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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

FRASER BROTH, MODIFIED

Cat. no. K98	Fraser Broth, Modified, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. U410	Fraser Broth, Modified, 500ml Polycarbonate Bottle, 225ml	10 bottles/box

INTENDED USE

Hardy Diagnostics Fraser Broth, Modified (without ferric ammonium citrate) is recommended for the selective enrichment of *Listeria* spp. in foods and environmental samples.

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

SUMMARY

First described by Murray, Webb, and Swann, *Listeria monocytogenes* is a widespread problem in the public health and food industry.[6,8] This microorganism can cause human illness and death, particularly in immunocompromised individuals and in the unborn fetus of pregnant women. The first reported food-borne outbreak of listeriosis was in 1985. 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*.[7,10]

Potential vehicles of transmission implicated in the spread of disease include turkey frankfurters, coleslaw, pasteurized milk, Mexican-style and other soft cheeses, paté and pickled pork tongue. Moreover, the organism has been isolated from commercial dairy and other food processing plants. It is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water. *Listeria* also possess the ability to grow over a pH range of 5.0-9.6 and can survive in food products with pH levels outside of these parameters.[10]

Hardy Diagnostics Fraser Broth, Modified is based on the formulation of Fraser and Sperber.[7] The media contains pancreatic digest of casein, proteose peptone, and yeast extract as nitrogen, vitamin, and mineral sources. Although the medium contains esculin, the modified formulation does not contain ferric ammonium citrate which normally produces the characteristic blackening when esculin is hydrolized. Ferric ammonium citrate may be added (Fraser Broth Supplement, Cat. no. Z210) to the tube of medium, if desired. More stable ELISA results are obtained when the ferric ammonium citrate is omitted. This media is made selective by the presence of lithium chloride, nalidixic acid, and acriflavine in the formula. The high salt concentration is used to inhibit the growth of enterococci.

FORMULA

Ingredients per liter of deionized water:*

Sodium Chloride	20.0gm
Disodium Phosphate	9.6gm
Proteose Peptone	5.0gm

Pancreatic Digest of Casein	5.0gm
Beef Extract	5.0gm
Yeast Extract	5.0gm
Lithium Chloride	3.0gm
Monopotassium Phosphate	1.35gm
Esculin	1.0gm
Nalidixic Acid	20.0mg
Acriflavine Hydrochloride	24.0mg

Final pH 7.2 +/- 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, 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.[1-4]

To isolate *Listeria monocytogenes* from processed meats and poultry, the following procedure is recommended by the U.S.D.A:

1. Add 25.0gm of test material to 225ml Modified Listeria Enrichment Broth (Cat. no. U167) and mix or blend thoroughly.

2. Incubate for 20-24 hours at 30ºC.

3. Transfer 0.1ml of the incubated broth to prepared Fraser Broth, Modified medium. Incubate at 35ºC. for 26 +/- 2.0 hours. If desired, add 0.1ml of a filter sterilized solution of 5% aqueous solution of ferric ammonium citrate (Fraser Broth Supplement, Cat. no. Z210).

4. At 24 and 48 hours, streak the prepared Fraser Broth, Modified culture to Modified Oxford Agar (Cat. no. G46) or PALCAM Agar (Cat. no. G149).

5. Incubate the Modified Oxford or PALCAM plates at 35ºC. for 24-48 hours.

INTERPRETATION OF RESULTS

Observe the Modified Oxford Agar plates for round 1mm colonies with a blackening of the surrounding medium. Suspect colonies may be confirmed by CAMP test on 5% Blood Agar (Cat. no. A10), by further biochemical testing, use of a macroscopic tube rapid slide test, or other means of definitive serological identification.

Observe PALCAM plates for the growth of round gray-green colonies with a black precipitate. Rapid slide and macroscopic tube tests can be used for definitive serological identification. Colonies of mannitol-fermenting contaminants, such as staphylococci and enterococci, appear as yellow colonies with a yellow halo.

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.

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

Modified Listeria Enrichment Broth (Cat. no. U167) is a partially selective medium. Growth of some contaminating strains will be markedly, but not completely, inhibited.

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, 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	24hr	35°C	Aerobic	Growth
Staphylococcus aureus					

ATCC ® 25923	B	24hr	35°C	Aerobic	Partial to complete inhibition
Escherichia coli ATCC ® 25922	B	24hr	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

Fraser Broth, Modified should appear clear, and bright yellow in color.

REFERENCES

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

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

3. Grau, F.H., et al. 1995. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. *J. Food Prot.;* 55:4-4.

4. International Dairy Federation. 1990. Milk and milk products - detection of *Listeria monocytogenes.* IDF Provisional International Standard No. 143. International Dairy Federation, Brussels.

5. Kramer, P.A., et al. 1969. Media selective for *Listeria monocytogenes. J. Appl. Bacteriology* ; 32:381-394.

6. Lovett, J.D., et al. 1987. *Listeria monocytogenes J. Food Prot.;* 50:188-192.

7. Monk, J.D. et al. 1987. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high-fat frozen and refrigerated ground beef. *J. Food Prot.;* 57:769-772.

8. Murray, E.G., et al. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus, *Bacillus monocytogenes. J. Path. Bact.;* 19:407-439.

9. U.S. Food and Drug Administration. *Bacteriological Analytical Manual,* AOAC, Arlington, VA.

10. Vanderzant, C. and D.F. Splittstoesser, (ed.). 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. APHA, Washington D.C.

11. Wehr, H.M. 1987. *Listeria monocytogenes* - a current dilemma Special Report. *J. Assoc. Anal. Chem.;* 80:769-7762.

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

HDQA 2207D [D]