

Specification

Sterile selective supplement used for *Listeria* enrichment according to ISO 11290-1:2006.

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 6 ± 0.1 g	23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 °C

Composition

Composition (g/vial)

Sodium Nalidixate.....	0.0050
Acriflavine.....	0.0062
Ammonium Ferric citrate.....	0.2500

Reconstitute the original freeze-dried vial
by adding :
Sterile Distilled Water.....6 ml

Description /Technique

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Reconstitute the vial with 6 ml of sterile diluent, pre-warmed to approx. 37°C and add to 500 ml of sterilized Fraser Broth base cooled to room temperature.

Once into tubes or bottles, inoculate with the appropriate volume of sample or dilution. Incubate the tubes/bottles at 37±1°C for 24±2h. (Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample, on the specifications,...)

After incubation, subculture onto secondary selective broth- Then inoculate plates either darkened tubes, either only turbid tubes.

Enumerate all the colonies that have appeared onto the surface of the agar.

Presumptive isolation of *Listeria sp.* must be confirmed by further microbiological and biochemical tests.
Listeria strains cause PALCAM and Oxford media get dark due to esculin hydrolysis.
Listeria strains develop characteristic haloes on ALOA medium.

Quality control**Physical/Chemical control**

Color : Dark Orange - Brown -

Microbiological control

Prepare tubes - Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity).

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

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

Aerobiosis. Incubation at 30 ± 1 °C during 25 ± 1 h.

Microorganism

Escherichia coli ATCC® 8739, WDCM 00012 (1)

Enterococcus faecalis ATCC® 19433, WDCM 00009 (2)

Listeria monocytogenes ATCC® 13932 + (1) + (2)

Listeria monocytogenes ATCC® 35152 + (1) + (2)

Growth

Inhibited. Confirm in TSA at $37^{\circ}\text{C} \pm 1$ reading 24 ± 3 h

Partial Inhibition. Confirm in TSA at $37^{\circ}\text{C} \pm 1$ reading 24 ± 3 h.

> 10 CFU. Blue-green coln. w. opaque halo (Ottaviani Agosti)

> 10 CFU. Blue-green coln. w. opaque halo (Ottaviani Agosti)

Sterility control

Incubation 24h at $30\text{-}35$ °C and 72 h at $20\text{-}25$ °C: NO GROWTH.

Incubation 7 days at 32.5 ± 2 °C and 7 days at 22.5 ± 2 °C: NO GROWTH.

Add 5mL of the sample to 100 mL of TSB and to 100 mL Thioglycollate.

Bibliography

- ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Florida.
- FRASER, J.A. & W.H. SPERBER (1988) Rapid detection of *Listeria* spp. In food and environmental samples by esculin hydrolysis. J. Food Prot. 51:762-765.
- 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.
- McCLAIN, D. & W.H. LEE (1988) Development of a USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J.AOAC 71:660-664.
- VANDERZANT, C & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC.