

OXFORD LISTERIA AGAR BASE (7428)

Intended Use

Oxford Listeria Agar Base is used with antimicrobics for the selective isolation of *Listeria* spp.

Product Summary and Explanation

Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann,¹ is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals.² Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.³ Implicated vehicles of transmission included turkey frankfurters,⁴ coleslaw, pasteurized milk, Mexican style cheese, and pate'. *Listeria* spp. are ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, and river water.⁵

Oxford Listeria Agar Base is prepared according to the formulation of Curtis et al.⁶ *Listeria* spp. grow over a pH range of 5.0 - 9.6, and survive in food products with pH levels outside these parameters.⁷

Principles of the Procedure

Columbia Blood Agar Base contains Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Yeast Enriched Peptone providing nitrogen, carbon, amino acids, and vitamins. 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. A blackening of the colony and surrounding medium in cultures containing esculin-hydrolyzing bacteria results from the formation of 6,7-dihydroxycoumarin which reacts with the ferric ions.⁸ Selectivity is provided by the presence of Lithium Chloride. The high salt tolerance of *Listeria* is used as a means to markedly inhibit growth of enterococci. Agar is the solidifying agent.

Selectivity is increased by adding various antimicrobial agents to the base. Incorporating these antimicrobial agents into Oxford Listeria Agar Base will completely inhibit Gram-negative organisms and most Gram-positive organisms after 24 hours of incubation. The most widely recognized antimicrobial agent combinations are the Oxford Medium formulation⁶ and the Modified Oxford Medium formulation.⁹

Modified Oxford Medium is recommended for isolating and identifying *Listeria monocytogenes* from processed meat and poultry products.⁹ Oxford Medium is recommended for isolating *Listeria* from enrichment broth cultures.¹⁰

Formula / Liter

Columbia Blood Agar Base	39 g
Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Lithium Chloride	15 g
Agar	2 g
Final pH: 7.0 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Antimicrobial Supplements

Oxford Listeria Supplement (7986)

Acriflavin	5 mg
Cefotetan	2 mg
Colistin Sulfate	20 mg
Cycloheximide	400 mg
Phosphomycin	10 mg

Modified Oxford Listeria Supplement (7991)

Colistin Sulfate	10 mg
Moxalactam	20 mg

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful if swallowed, inhaled, or absorbed through the skin. Irritating to eyes, respiratory system, and skin. Skin irritation may be severe. May cause central nervous system effects.

Directions

Oxford Medium Base

1. Suspend 57.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 10 – 15 minutes. Cool to 45 - 50°C.

Oxford Medium

Aseptically add a filtered sterilized aqueous solution of 5 mg acriflavin, 2 mg cefotetan, 20 mg colistin sulfate, 400 mg cycloheximide*, and 10 mg phosphomycin. Note:*Natamycin may be used in place of cycloheximide at 25 mg.

Modified Oxford Medium

Aseptically add a filtered sterilized aqueous solution of 10 mg colistin sulfate and 20 mg moxalactam.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light to medium amber and slightly hazy.

Expected Cultural Response: Cultural response in Oxford Listeria Agar and Modified Oxford Listeria Agar at 35 ± 2°C after 24 - 48 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Response	
		Oxford	Modified Oxford
<i>Escherichia coli</i> ATCC® 25922	~ 1000	Inhibited	Inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	10 - 300	Growth; brown to black colonies	Growth; brown to black colonies
<i>Listeria monocytogenes</i> ATCC® 19111	10 - 300	Growth; brown to black colonies	Growth; brown to black colonies
<i>Staphylococcus aureus</i> ATCC® 25923	~ 1000	Suppressed to inhibited	Inhibited

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

Test Procedure

The USDA method⁹ involves enrichment of the food sample in UVM Modified Listeria Enrichment Broth (one part sample to nine parts broth) at 30°C. After incubation, a portion of the enrichment mixture is plated onto Oxford or Modified Oxford Medium. The FDA Method¹⁰ involves adding 25 mL of liquid or 25 g of solid material to 225 mL Listeria Enrichment Broth and incubating at 30°C for two days. After enrichment, the broth is plated onto Oxford Medium. For further information consult appropriate references.^{7, 9, 10}

Results

Select esculin-positive colonies and confirm their identity through biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to 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. An identification of *L. monocytogenes* must be confirmed through biochemical and serological testing.
2. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

Packaging

Oxford Listeria Agar Base	Code No.	7428A	500 g
		7428B	2 kg
		7428C	10 kg
Oxford Listeria Supplement, 5 mL	Code No.	7986	10 vials/pkg
Modified Oxford Listeria Supplement, 5 mL	Code No.	7991	10 vials/pkg

References

1. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bacteriol. **29**:407-439.
2. Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. Bremer, P. J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58**:604-608.
4. Graud, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
5. Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**: 244-250.

6. **Curtis, G. D. W., R. G. Mitchell, A. F. King, and J. Emma.** 1989. A selective differential medium for the isolation of *Listeria monocytogenes*. *Appl. Microbiol.* **8**:95-98.
7. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Fraser, J., and W. Sperber.** 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. *J. Food Prot.* **51**:762-765.
9. **Lee, W. H., and D. McClain.** 1989. Laboratory Communication No. 57 (revised May 24, 1989). U.S.D.A., F.S.I.S. Microbiology Division, Beltsville, MD.
10. **U.S. Food and Drug Administration.** Bacteriological analytical manual. 1995, 8th ed., AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.