

## Specification

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

## Presentation

20 Prepared Plates  
90 mm  
with: 21 ± 2 ml

### Packaging Details

1 box with 2 packs of 10 plates/pack. Single cellophane.

### Shelf Life

3 months

### Storage

2-14°C

## Composition

Composition (g/l):

Tryptone.....	23.0
Lithium chloride.....	15.0
Mannitol.....	10.0
Sodium chloride.....	5.00
Starch.....	1.00
Esculin.....	0.80
Ferric Ammonium citrate.....	0.50
D(+) Glucose.....	0.50
Phenol red.....	0.08
Agar.....	13.0
Polymixin B.....	0.01
Acriflavine.....	0.005
Ceftazidime.....	0.023
Yeast extract.....	3.00

## Description /Technique

### 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.

### 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 48 hours at 37°C.

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

### Physical/Chemical control

Color : Reddish                      pH: 7.2 ± 0.2 at 25°C

### Microbiological control

Loop spreading

According to ISO 11290-1

Aerobiosis. Incubation at 37 °C±1, reading after 24-48±2h

### Microorganism

*Escherichia coli* ATCC® 25922, WDCM 00013

*L. monocytogenes* ATCC® 13932, WDCM 00021

*L. monocytogenes* ATCC® 35152, WDCM 00109

*Enterococcus faecalis* ATCC® 29212, WDCM 00087

### Growth

Inhibited

Good- Esculin +. Black medium

Good- Esculin +. Black medium

Inhibited

### 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

(Base sterilized by autoclaving with continuous stirring at 121°C for 15' - Sterile supplements added at 50°C)

## Bibliography

- 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/ Adm 1:2018. 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.