
Buffered Listeria Enrichment Broth Base

Intended Use

Buffered Listeria Enrichment Broth Base is recommended by FDA Committee for enrichment in isolation of *Listeria monocytogenes*.

Typical Composition (g/litre)

Casein enzymic hydrolysate 17.0; Papaic digest of soya bean meal 3.0; Sodium chloride 5.0; Dipotassium hydrogen phosphate 2.50; Dextrose 2.50; Yeast extract 6.0; Monopotassium phosphate, anhydrous 1.350; Disodium phosphate, anhydrous 9.60; Sodium pyruvate 1.0

Mode of Action

Listeria species grow over a pH range of 4.4-9.6, and survive in food products with pH levels outside these parameters. *Listeria* species are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Casein enzymic hydrolysate and papaic digest of soya bean meal provide amino acids and other complex nitrogenous substances. Dextrose is the energy source. Sodium pyruvate aids in resuscitation of organisms. The phosphates provide buffering capacity. Sodium chloride maintains the osmotic equilibrium. Yeast extract provides vitamin B complex. The medium is rendered selective due to the inclusion of antimicrobial agents. Cycloheximide inhibits the growth of saprophytic fungi. Nalidixic acid inhibits the growth of gram-negative organisms, whereas acriflavin suppresses growth of gram-positive microorganisms.

Preparation

Suspend 23.97 grams in 500 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C. Aseptically add the rehydrated contents of 1 vial of Listeria Selective Supplement. Mix well and dispense as desired.

Final pH (at 25°C) 7.3±0.2

Storage

Store below 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Experimental Procedure and Evaluation

L. monocytogenes is a well-documented food borne pathogen because of its high morbidity on infection to animals and humans and also due to its psychrotrophic nature exhibiting high tolerance to heat, cold and desiccation. The organism has been isolated from commercial dairy and other food processing plants, and is

ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water. According to FDAs enrichment procedure for isolation of *L. monocytogenes* from dairy products, the sample to be tested is inoculated in enrichment broth and incubated at 30°C for 4 hours without the selective supplement. After 4 hours' selective supplement is added and further kept for incubation for additional 44 hours at 30°C. After 24 hours and 48 hours the enriched culture is streaked on Oxford Listeria Medium Base and LPM Agar / Listeria Identification Agar Base, and incubated at 35°C for 24-48 hours. Presumptive Listeria colonies are selected and colonies are further purified on Tryptone Soya Yeast Extract Agar. Purified isolates are then subjected to a variety of biochemical tests to confirm the presence of *L. Monocytogenes*.

Quality Control

| Organism | Inoculum (CFU) | Growth |
|---|----------------|----------------|
| <i>E.coli</i> ATCC 25922 | $\geq 10^3$ | Inhibited |
| <i>Listeria innocua</i> ATCC 33090 | 50 - 100 | Good-Luxuriant |
| <i>Listeria monocytogenes</i> ATCC 19111 | 50 - 100 | Good-Luxuriant |
| <i>Listeria monocytogenes</i> ATCC 19112 | 50 - 100 | Good-Luxuriant |
| <i>Listeria monocytogenes</i> ATCC 19118 | 50 - 100 | Good-Luxuriant |
| <i>Staphylococcus aureus</i> ATCC 25923 | 50 - 100 | None -Poor |
| <i>Saccharomyces cerevisiae</i> ATCC 9763 | 50 - 100 | None -Poor |

Reference

1. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
2. Bremer and Osborne, 1995, J. Food Prot., 58:604.
3. Hitchens, 1995, FDA Bacteriological Analytical Manual, 8th Ed. AOAC International, Gaithersburg, Md
4. Murray, Webb and Swann, 1926, J. Pathol. Bacteriol., 29:407.