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..... 10.00
Lithium chloride..... 15.00
Proteose peptone..... 10.00
Sodium chloride.....5.00
Yeast extract..... 3.00
Starch..... 1.00
Esculin.....1.00
Ammonium iron(III) citrate..... 0.50
Agar.....13.00

Final pH 7.0 ±0.2 at 25 °C

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

Directions

Suspend 58,5 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 the Oxford Agar Selective Supplement (Ref. DSHB3051) to each flask. Mix well and pour into Petri dishes.
Note: Prepared medium (Agar + supplement) must be kept away from light, since it helps the production of acriflavine oxidised photocomplexes that can repress *Listeria* growth.

Description

Oxford Agar is a derivative of the original formulation used by Curtis *et al.* which had a high nutritive capability equivalent to Columbia agar with the addition of. Inhibitor agents helps reduce undesirable companion bacteria.
The current formulation retains the high capacity to support growth and inhibit both Gram negative and most Gram positive, bacteria including yeast. Thanks to the inhibitors incorporated in the selective supplement: cycloheximide, acriflavine, colistin, phosphomycin and cefotaxime and in a combination with lithium chloride the growth of all other bacteria except *Listeria* is inhibited.
Listeria colonies are easily recognizable since they hydrolyze esculin to free esculetin that reacts with the ferric ions and produces a dark precipitate around the colonies, which typically present as a grey-blue colour with a very dark core.

Necessary supplements

Oxford Agar Selective Supplement (Ref. DSHB3051)
Vial contents:
Necessary amount for 500 mL of complete medium.
Acriflavine.....2,5 mg
Phosphomycin.....5,0 mg
Sodium cefotaxime.....1,0 mg
Colystin.....10,0 mg
Cycloheximide..... 200,0 mg

Distilled water (Solvent)

Technique

Although the selectivity of the medium is enough to allow the isolation and differentiation by direct surface inoculation, a previous dilution of the inoculum is advisable, using greater dilutions when the sample is highly polluted.
Most authors prefer one or two prior cultures in any of the primary enrichment broth (UVM I or Lovett) or a secondary enrichment broth (UVM II or Fraser) before inoculating in Oxford Agar.
Incubation is carried out at 37 ºC ±1, and after 24 h typical colonies of *Listeria monocytogenes* are visible. However, it extending the incubation for another 44±4 h is recommended in order to detect slow growing strains even though this may allow staphylococci or streptococci development.

Quality control

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

Inoculum: Practicalrange 100 ±20 CFU. Min. 50 CFU (productivity)/10□-10□ CFU (selectivity), according to ISO 11133:2014.

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC® 29212	Inhibited	-
Escherichia coli ATCC® 25922	Inhibited	-
Listeria monocytogenes ATCC® 13932	Productivity > 0.50	Esculin (+). Black medium
Listeria monocytogenes ATCC® 35152	Productivity > 0.50	Esculin (+). Black medium

References

· 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. 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.

Storage

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