

Listeria Oxford HiCynth™ Medium Base

MCD1145

Intended use

Recommended for isolation of *Listeria* species from various samples.

Composition**

Ingredients	g/ L
HiCynth™ Peptone No.1*	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

* Chemically defined peptone

Directions

Suspend 27.75 gram 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 Selective Supplement (FD071) or 1 vial of CM Selective Supplement (FD126). Mix well and pour 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 (1) for isolation of *L. monocytogenes* from clinical and food specimens. Listeria Oxford HiCynth™ Medium Base is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones.

HiCynth™ Peptone No.1 serves as the source of carbon, nitrogen, vitamins and other 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. Oxford Selective Supplement (FD071) reduces fungal and bacterial contamination and lithium chloride inhibit bacteria other than *Listeria* species. Alternatively CM Selective Supplement (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 Tryptone Soya HiCynth™ Agar (MCD290). Incubate for 24 hours and then use these colonies for biochemical confirmation.

Type of specimen

Food and dairy samples

Specimen Collection and Handling:

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

Warning and Precautions :

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 specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. 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 Selective Supplement (FD071) or CM Selective Supplement (FD126), after an incubation at 35-37°C for 24-48 hours.

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

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

Key : (*) Corresponding WDCM numbers, **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

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 in order 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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

References

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8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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