Listeria Chromogenic Agar Base according to Ottaviani and Agosti (ALOA) ISO

Cat. 1345

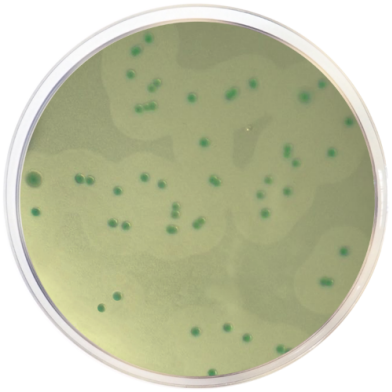
Selective medium for the detection and enumeration of Listeria monocytogenes.

Practical information

Aplications	Categories
Selective enumeration	Listeria
Detection	Listeria

Industry: Clinical / Food

Regulations: ISO 11133 / ISO 11290



Principles and uses

Listeria Chromogenic Agar Base acc. to Ottaviani and Agosti (ALOA) is a selective medium for the presumptive isolation and identification of Listeria monocytogenes and Listeria spp. in food and clinical samples. It is used for confirmation after using Listeria Enrichment Broth Base Fraser (Cat. 1120). This medium is also recommended by ISO 11290-1 for the detection and enumeration for Listeria monocytogenes.

Enzymatic digest of animal tissues and enzymatic digest of casein provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium pyruvate is a source of energy for bacterial metabolism and aids in the resuscitation of stressed organisms. Glucose is the fermentable carbohydrate providing carbon and energy. Magnesium glycerophosphate is a buffering compound. Magnesium sulphate is a magnesium ion required for a large variety of enzymatic reactions, including DNA replication. The differential activity of the medium is due to two factors. Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Cycloheximide provide the medium's selectivity. Bacteriological agar is the solidifying agent.

The presence of the chromogenic component X-glucoside, a substrate for the detection of the enzyme ß-glucosidase, is common to all Listeria species giving the colonies their blue colour. Other organisms that possess this enzyme, for example, Enterococci, are inhibited by the selective agents within the medium and by the selective supplement. The differential activity is also obtained by lipase C substrate, upon which the specific enzyme for L. monocytogenes acts. The lipase is responsible for the opaque white halo which surrounds L. monocytogenes.

The combination of both substrates allows us to differentiate the colonies of Listeria monocytogenes from the rest of Listeria spp. since, although all are blue in colour, L. monocytogenes present an opaque white halo surrounding them.

It has been observed that some strains of Listeria ivanovii, mostly pathogenic to animals although some have caused infections in humans, also possess lipase activity.

Formula in g/L

Enzymatic digest of casein	6	Glucose	2
Bacteriological agar	13,5	Magnesium sulfate	0,5
Sodium chloride	5	Sodium hydrogen phosphate	2,5
Sodium pyruvate	2	Yeast extract	10
Enzymatic digest of animal tissues	18	Lithium chloride	10
Magnesium glycerophosphate	1	5-Bromo-4-chloro-3-indolyl-ß-D-glucopyranoside	0,05

Preparation

Suspend 35,275 grams of the medium in 470 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. To prepare more quantity of 500 ml, it is recommended to sterilize at 115 ºC for 10

minutes. Cool to 70 ºC to aseptically add one bottle of Listeria Lipase C Supplement (24 ml) (Cat. 6031) and shake it strongly. Then, cool to 47-50 ºC and aseptically add one vial of Listeria Chromogenic Selective Supplement (Cat. 6040). Homogenize gently and dispense into Petri dishes.

Instructions for use

Dection and enumeration of Listeria monocytogenes and Listeria spp. according to ISO 11290:

Detection method:
- Weigh 25 g (or 25 ml) of the sample and add 225 ml of Half Fraser Broth (Cat.1183). Homogenize and incubate at 30 °C for 25±1 hours.
- Inoculate 0,1 ml of incubated Half Fraser Broth culture (regardless of its colour) into 10 ml of Fraser Broth (Cat.1182).
Incubate at 37 ºC for 24±2 hours in aerobic conditions.
- From the primary enrichment culture inoculate the surface of the Agar Listeria according to Ottaviani and Agosti and the other selective medium at the choice of the laboratory, to obtain well-separated colonies.
From the secondary enrichment culture, repeat the procedure, inoculate the surface of the Agar Listeria according to Ottaviani and Agosti and the other selective medium.
For Agar Listeria according to Ottaviani and Agosti incubate for a total of 48±2 h.
- Select the presumptive colonies and carry out the confirmation tests for L. monocytogenes or Listeria spp.

Enumeration method:
- Prepare an initial suspension 1:10 of sample and Buffered Peptone Water for analysis. Listeria 1/2 Fraser Broth (Cat. 1183) can be used as a diluent if the detection and enumeration procedures are carried out simultaneously.
- Inoculate 0,1 ml on the surface of Listeria Chromogenic Agar according to Ottaviani and Agosti.
- Incubate at 37 ºC for 24 ± 2 h. Incubate for an additional 24 hours in case no microbial growth is detected.
- Select the presumptive colonies and carry out confirmation tests for L. monocytogenes or Listeria spp.
- Calculate from the confirmed colonies the number of L. monocytogenes or Listeria spp colonies.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber slightly opalescent	7,2 ± 0,2

Microbiological test

According to ISO 11133:
Incubation conditions: Productivity, Selectivity and Specificity (37±1 ºC / 48±4 h).
Inoculation conditions: Productivity qualitative (10^3-10^4 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).
Reference media: TSA

Microorganisms	Specification	Characteristic reaction
Listeria monocytogenes 4b ATCC 13932	Good growth (2) >50%	Blue green colonies with opaque halo
Enterococcus faecalis ATCC 29212	Total inhibition (0)	
Listeria innocua ATCC 33090		Blue green colonies without opaque halo
Listeria monocytogenes 1/2a ATCC 35152	Good growth (2) >50%	Blue green colonies with opaque halo
Escherichia coli ATCC 8739	Total inhibition (0)	

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Ottaviani, F., Ottaviani, M. and Agosti, M (1987) Quimper Froid Symposium Proceedings, P6 A.D.R.I.A Quimper (F) 16-18 June.
ISO 11290 Horizontal method for the detection and enumeration of Listeria monocytogenes.