# FRASER BROTH BASE (7502)

# Intended Use

Fraser Broth Base is used with ferric ammonium citrate for the selective enrichment of Listeria species.

# **Product Summary and Explanation**

*Listeria monocytogenes*, described in 1926 by Murray, Webb and Swann, is a widespread problem in public health and food industries.<sup>1</sup> This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and susceptible pregnant women.<sup>2</sup> Epidemiological evidence from outbreaks of listeriosis indicate the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.<sup>3</sup> Implicated vehicles of transmission include turkey frankfurters, coleslaw, pasteurized milk, Mexican style cheese and pate'.<sup>4</sup> *Listeria* species are ubiquitous in nature, present in a wide range of unprocessed foods and in soil, sewage, and river waste.<sup>5</sup>

Fraser Broth Base is based on the formulation of Fraser and Sperber.<sup>6</sup> This medium is used in rapid detection of *Listeria* from food<sup>7</sup> and environmental samples. *Listeria* species grow over a pH range of 5.0 - 9.6, and can survive in food products with pH levels outside these parameters.<sup>8</sup> *Listeria* species are microaerophilic, Gram-positive, asporogenous, non-encapsulated, non-branching, short, motile rods. Motility is pronounced at 20°C. Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization, and serological confirmation.

#### **Principles of the Procedure**

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, Beef Extract, and Yeast Extract provide nitrogen, vitamins, and minerals in Fraser Broth Base. The Phosphates are the buffering agents. Sodium Chloride maintains osmotic balance. Differentiation is aided by including Ferric Ammonium Citrate in the final medium. Since all *Listeria* species hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the medium by cultures containing esculin hydrolyzing bacteria is the result of formation of 6,7-dihydroxycoumarin that reacts with ferric ions.<sup>6</sup> Selectivity is provided by the presence of Lithium Chloride, Nalidixic Acid, and Acriflavin in the formula. The high salt tolerance of *Listeria* is used to inhibit growth of enterococci.

# Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue	
Beef Extract	5 g
Yeast Extract	5 g
Sodium Chloride	20 g
Disodium Phosphate	9.6 g
Monopotassium Phosphate	1.35 g
Esculin	1 g
Acriflavin	0.024 g
Nalidixic Acid	0.020 g
Lithium Chloride	3 g
Final pH: 7.2 ± 0.2 at 25°C	

Supplement / 10 mL 5% Ferric Ammonium Citrate, 10 mL filtered sterilized aqueous solution /L

Formula may be adjusted and/or supplemented as required to meet performance specifications.

# **Precautions**

- 1. For Laboratory Use.
- 2. TOXIC. IRRITATING TO EYES, RESPIRATORY SYSTEM, AND SKIN.

#### **Directions**

- 1. Dissolve 55 g of the medium in one liter of purified water.
- 2. Heat gently in a 50°C waterbath to completely dissolve the medium. DO NOT OVERHEAT.
- 3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
- 4. Aseptically add 10 mL of a filtered sterilized 5% aqueous solution of ferric ammonium citrate.

# Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and beige.

**Prepared Appearance:** Prepared medium is golden yellow with an opalescent top and clear to slight hazy.

**Expected Cultural Response:** Cultural response in Fraser Broth Base at 35°C after 18 - 48 hours incubation.

Microorganism	Response
Escherichia coli ATCC® 25922	inhibited
Listeria monocytogenes ATCC® 7644	growth w/blackening
Listeria monocytogenes ATCC® 15313	growth w/blackening
Staphylococcus aureus ATCC® 25923	inhibited (@ 18-24 hrs)

The organisms listed are the minimum that should be used for quality control testing.

# **Test Procedure**

To isolate *Listeria monocytogenes*, the following procedure is recommended by U.S.D.A.<sup>7</sup>

- 1. Add 25 g of test material to 225 mL of UVM Modified Listeria Enrichment Broth and mix or blend thoroughly. Incubate for 20 24 hours at 30°C.
- 2. Transfer 0.1 mL of the incubated broth to Fraser Broth. Incubate at  $35^{\circ}$ C for 26 ± 2 hours.
- 3. At 24 and 48 hours, streak the Fraser Broth culture to Modified Oxford Agar. Incubate the Modified Oxford plates at 35°C for 24 48 hours.

# <u>Results</u>

For further identification and confirmation of *Listeria* species, consult appropriate references.<sup>7,8,9,10</sup> Rapid slide and macroscopic tube tests can be used for definitive serological identification.

#### Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

#### **Expiration**

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in intact container when stored as directed.

# Limitations of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

# <u>Packaging</u>

Fraser Broth Base	Code No.	7502A	500 g
		7502B	2 kg
		7502C	10 kg

#### **References**

- 1. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bacteriol. **29:**407-439.
- 2. Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
- Bremer, P. J., and C. M. Osborne. 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. 58:604-608.
- 4. Grau, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuumpackaged processed meats. J. Food Prot. 55:4-7.
- 5. Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monoytogenes*. J. Food Prot. **58**:244-250.
- 6. Fraser, J., and W. Sperber. 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. 51: 762-765.
- 7. Lee, W. H., and D. McClain. 1994. Laboratory Communication No. 57, U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
- 8. **Vanderzant, C., and D. F. Splittstoesser (eds).** Compendium of methods for the microbiological examination of foods, 3<sup>rd</sup> ed. American Public Health Association, Washington, D.C.
- 9. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.
- 10. **Marshall, R. T. (ed.).**, Standard methods for the examination of dairy products,16<sup>th</sup> ed. American Public Health Association, Washington, D.C.

#### Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.