Listeria Oxford Medium Base , Modified

M1781

Listeria Oxford Medium Base, Modified is recommended for isolation and differentiation of *Listeria* species from clinical specimens.

Composition**

Ingredients	Gms / Litre
Tryptone	8.900
Meat extract #	2.700
Proteose Peptone, B	4.400
Yeast Extract	4.400
Lithium Chloride	15.000
Sodium Chloride	4.400
Corn Starch	0.900
Esculin	1.000
Ammonium Ferric Citrate	0.500
Agar	15.300
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Beef heart extract

Directions

Suspend 57.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. Cool to 45-50°C and aseptically add the rehydrated contents of 2 vials of Oxford Listeria Supplement (FD071) or 2 vials of Listeria Moxalactam Supplement Modified (FD266).Mix well and pour into sterile Petri plates.

Caution: Lithium chloride is harmful.Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately .

Principle And Interpretation

Listeria monocytogenes is the only species of the genus *Listeria* that is important as a human pathogen. *Listeria seeligeri, Listeria welshimeri* and *Listeria ivanovii* have been related with animal diseases. In any case, all the species are pathogenic between the ovine and bovine cattle. Positive diagnosis of listeriosis can be obtained only by the isolation and cultivation of the responsible bacteria from blood or CSF samples of the affected organisms. Listeria Oxford Medium Base is based on the formulation described by Curtis et al (1) for isolation of *L. monocytogenes* from clinical and food specimens.

Tryptone, meat extract, proteose peptone B and yeast extract serves as the source of essential nutrients to the organisms. Corn starch serves to neutralize the toxic metabolites formed. Lithium chloride and the antibiotics inhibit gram-negative bacteria and most gram-positive organisms but certain strains of Staphylococci may grow as esculin negative colonies. Cycloheximide is used to reduce fungal contamination; cefotetan and fosfomycin are inhibitors of bacterial overgrowth. Acriflavin, colistin sulphate and lithium chloride inhibit bacteria other than *Listeria* species. Alternatively moxalactam (FD266) can be added which inhibits both gram-positive and gram-negative bacteria. *L. monocytogenes* hydrolyzes esculin to esculetin and dextrose. Esculetin reacts with ferric ions and produces black zones around the colonies. 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 or even more when the sample is highly polluted.

The techniques for isolation vary with the material under examination (2). For all specimens selective and cold enrichment is recommended (3, 4). For faecal and biological specimens, the sample is homogenized in 0.1% Peptone Water (M028) and 0.1 ml amount is either directly plated on Listeria Selective Medium or inoculated into the Selective Enrichment Broth and incubated at 30°C for 7 days and then further inoculated on Listeria Selective Medium. For food and environmental samples selective enrichment is generally used.

For isolation of Listeria from food (milk and milk products), add 25 ml or 25 grams of sample to 225 ml of Listeria Enrichment Broth, UVM (M890A). Homogenize and mix carefully. Incubate for 48 hours at 30°C. Streak the enriched cultures onto Listeria Oxford medium Base and incubate aerobically for 48 hours at 37°C. Take 5 typical colonies (esculin positive) and inoculate onto Soyabean Casein Digest Medium (M290). Incubate for 24 hours and then use these colonies for biochemical confirmation.

Quality Control

Appearance
Light yellow to dark yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium
Dark amber coloured clear to slightly opalescent gel with a blue cast forms in Petri plates

Reaction
Reaction of 5.55% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed with added Oxford Listeria Supplement (FD071) or Listeria Moxalactam supplement Modified(FD266), after an incubation at 35-37°C for 24-48 hours.

Cultural Response

Organism	Growth	Inoculum (CFU)	Recovery	Esculin Hydrolysis
Cultural Response				
Bacillus subtilis ATCC 6633	inhibited	>=10^3	0%	
Enterococcus faecalis ATCC 29212	inhibited	>=10^3	0%	
Enterococcus hirae ATCC 10541	inhibited	>=10^3	0%	
Escherichia coli ATCC 25922	inhibited	>=10^3	0%	
Listeria monocytogenes ATCC 19111	luxuriant	50-100	>=50%	positive reaction, blackening of medium around the colony
Listeria monocytogenes ATCC 19112	luxuriant	50-100	>=50%	positive reaction, blackening of medium around the colony
Listeria monocytogenes ATCC 19117	luxuriant	50-100	>=50%	positive reaction, blackening of medium around the colony
Staphylococcus aureus ATCC 25923	good	50-100	40-50%	negative reaction

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8 °C. Use before expiry date on label.

Reference

1. Curtis G. D. W., Mitchell R. G, King A. F., Griffin E. J., 1989,Lett. Appl. Microbiol.,8:95
2. Van Netten P., Peroles I., Van de Mosdik A., Curtis G. D. W., Mossel D. A. A, 1988, Int. J. Food Microbiol., 6:187.
3. Hayes P. S, Feeley J. L, Groves L. M, Ajello G. W. and Fleming D. W, 1986, Appl. Environ. Microbiol., 51:438.
4. Fernandez G. J. F., Dominguez R. L., Vazzuez B. J. A., Rodriguez F.E. F., Briones D. V., Blanco L. J. L., Suarez F. G., 1986, Can. J. Microbiol., 32:149.

Revision :02 / 2015

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