Buffered Listeria Enrichment Broth Base (NCM0164)

Effective Date: 4/30/2019
Revision: PI- NCM0164

Intended Use
Buffered Listeria Enrichment Broth Base is used with supplements for selective enrichment of *Listeria* spp. in foods and is not intended for use in the diagnosis of human disease.

Description
Listeria monocytogenes, described first in 1926 by Murray, Webb, and Swann, is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women. Epidemiological evidence from outbreaks of listeriosis has indicated that the principle route of transmission is via consumption of foodstuffs contaminated with *Listeria monocytogenes*. Implicated vehicles of transmission include turkey, frankfurters, coleslaw, pasteurized milk, Mexican style cheese, and pate.

Buffered Listeria Enrichment Broth, a modification of the formula by Lovett et al. was developed after research concluded that enrichment properties can be improved by increasing the buffering capacity of the medium with the addition of disodium phosphate. BLEB Base is based upon the FDA/BAM recommendations where the medium is supplemented with selective agents after an initial four hour, non-selective, pre-enrichment.

Typical Formulation

Enzymatic Digest of Casein	17.0 g/L
Enzymatic Digest of Soybean Meal	3.0 g/L
Yeast Extract	6.0 g/L
Dextrose	2.5 g/L
Sodium Chloride	5.0 g/L
Monopotassium Phosphate	1.35 g/L
Dipotassium Phosphate	2.5 g/L
Disodium Phosphate	9.6 g/L

Final pH: 7.3 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

BLEB Supplement (7980)
Acriflavine HCl, 4.5 mg
Nalidixic Acid, 18 mg
Cycloheximide, 22.5 mg

Precautions
Refer to SDS

Preparation
1. Dissolve 47 g of the medium in 1000 mL of purified water.
2. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add 11.1 mL of a 10% filter sterilized solution of sodium pyruvate.
5. After four hours of incubation at 30 ± 2°C, aseptically add 2.5 mL Buffered Listeria Enrichment Supplement (7980) to 225 mL of Buffered Listeria Enrichment Broth Base containing 25 grams of the sample. OR, aseptically add 0.455 mL of a 0.5% aqueous solution of acriflavine, 1.8 mL of a 0.5% aqueous solution of nalidixic acid, and 1.15 mL of a 1.0% solution of cycloheximide in 40% ethanol to 225 mL of medium containing 25 g or 25 mL of food to be tested.

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Test Procedure
Use recommended laboratory procedures for isolating Listeria in food samples.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is clear, medium amber with none to moderate precipitate.

Expected Cultural Response: Cultural response in Buffered Listeria Enrichment Broth Base, supplemented with selective agents incubated aerobically after 4 hours at 30 ± 2°C, and incubated an additional 18 - 44 hours. Cultures were examined for growth at 18 – 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Growth
Escherichia coli ATCC® 25922	10^4- 10^6	Inhibited
Listeria monocytogenes ATCC® 19111	10-100	Good growth
Listeria monocytogenes ATCC® 13932	10-100	Good growth
Staphylococcus aureus ATCC® 25923	10-100	Suppressed at 18-24 hours

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

Results
Refer to appropriate references and procedures for results.

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

Limitation of the Procedure
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage
Store dehydrated culture media at 2-30°C away from direct sunlight. 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.

References
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3. 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 vacuum-packaged processed meats. J. Food Prot. 55:4.7.
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6. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

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