

Specification

Sterile selective supplement used for *Listeria* isolation in food samples.

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials			
Vial	22±0,25 x 55±0,5 mm glass vials, tag labelled, White	49 months	2-25°C
with: 3 ± 0,1 g	plastic cap - 10 vials per box.		

Composition

Composition (g/vial)

Cycloheximide.....	0.2000
Colistin sulphate.....	0.0100
Acriflavine.....	0.0025
Cefotetan.....	0.0010
Phosphomycin sodium salt.....	0.0050

Note : Each vial is enough to supplement
500 ml of Oxford medium Base.

Reconstitute the original freeze-dried vial
by adding:
Sterile solvent (50% Ethanol/water) 9 ml

Description /Technique

Description:

Listeria Selective Supplement (Oxford Formulation) is added to Oxford agar base in order to obtain a complete selective medium for the detection of *Listeria monocytogenes* from clinical and food specimens.

Listeria monocytogenes plays an important role in human and animal illness and the sources of infections are numerous.

In the last years the lack of an effective selective medium has been a gap in the detection of *Listeria*, as it can be easily and completely overgrown by competing flora.

With his supplement, which supplies the selective inhibitory components acriflavine, colistin sulphate, cefotetan, cycloheximide and fosfomycin, the competing flora is inhibited. *Listeria monocytogenes* is differentiated because it hydrolyses aesculin, producing black zones around the colonies.

Gram-negative bacteria are completely inhibited and also most of the unwanted Gram-positive species. Some strains of enterococci grow poorly and exhibit a weak aesculin reaction, usually after 40 hours incubation. Some staphylococci may grow as aesculin-negative colonies.

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 9 ml of sterile diluent (50% Ethanol:water) in aseptic conditions and add it to 500 ml of Oxford Agar base cooled to 50°C.

Do not overheat once supplemented.

Pour the complete medium into Petri dishes and, once solidified on a flat surface, spread the plates by streaking or spiral method.

Incubate the plates in aerobic atmosphere at 37 ± 1°C for 44 ± 4h.

Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample or the specifications.

After incubation, enumerate all the colonies that have appeared onto the surface of the agar, observing any blackening of the medium due to esculin hydrolysis, typical for *Listeria* strains.

Presumptive isolation of *Listeria* must be confirmed by further microbiological and biochemical tests.

Quality control**Physical/Chemical control**

Color : Yellow- orangey pH: at 25°C

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Distribute the complete medium, cooled to 50°C, into 90 mm plates

Microorganism*L. monocytogenes* ATCC® 13932, WDCM 00021*Escherichia coli* ATCC® 25922, WDCM 00013*Enterococcus faecalis* ATCC® 29212, WDCM 00087*L. monocytogenes* ATCC® 35152, WDCM 00109**Growth**

Good - Esculin Positive reaction

Inhibited

Inhibited

Good - Esculin Positive reaction

Sterility Control

Add 5ml of the sample to 100ml of TSB and to 100ml Thioglycollate

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

Bibliography

· ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Florida.

· CURTIS, G.D, R.G. MITCHELL, A.F. KING & E.J. GRIFFIN (1989) A selective differential medium for the isolation of *Listeria monocytogenes*. Letters Appl. Microbiol. 8:95-98.

· ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

· 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

· VANDERZANT, C. & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington DC.

· UNE-EN ISO 11133 (2014). Microbiología de los alimentos para consumo humano, alimentación animal y agua.-Preparación, producción, conservación y ensayos de rendimiento de los medios de cultivo.