.000		
Tryptone.....	6.000	Lithium chloride.....	10.000
Yeast extract.....	10.000	Disodium phosphate anhydrous.....	2.500
Sodium pyruvate.....	2.000	5-bromo-4-chloro-3-indolyl-	
Dextrose.....	2.000	β-D-glucopyranoside.....	0.050
Magnesium glycerophosphate.....	1.000	Agar.....	13.000
Magnesium sulphate.....	0.500		
Sodium chloride.....	5.000	Final pH 7.2 ± 0.2 at 25 °C	

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 35 g of powder in 476 ml of purified water and bring to the boil with constant stirring. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 45-50°C and add 1 bottle of Listeria enrichment supplement Ottaviani & Agosti (Art. DSHB3072) and 1 vial of Listeria selective supplement Ottaviani & Agosti (Art. DSHB3071). Homogenize by mixing and distribute in Petri dishes. The solidified medium appears turbid.

Description

Completed with all its supplements the Agar Listeria O&A 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 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 (Ramnosa / Xylose sugar fermentation, hemolysis tests, CAMP test, etc.) or any test confirming the species without hesitation.

Remarks:

Listeria enrichment supplement Ottaviani & Agosti (Art. DSHB3072):

1 vial sufficient amount for 500ml complete medium

L-α-phosphatidylinositol..... 1.0 g

Steril distilled water..... 24.0 ml

Listeria selective supplement Ottaviani & Agosti (Art. DSHB3071):

1 vial sufficient amount for 500ml complete medium

Nalidixic acid..... 10.0 mg

Ceftazidime..... 10.0 mg

Cycloheximide..... 25.0 mg

Polymyxin B sulphate..... 38350 ui

Technique

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



Quality control

Incubation temperature:	37 ± 1 °C	Incubation time:	44±4 h
Inoculum:	Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10 ⁴ -10 ⁶ CFU (selectivity)/ 10 ³ -10 ⁴ CFU (specificity), according to ISO 11133:2014/Amd 1:2018.		
Microorganism	Growth	Remarks	
<i>Escherichia coli</i> ATCC® 25922	Inhibited	-	
<i>Listeria monocytogenes</i> ATCC® 13932	Productivity > 0.50	green-blue colonies surrounded by opaque halo	
<i>Listeria monocytogenes</i> ATCC® 35152	Productivity > 0.50	green-blue colonies surrounded by opaque halo	
<i>Listeria innocua</i> ATCC® 33090	Good (specificity)	green-blue colonies without opaque halo	
<i>Enterococcus faecalis</i> ATCC® 29212	Inhibited	-	

References

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- 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
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).