IFU

Instructions for Use

BUFFERED LISTERIA ENRICHMENT BROTH

Cat. no. U233	Buffered Listeria Enrichment Broth Base, 12oz Wide Mouth Jar, 225ml	12 jars/box
Cat. no. K183	BLEB Selective Supplement, 13x100mm Tube, 3.4ml**	3.4ml
** Sold separately.		

INTENDED USE

Hardy Diagnostics Buffered Listeria Enrichment Broth is recommended for the selective enrichment of *Listeria monocytogenes* from food samples.

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.[1,2]

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 to 9.6) further 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 Buffered Listeria Enrichment Broth follows the *Food and Drug Adminstration Bacteriological Analytical Manual* 8th edition formulation.[5] This medium contains digests of soybean meal and casein, which provide amino acids and other nitrogenous substances. Sodium chloride is added to maintain the osmotic equilibrium. Dextrose is incorporated as an energy source. The dipotassium phosphate, monopotassium phosphate and disodium phosphate are included in the formulation as buffers to maintain the pH. Protein, B vitamins and other growth nutrients are supplied by yeast extract. Pyruvic acid aids in resuscitating organisms that are stressed or injured. Cycloheximide inhibits saprophytic fungi while the selective agents, nalidixic acid, and acriflavine act to inhibit gram-negative and gram-positive organisms, respectively.

FORMULA

Ingredients per liter of deionized water:*

Buffered Listeria Enrichment Broth (Cat. no. U233):	
Pancreatic Digest of Casein	17.0gm
Sodium Chloride	5.0gm
Papaic Digest of Soybean Meal	3.0gm
Dextrose	2.5gm
Dipotassium Phosphate	2.5gm
Yeast Extract	6.0gm
Monopotassium Phosphate	1.35gm
Disodium Phosphate	9.6gm
Pyruvic Acid	1.1gm

Final pH 7.3 +/- 0.2 at 25ºC.

Ingredients per liter (after addition to the Buffered Listeria Enrichment Broth):*

BLEB Selective Supplement (Cat. no. K183) not included:**	
Nalidixic Acid	40.0mg
Cycloheximide	50.0mg
Acriflavine HCl	10.0mg

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

**** Sold separately.**

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, 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[5]

1. Sample possibly contaminated food lots.

2. If required by FDA field laboratory instructions, sub-samples may be composited.

3. Pre-enrich by placing the 25gm food portion in Hardy Diagnostics Buffered Listeria Enrichment Broth Base for four hours at 30ºC.

4. At the fourth hour, add 3.4ml of the BLEB Selective Supplement (Cat. no. K183 sold separately).

5. Continue incubation for selective enrichment at 30ºC. for a total of 48 hours.

6. At 24 and 48 hours, streak BLEB culture onto one of the following esculin-containing selective isolation agars: either Modified Oxford Medium (Cat. no. G46) or PALCAM (Cat. no. G149) or LPM (Cat. no. G92).

INTERPRETATION OF RESULTS[5]

Listeria colonies will appear black with a black halo on esculin-containing media.

For further identification of purified isolates, consult listed references for details on the following optional tests:

1. Perform a motility test either by wet mount or using Motility Test Medium (Cat. no. Q10) or SIM medium (Cat. no. Q30). The motility pattern should give a typical umbrella-shaped growth pattern. Pick colonies from cultures incubated at 30 degrees C. or less. *Listeria* spp. are slim, short rods with slight rotating or tumbling motility. Always compare with known culture. Cocci, large rods, or rods with rapid, swimming motility are not *Listeria* spp.

2. Test for catalase using catalase reagent (Cat. no. Z62 and Z76). *Listeria* species are catalase-positive.

3. Gram stain 16 to 24 hour cultures. All *Listeria* spp. are short, gram-positive rods; however, with cultures older than 24 hours, the Gram stain reaction can be variable.

4. Perform xylose-rhamnose reaction tests. Inoculate the following carbohydrates as 0.5% solutions in purple

carbohydrate broth (with Durham tubes): dextrose (Cat. no. Y104), maltose (Cat. no. Y109), rhamnose (Cat. no. Y112), mannitol (Cat. no. Y110), and xylose (Cat. no. Y117). Incubate 7 days at 35ºC. *Listeria* spp. produce acid with no gas and a positive reaction. All species should be positive for dextrose and maltose. All *Listeria* spp. except *L. grayi* should be mannitol-negative. *L. monocytogenes* does not utilize xylose and is positive for rhamnose utilization.

5. Perform CAMP (Christie-Atkins-Munch-Peterson) test. Other organisms needed are a beta-hemolytic *Staphylococcus aureus* and/or *Rhodococcus equi*. Examine plates for hemolysis after incubation. *L. monocytogenes* and *L. eligeri* should show increased hemolysis near the *S. aureus* streak. *L. ivanovii* has enhanced hemolysis near the *R. equi* streak.The other species are non-hemolytic. Quality control with known a isolate of *Listeria* spp. on a separate sheep blood agar plate is encouraged to verify the procedure was done correctly.

6. Consult list of references for approved rapid methods of identification.

7. *Listeria monocytogenes* isolates may require subtyping by the FDA (serological or genetic).

LIMITATIONS

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.

Other bacterial species may exhibit similar black to brown coloring on the esculin-containing media but will take longer than 48 hours to do so.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, BLEB Selective Supplement (Cat. no. K183), 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	24-48hr	35°C	Aerobic	Growth
Enterococcus faecalis ATCC® 29212	B	18-24hr	35°C	Aerobic	Partial inhibition
Escherichia coli ATCC® 25922	B	24-48hr	35°C	Aerobic	Inhibited
Saccharomyces cerevisiae ATCC® 9763	B	24-48hr	35°C	Aerobic	Inhibited

* 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

Buffered Listeria Enrichment Broth should appear clear, and light to medium amber in color, with a slight precipitate.

BLEB Selective Supplement should appear clear and yellow in color, with a possible precipitate. (sold separately)

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. 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 2207D [D]