

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 and pregnant woman.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 *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

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 The Oxford Medium formulation contain cycloheximide, colistin sulfate, acriflavin, cefotetan, and fosfomycin. The Modified Oxford Medium formulation contains moxalactam and colistin sulfate.

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: 7.2 ± 0.2 at 25°C	

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

Antimicrobics

Oxford Medium

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

Precautions

1. For Laboratory Use.
2. **Oxford Medium Base**
HARMFUL. Harmful if swallowed, inhaled, or absorbed through the skin.

Modified Oxford Medium

Colistin Sulfate	10 mg
Moxalactam	20 mg

Directions

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 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 fosfomycin.

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 Base and Modified Oxford Listeria Agar at 35°C after 24 - 48 hours incubation.

Microorganism	Response	
	Oxford	Modified Oxford
<i>Escherichia coli</i> ATCC® 25922	inhibited	inhibited
<i>Listeria monocytogenes</i> ATCC® 7644	good growth	good growth
<i>Listeria monocytogenes</i> ATCC® 19111	good growth	good growth
<i>Staphylococcus aureus</i> ATCC® 25923	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, 11}

Results

Select esculin-positive colonies and confirm their identity through biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification. For additional information, refer to appropriate references.^{7, 9-11}

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 by keeping container tightly closed.

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.¹¹
2. Poor growth and a weak esculin reaction maybe seen after 40 hours incubation for some enterococci.

Packaging

Oxford Listeria Agar Base	Code No.	7428A	500 g
		7428B	2 kg
		7428C	10 kg

References

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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.
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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.
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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.
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Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.