IFU

Instructions for Use

OXFORD MEDIUM, MODIFIED

Cat. no. G46	Oxford Medium, Modified, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. GA46	Oxford Medium, Modified, 15x100mm Plate, no logo, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Oxford Medium, Modified is recommended for the selective isolation of *Listeria monocytogenes* in food.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Listeria spp. are microaerophilic, gram-positive regular, short motile rods or coccobacilli that are asporogenous, non-encapsulated, and non-branching. Motility is best observed at 20-25ºC. *Listeria monocytogenes* is a pathogenic organism for humans and a large number of animal species. The members of the population most at risk are neonates, the elderly and those compromised by pregnancy or an underlying illness such as malignancy, alcoholism or some condition which requires immunosuppressive procedures. Intrauterine infection of the fetus results in death, or an acutely ill infant with a septic disseminated form of listeriosis. Papular lesions of the skin may be found in listeriosis of the newborn. A similar cutaneous form has been reported in veterinarians working with infected animals.(1)

A common vehicle for *Listeria monocytogenes* is pasteurized milk; since the induction of the Pasteurized Milk Ordinance in 1924, there have been fewer reported cases of milk contaminants other than *Listeria* spp. In Massachusetts in 1983, pasteurized milk spread *Listeria monocytogenes* to forty-nine people, 14 of which died of septicemia. Another incidence in California in 1985, was due to contamination of a soft Mexican cheese which caused 85 deaths of 300 infected patients. This led to a re-evaluation of pasteurization and aging techniques; however, the ability of *L. monocytogenes* to grow between 4 and 10ºC. and over a wide pH range (4.4-9.6) complicates the issue. The most effective containment still involves post-pasteurization pathogen detection.(2)

Other types of food that have been found to contain *Listeria* species as a contaminant are raw milk, raw vegetables, fish, poultry, and both fresh and processed meats. Ice cream has also served as a vehicle of transmission and in 1994 shrimp from a party in New York City infected ten people including two pregnant women. The CDC recommends, for immunocompromised, pregnant or elderly individuals, that foods to avoid are: soft cheeses, cold cuts and salami. There are also some reports of nosocomial infections of *Listeria monocytogenes* usually among infants or immunosuppressed adults.(1)

Listeria monocytogenes is ubiquitous in nature and has been isolated from soil, mud, sewage, decaying vegetation, silage, feces, and river water. Many animal species are vulnerable to infection by *Listeria* species and some lactating mammals can function as carriers (with no visible symptoms) while still excreting the organisms in their milk. Sheep, cattle and goats have also been found to shed *Listeria monocytogenes* in their feces. Listeriosis was caused by a meat product (hot dogs) in 1999 in the United States when 101 infections caused 21 deaths. Other contaminated foods include: coleslaw, pate, jellied pork tongue, cooked chicken and smoked mussels.(3)

This Modified Oxford Medium formulation contains moxalactam (to inhibit staphylococci, bacilli and *Proteus* species) and colistin sulfate (to inhibit gram-negative bacilli); this is especially useful when attempting to isolate *Listeria monocytogenes* from samples containing a mixed bacterial flora. The selectivity is also enhanced by lithium chloride which negatively affects the growth of enterococci. The Columbia Agar Base provides amino acids, carbon, vitamins and nitrogen. The esculin in the media is hydrolyzed by *Listeria* spp. and the resulting compound reacts with ferric ions (from the ferric ammonium citrate) to produce 6,7-dihydroxycoumarin and blackening of the media surrounding the colonies. Agar is used in solidification of the media.

FORMULA

Ingredients per liter of deionized water:*

Columbia Agar Base	39.0gm
Lithium Chloride	15.0gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm
Moxalactam	20.0mg
Colistin Sulfate	10.0mg
Agar	2.0gm

Final pH 6.8 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Consult listed references for information regarding sample preparation and processing.[3,4]

USDA METHOD

For raw meat and poultry samples:

1. Add 25gm of meat sample to 225ml of primary enrichment broth in a stomacher bag.

2. Mix in a stomacher for 2 minutes with some air trapped in the bag.

3. Incubate for 20 to 24 hours at 28 to 32ºC.

4. Streak onto Hardy Diagnostics Modified Oxford Medium and incubate at 35ºC.

5. Examine plates at 26-28 and 48 hours.

As a concurrent secondary test, 0.08 to 0.12ml of the enrichment broth may be pipetted into 10ml of Fraser Broth and examined for blackening after 24 and 48 hours of incubation at 35ºC. Positive cultures are then subcultured onto Oxford Medium, Modified and incubated for another 24 hours.

INTERPRETATION OF RESULTS

Observe plates for round 1mm colonies with a blackening of the media around them. Suspect colonies may be confirmed by CAMP test on 5% Blood Agar, by further biochemical testing, use of a macroscopic tube rapid slide test, or other means of definitive serological identification.

LIMITATIONS[3]

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Since the nutritional requirements of organisms vary, some strains of *Listeria* may fail to grow or grow poorly on Oxford Medium, Modified.

Oxford Medium, Modified is a partially selective medium. Growth of some contaminating strains will be markedly but not totally inhibited. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as stomacher, stomacher bags, loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following

microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Listeria monocytogenes ATCC® 7644	A	24-48hrs	35°C	Aerobic	Growth; blackening of media around colonies
Escherichia coli ATCC® 25922	B	24-48hrs	35°C	Aerobic	Partial to complete inhibition
Staphylococcus aureus ATCC® 25923	B	24-48hrs	35°C	Aerobic	Partial to complete inhibition

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Hardy Diagnostics Oxford Medium, Modified should appear clear, slightly opalescent, and amber in color.

REFERENCES

1. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

2. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.

3. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

4. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

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Ordering Information

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California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207D [D]