Listeria Oxford Medium Base

M1145

Intended use

Recommended for isolation of *Listeria* species from pathological 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

Suspend 27.75 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add the rehydrated contents of 1 vial of Oxford Listeria Supplement (FD071) or 1 vial of Listeria Moxalactam Supplement (FD126). Mix well before pouring into sterile Petri plates.

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 (2) 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 (8). 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.

Type of specimen

Clinical samples - Body tissues or body fluids, Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Further biochemical tests are needed for a final identification of the isolated organisms.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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 : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added Oxford Listeria Supplement (FD071) or Listeria Moxalactam supplement(FD126), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Bacillus subtilis ATCC 6633 (00003*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Enterococcus hirae ATCC 10541	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Listeria monocytogenes serovar 1 ATCC 19111 (00020*)	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Listeria monocytogenes ATCC 19112	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony

Listeria monocytogenes ATCC 19117	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good	40-50%	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

References

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5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Van Netten P., Peroles I., Van de Mosdik A., Curtis G. D. W., Mossel D. A. A, 1988, Int. J. Food Microbiol., 6:18
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IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, 23 Vadhani Industrial Estate, LBS Marg,Mumbai-86,MS,India
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

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