Specification

Solid, selective and differential medium for the detection, enumeration and isolation of *Listeria spp.,* according to ISO standards 11290-1 and 11290-2.

Formula * in g/L

Tryptone	23.00	Ammonium iron(III) citrate	0.50
Lithium chloride	15.00	Dextrose	0.50
Mannitol	10.00	Phenol red	0.08
Sodium chloride	5.00	Aga	13.00
Yeast extract	3.00		
Starch	1.00		
Esculin	0.80		

Final pH 7.2 ±0.2 at 25 °C

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

Directions

Suspend 72 g of powder in 1 L of distilled water and let it soak. Bring to the boil and distribute 500 mL per flask. Sterilize in the autoclave at 121ºC for 15 minutes. Cool to 50ºC and aseptically add one vial of Palcam Agar Selective Supplement (Ref. DSHB3052) to each flask. Mix well and pour into Petri dishes.
Note: Prepared medium (Agar + supplement) must be kept away from light, since it promotes the production of acriflavine oxidised photocomplexes that can repress *Listeria* growth.

Description

Palcam Agar is based on the formulation described initially by van Netten *et al.* which has a high selectivity and produces good colonial differentiation. Selectivity is achieved by the inclusion of lithium chloride, acriflavine, polymyxin B and ceftazidime, since they inhibit the growth of almost all the Gram negative and most of the Gram positive companion bacteria.
Listeria hydrolyze esculin to esculetin, which reacts with ferric ammonium citrate producing a dark precipitate and green-grey colonies with beige halos. If colonies of enterococci or staphylococci do grow on this medium they can be easily recognized, since they utilise mannitol and produce yellow colonies and haloes, contrasting with the cherry-red colour of medium.
However, when there are many *Listeria* colonies, the entire medium darkens, which can cause interference in differentiation. In these cases it is advisable to perform the inoculation with a more diluted sample.

Necessary supplements

Palcam Agar Selective Supplement (Ref. DSHB3052)
Vial contents:
Necessary amount for 500 mL of complete medium.

Acriflavine	2,50 mg
Polymyxin B sulfate	5,00 mg
Sodium cephtazidime	10,00 mg

Distilled water (Solvent)

Technique

Seed the Palcam Agar with growth from a primary enrichment broth (UVM I or Lovett) or a secondary enrichment broth (UVM II or Fraser). Incubate in a microaerophilic atmosphere for 44 ± 4h at 37 °C ±1.
In these conditions, Listeria colonies have a size approx. 2 mm in diameter, and are green-grey in colour with a black core and halo. Enterococcus and Staphylococcus colonies are bigger, grey with a green-brown halo if they do not ferment mannitol and form yellow colonies with a yellow halo if they do. Presumptive Listeria colonies must be confirmed biochemically and serologically.

Quality control

Incubation temperature: 37°C ±1,0 **Incubation time:** 44±4 h

Inoculum: Loop spreading (Specificity) /10^4-10^6 CFU (selectivity), according to ISO 11133.

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC® 29212	Inhibited	-
Escherichia coli ATCC® 25922	Inhibited	-
Listeria monocytogenes ATCC® 13932	Good	Esculin (+). Black medium
Listeria monocytogenes ATCC® 35152	Good	Esculin (+). Black medium

References

· ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press Boca Raton Florida.
· ISO 11290 standard (1996) Microbiology of food and animal feeding stuff. Horizontal method for the detection and enumeration of Listeria monocytogenes. Part 1 - Detection method. Part 2 - Enumeration method.
· 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
. ISO 11133:2014. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· VANDERZANT, C. & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington DC.
· Van NETTEN, P., J. PERALES, A.van deMOOSDUCK, G.D.W. CURTIS & D.A.A. MOSSEL (1989) Liquid and solid selective differential media for the detection and enumeration of Listeria monocytogenes. Int. J. Food Microbiol. 8:299-316.

Storage

Keep tightly closed, away from light, in a dry place (4-30 °C).