PRODUCT SPECIFICATION SHEET

Listeria Oxford Medium Base (DM1078)

Intended Use
Listeria Oxford Medium Base (DM1078) is recommended for isolation of *Listeria* species from pathological specimen.

Product Summary and Explanation
Listeria species are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C. The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[1] *Listeria* species grow over a pH range of 4.4-9.6, and survive in food products with pH levels outside these parameters.[2] Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.
Among the *Listeria* species only *Listeria monocytogenes* is reported to cause infection in humans. In 1926 Murray, Webb and Swann,[3] first described that *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness such as meningitis, encephalitis or septicemia and the tropism of *L. monocytogenes* for the central nervous system leads to severe disease, often with high mortality or with neurologic disorders among survivors,[4] particularly in immunocompromised individuals and pregnant women.[5] The first food-borne outbreak of listeriosis was reported in 1985.[6] Since then, microbiological and epidemiological evidence from both sporadic and epidemiccases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[7] Concerned vehicles of transmission include Mexican-style cheese, coleslaw, turkey frankfurters, pasteurized milk and pickled pork tongue.[8] The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[9]
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[10] for isolation of *L. monocytogenes* from clinical and food specimens.

Principles of the Procedure
Listeria Oxford Medium Base contains peptone special which serves as the source of essential nutrients to the organisms. Corn starch serves to neutralize the toxic metabolites formed. Lithium chloride inhibits gram-negative bacteria and most gram-positive organisms but certain strains of *Staphylococci* may grow as esculin negative colonies. Sodium chloride maintains the osmotic balance. Ferric ammonium citrate aids in the differentiation of *Listeria* spp. Since all *Listeria* spp. hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. Selectivity is increased by adding various antimicrobial agents to the base. Incorporating these agents into the Listeria Oxford Medium Base will completely inhibit gram-negative organisms and most gram-positive organisms. Cycloheximide is used to reduce fungal contamination; cefotetan and hosphomycin are inhibitors of bacterial overgrowth. Acriflavin, colistin sulphate and lithium chloride inhibit bacteria other than *Listeria* species. Alternatively, moxalactam (MS172) 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.

Formula / Liter

Ingredients	Gms / Liter
Peptone, special	23.00
Lithium chloride	15.00
Sodium chloride	5.00
Corn starch	1.00
Esculin	1.00
Ammonium ferric citrate	0.50
Agar	10.00
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately

Directions

1. Suspend 27.75 grams in 500 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C and aseptically add the rehydrated contents of 1 vial of Oxford Listeria Supplement (MS171) or 1 vial of Listeria Moxalactam Supplement (MS172).
5. Mix well before pouring into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to dark yellow homogeneous free flowing powder
Prepared Medium	Dark amber coloured clear to slightly opalescent gel with a blue cast forms in Petri plates
Reaction of 5.55% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel

Expected Cultural Response: Cultural characteristics observed with added Oxford Listeria Supplement (MS171) or Listeria Moxalactam Supplement (MS172), after an incubation at 35-37°C for 24-48 hours.

Sr. No.	Organisms		Results to be achieved		
		Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
1.	*Bacillus subtilis ATCC 6633*	>=10^3	inhibited	0%	--
2.	*Enterococcus faecalis ATCC 29212*	>=10^3	inhibited	0%	--
3.	*Enterococcus hirae ATCC 10541*	>=10^3	inhibited	0%	--
4.	*Escherichia coli ATCC 25922*	>=10^3	inhibited	0%	--
5.	*Listeria monocytogenes ATCC 19111*	50 - 100	good-luxuriant	>=50%	positive reaction, blackening of medium around the colony
6.	*Listeria monocytogenes ATCC 19112*	50 - 100	good-luxuriant	>=50%	positive reaction, blackening of medium around the colony
7.	*Listeria monocytogenes ATCC 19117*	50 - 100	good-luxuriant	>=50%	positive reaction, blackening of medium around the colony
8.	*Staphylococcus aureus ATCC 25923*	50 - 100	good	40-50%	negative reaction

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. 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.
2. For all specimens selective and cold enrichment is recommended.
3. For faecal and biological specimens, the sample is homogenized in 0.1% Peptone Water 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.
4. For food and environmental samples selective enrichment is generally used.
5. 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 (DM521).
6. 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.

7. Take 5 typical colonies (esculin positive) and inoculate onto Soyabean Casein Digest Medium (DM277). Incubate for 24 hours and then use these colonies for biochemical confirmation.

Results

Select esculin-positive colonies and confirm their identity by further biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification. Refer to appropriate references for additional information.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Since *Listeria* spp. other than *L. monocytogenes* can grow on these media, an identification of *L. monocytogenes* must be confirmed by biochemical and serological testing.
2. Use freshly prepared antimicrobial agent solutions or aliquot portions and store at -20°C or below.
3. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Listeria Oxford Medium Base
Product Code : DM1078
Available Pack sizes : 100gm/ 500gm

References

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Further Information

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