IFU

Instructions for Use

CRITERION™ OXFORD LISTERIA AGAR BASE

Cat. no. C6520	CRITERION™ Oxford Listeria Agar Base	118.5gm
Cat. no. C6521	CRITERION™ Oxford Listeria Agar Base	500gm
Cat. no. C6522	CRITERION™ Oxford Listeria Agar Base	2kg
Cat. no. C6523	CRITERION™ Oxford Listeria Agar Base	10kg
Cat. no. C6524	CRITERION™ Oxford Listeria Agar Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Oxford Listeria Agar Base is used for the selective isolation of *Listeria monocytogenes* from food.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

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.[1,2]

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.[3]

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.[4]

The Oxford formulation contains 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.

Selectivity is enhanced by adding various antimicrobial agents to the base. Adding these agents into Oxford Medium Base will completely inhibit gram-negative organisms and most gram-positive organisms after 24 hours of incubation. The combinations available are the Oxford Medium formulation and the Modified Oxford Medium formulation. The Oxford Medium formulation contains cycloheximide, colistin sulfate, acriflavine, cefotetan and fosfomycin (available as Oxford Antimicrobic Supplement), The Modified Oxford Medium formulation contains moxalactam and colistin sulfate (available as Modified Oxford Antimicrobic Supplement).

FORMULA

Gram weight per liter:	57.5gm/L
Columbia Agar Base	39.0gm
Lithium Chloride	15.0gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm
Agar	2.0gm

Final pH 7.2 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light beige.

Store the prepared culture media at 2-8ºC.

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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 59.3gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 10 minutes.

4. Cool to 45-50ºC. in a waterbath.

5. Aseptically add selective supplements as desired.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. G46.

LIMITATIONS[4-6]

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Since *Listeria* spp. other than *L. monocytogenes* can grow in these media, an identification of *L. monocytogenes* must be confirmed by biochemical and serological testing.

Use freshly prepared antimicrobial agent solutions or aliquot portions and store at -20 degrees C. or below.

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 autoclaves, incinerators, and incubators, etc., 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	24-48hr	35°C	Aerobic	Growth; blackening of media around colonies
Escherichia coli ATCC® 25922	B	24-48hr	35°C	Aerobic	Inhibited
Staphylococcus aureus ATCC® 25923	B	24-48hr	35°C	Aerobic	Partial to complete inhibition

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

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Oxford Listeria Agar Base powder should appear homogeneous, free-flowing, and light beige in color. The prepared media should appear very slightly to slightly opalescent, and light to medium amber in color.

REFERENCES

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

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

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

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

5. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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

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

HDQA 2207B [D]