

Oxford Medium Base

Modified Oxford Antimicrobial Supplement

Intended Use

Oxford Medium Base is used to prepare Oxford Medium¹ or Modified Oxford Medium² for isolating and differentiating *Listeria monocytogenes*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,³ *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.⁴ The first reported foodborne outbreak of listeriosis was in 1985.⁵ Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.⁶

Implicated vehicles of transmission include turkey frankfurters,⁷ coleslaw, pasteurized milk, Mexican-style cheese, pâté and pickled pork tongue. 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.⁸

Listeria spp. grow over a pH range of 4.4-9.6 and survive in food products with pH levels outside these parameters.⁹ *Listeria* spp. 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 and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.¹⁰

User Quality Control

Identity Specifications

Difco™ Oxford Medium Base

Dehydrated Appearance: Tan, free-flowing, homogeneous (may contain small dark particles).

Solution: 5.75% solution, soluble in purified water upon boiling. Solution is medium amber, slightly to moderately opalescent with a blue ring at the surface of the liquid.

Prepared Appearance: Light to medium amber, very slightly to slightly opalescent.

Reaction of 5.75% Solution at 25°C: pH 7.2 ± 0.2

Difco™ Modified Oxford Antimicrobial Supplement

Appearance: White cake may be broken; colorless solution with a pale yellow tint upon rehydration.

Cultural Response

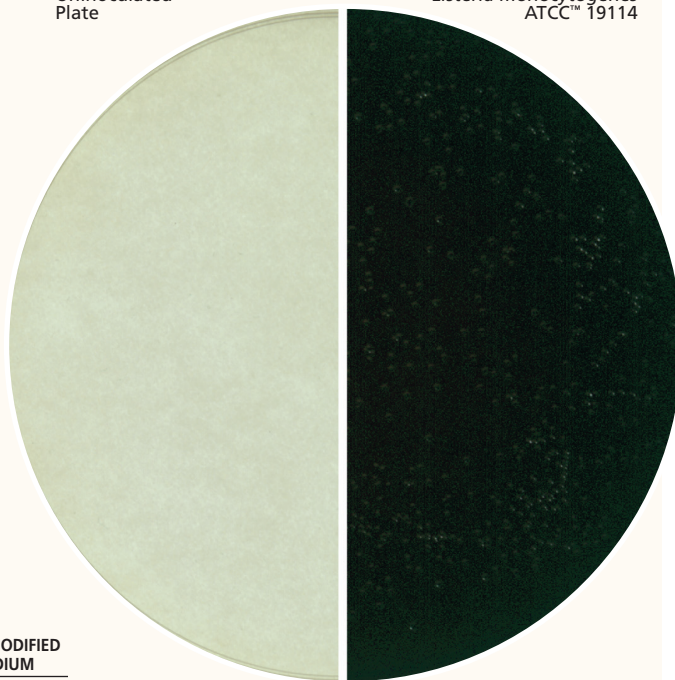
Difco™ Oxford Medium or Modified Oxford Medium

Prepare the medium with corresponding supplement. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY ON OXFORD MEDIUM	RECOVERY ON MODIFIED OXFORD MEDIUM
<i>Enterococcus faecalis</i>	29212	10 ³ -2×10 ³	Marked to complete inhibition	Marked to complete inhibition
<i>Escherichia coli</i>	25922	10 ³ -2×10 ³	Marked to complete inhibition	Marked to complete inhibition
<i>Listeria monocytogenes</i>	19114	10 ² -10 ³	Good at 40-48 hours, black colonies	Good at 40-48 hours, black colonies

Uninoculated Plate

Listeria monocytogenes ATCC™ 19114



Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Oxford Medium Base is prepared according to the formulation of Curtis et al.¹¹ who originally described the medium and its use in the selective isolation of *Listeria* from mixed cultures.

Principles of the Procedure

Peptones and beef heart digest provide nitrogen, carbon, amino acids and vitamins. Agar is the solidifying agent. 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. 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.¹²

Selectivity is provided by the presence of lithium chloride in the formula. The high salt tolerance of *Listeria* is used as a means to markedly inhibit growth of enterococci.

Selectivity is increased by adding various antimicrobial agents to the base. Incorporating these agents into Oxford Medium 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 formulation¹¹ and the Modified Oxford Medium formulation.² The Oxford Medium formulation contains cycloheximide, colistin sulfate, acriflavine, cefotetan and fosfomycin. The Modified Oxford Medium formulation contains moxalactam and colistin methane sulfonate or colistin sulfate (available as Modified Oxford Antimicrobial Supplement).

Modified Oxford Medium is recommended for isolating and identifying *Listeria monocytogenes* from processed meat and poultry products.² Oxford Medium is recommended for isolating *Listeria* from enrichment broth cultures.¹³

Formulae

Difco™ Oxford Medium Base

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	8.9 g
Proteose Peptone No. 3	4.4 g
Yeast Extract	4.4 g
Tryptic Digest of Beef Heart	2.7 g
Starch	0.9 g
Sodium Chloride	4.4 g
Esculin	1.0 g
Ferric Ammonium Citrate	0.5 g
Lithium Chloride	15.0 g
Agar	15.3 g

Difco™ Modified Oxford Antimicrobial Supplement

Formula Per 10 mL Vial	
Colistin Sulfate	10.0 mg
Moxalactam	20.0 mg

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 57.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 10 minutes. Cool to 45-50°C.
4. **To prepare Oxford Medium:** Dissolve acriflavine (5.0 mg), cefotetan (2.0 mg), colistin sulfate (20.0 mg), cycloheximide (400.0 mg) and fosfomycin (10.0 mg) in 5 mL reagent grade ethanol and 5 mL purified water. Filter sterilize supplement before use. Add 10 mL of antimicrobial supplement to 1 L of molten Oxford Medium Base (45-50°C). Mix thoroughly.
To prepare Modified Oxford Medium: Aseptically rehydrate one vial of Modified Oxford Antimicrobial Supplement with 10 mL of sterile purified water. Rotate in an end-over-end motion to dissolve the contents completely. Add 10 mL of rehydrated Modified Oxford Antimicrobial Supplement to 1 L of Oxford Medium Base (45-50°C). Mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The USDA method² 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 method¹ involves adding 25 mL of liquid or 25 g of solid material to 225 mL *Listeria* Enrichment Broth and incubating at 30°C for 2 days. After enrichment, the broth is plated onto Oxford Medium.

For further information when testing food samples or clinical specimens for *Listeria*, consult appropriate references.^{1,2,9,13,14}

Expected Results

Select esculin-positive colonies and confirm their identity by further biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification. For additional information, refer to appropriate references.^{1,2,9,13,14}

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.¹⁴
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.

References

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3. Murray, Webb and Swann. 1926. J. Pathol. Bacteriol. 29:407.
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12. Fraser and Sperber. 1988. J. Food Prot. 51:762.
13. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
14. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Oxford Medium Base

AOAC BAM CCAM COMPF ISO SMD USDA

Cat. No. 222530 Dehydrated – 500 g
222510 Dehydrated – 2 kg

Difco™ Modified Oxford Antimicrobial Supplement

AOAC CCAM COMPF USDA

Cat. No. 211763 Vial – 6 × 10 mL*

*Store at 2-8°C.