HiCulture™ Listeria Isolation and transport Swabs

MS1145

Recommended for transportation of *Listeria* species from clinical specimens.

Composition**

Ingredients	Gms / Litre
Peptone special	23.000
Lithium chloride	15.000
Sodium chloride	5.000
Corn starch	1.000
Esculin	1.000
Ammonium ferric citrate	0.500
Agar	10.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Using the capped swab, provided along with the media containing tube, collect the sample to be transported.Discard the cap of the tube and insert the capped swab with the sample till the bottom of the medium. Tighten the cap firmly. The specimen will be preserved during transportation and also the viability of the organisms will be maintained but it will diminish over the time. Some growth of contaminants may occur during longer period of transport. After the transportation, the specimen should be inoculated in proper medium as soon as possible. The cultures on transport swabs must not be kept at room temperature for more than 24 hours.

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.

Peptone special 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 phosphomycin are inhibitors of bacterial overgrowth. Acriflavin, colistin sulphate and lithium chloride inhibit bacteria other than *Listeria* species.

Alternatively moxalactam (FD126) 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
Sterile Listeria Oxford Medium in tube with Sterile Cotton Swabs.

Colour
Dark amber coloured medium with blue cast

Quantity of Medium
8ml of medium in tubes

Reaction
6.80- 7.20

Sterility test
Passes release criteria

Cultural response
Viability of following was established for a period of 48 hours. Following results were observed when recovered on Listeria Oxford Medium (M1145) after incubation at 35-37°C for 24-48 hours.

Organism	Recovery
Enterococcus faecalis ATCC 29212	Poor-good
Enterococcus hirae ATCC 10541	Poor-good
Bacillus subtilis ATCC 6633	None-poor
Escherichia coli ATCC 25922	None-poor
Listeria monocytogenes ATCC 19111	Good-luxuriant
Listeria monocytogenes ATCC 19112	Good-luxuriant
Listeria monocytogenes ATCC 19117	Good-luxuriant
Staphylococcus aureus ATCC25922	Fair-good

Storage and Shelf Life

Store between 5 – 25°C with caps firmly screwed. DO NOT FREEZE. 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.

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