Technical Information

Listeria Oxford Agar Base w/ 1.2% Agar

Product Code: DM 2145F

Application: - Listeria Oxford Agar Base w/1.2% Agar with supplements is used for isolation of specimen *Listeria* species from food samples in accordance with FDA BAM, 1998.

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	12.000
Final pH (at 25°C)	7.2±0.1

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The genus Listeria contains six species; *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, L. ivanovii @, and L. grayi @. *Listeria monocytogenes* is the only species of the genus Listeria that is important as a human pathogen. The universal occurrence of L. monocytogenes in food (1,2,3) and the risk of contracting food-borne *L. monocytogenes* listeriosis has been thoroughly reviewed recently and *L.monocytogenes* has been detected in the food processing environment, such as on food contact surfaces and equipment(4). Other species; *Listeria seeligeri*, *Listeria welshimeri* and *Listeria ivanovii* have been related with animal diseases. *L. ivanovii* and *L. monocytogenes* are pathogenic for mice and other animals. However, only *L. monocytogenes* is commonly associated with human listeriosis. Listeriosis associated infection by *L. ivanovii* , and even *L. seeligeri* is extremely rare in humans (5).

The preferred standard methodology, and permitted alternative rapid methodologies, to be used for detection and isolation of *Listeria monocytogenes* are as follows. Presumptive contaminated food lots are sampled. Generally, sub-samples are composited if required by FDA field laboratory instructions. Analytical portions (25 g) are pre-enriched for Listeria species at 30° C for 4 h in buffered Listeria Enrichment broth (BLEB) (DM2578) , equivalent to AOAC/IDF dairy products enrichment broth base containing sodium pyruvate (6,7,8). At the fourth hour of the incubation, the selective agents (acriflavin, 10 mg/ L; sodium nalidixate, 40 mg/L; optional antifungal, e.g. cycloheximide 50 mg/L) are added. Incubation for selective enrichment is continued at 30° C for a total of 48 h. The enrichment culture is streaked at 24 and 48 h on one of the prescribed differential selective-agars in order to isolate Listeria species. The selective and differential media containing esculin and ferric citrate aids in Listeria isolation and detection based on esculin hydrolysis. Listeria Oxford Medium Base is based on the formulation described by Curtis et al (9) for the selective isolation of *L. monocytogenes* from food specimens, and is recommended by FDA BAM for the same purpose (10).

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. Colistin sulphate, moxalacatam and lithium chloride inhibit bacteria other than *Listeria* species. Alternatively moxalactam (MS2126F) 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.

For food and environmental samples selective enrichment is generally used (10, 11, 12). For isolation of Listeria from food (milk and milk products), add 25 ml or 25 grams of sample is enriched to 225 ml of Buffered Listeria Enrichment Broth (DM2578). Homogenize and mix carefully. Incubate for 4 hours at 30°C. After 4 hours add Selective agents (MS2063I) containing nalidixic acid, cycloheximide, acriflavine hydrochloride is added to enhance selectivity. Incubate for 48 hours at 30°C. The enriched cultures are streaked onto Listeria Oxford medium Base at 24 and 48 hours. Incubate aerobically for 24-48 hours at 35°C. Take 5 esculin positive colonies that appear typical black coloured and inoculate onto Tryptone Soya Yeast Extract agar (DM2214/DM2214F). Incubate for 24-48 hours at 30°C and then confirm colonies for using Henrys illumination light.

Methodology

Suspend 28.75 grams of dehydrated powder media in 500 ml distilled water. Mix thoroughly & 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 Listeria Moxalactam Supplement, Modified (MS1126F). Mix well before pouring into sterile Petri plates.

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

Quality Control

Appearance
Light yellow to dark yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.2% Agar gel.

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

Reaction
Reaction of 5.75% w/v aqueous solution at 25°C. pH : 7.2±0.1

pH Range
7.10-7.30

Cultural Response
DM2145F: Cultural characteristics observed with added Listeria Moxalactam supplement, Modified (MS226F), after an incubation at 35°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Cultural Response				
Bacillus subtilis ATCC 6633	>=10^3	inhibited	0%	-
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	-
Enterococcus hirae ATCC 10541	>=10^3	inhibited	0%	-
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	-
Listeria monocytogenes ATCC 19111	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 ATCC 25923	50-100	good	40-50%	negative reaction

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and prepared medium at 2-8°C.Use before expiry period on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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7. Hitchins A. D., and Duvall R. E., 2000, J. Food Protect. 63:1064-1070.
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10. FDA US. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD. : AOAC International; 1998.
11. Asperger H., Heistinger H., Wagner M., Lehner A. and Brandl E., 1999. Microbiology, 16:419-431.
12. Hayes P. S, Feeley J. L, Groves L. M, Ajello G. W and Fleming D. W.,1986, Appl Environ Microbiol. 51:438.

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