Granulated® HIMEDIA®

Technical Data

Buffered Listeria Enrichment Broth Base (BLE Broth Base),Granulated®

GM1578

Intended Use:

Recommended by FDA Committe for enrichment procedure for isolation of *Listeria monocytogenes.*

Composition**

Ingredients	g / L
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Dextrose (Glucose)	2.500
Yeast extract	6.000
Potassium dihydrogen phosphate	1.350
Disodium hydrogen phosphate	9.600
Sodium pyruvate	1.11
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.03 grams in 500 ml purified/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 - 50°C. Aseptically add the rehydrated contents of 1 vial of ANC Selective Supplement (FD063I) or 1 vial of ANA Selective Supplement, Modified (FD163I) as desired. Mix well and dispense into sterile tubes or flasks as desired.

Principle And Interpretation

Listeria monocytogenes is the only species of the *Listeria* genus that causes Listeriosis in human, however occasionally *L.seeligeri, L. welshimeri* and *L. ivanovii* have been related with human diseases. Microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has show that the principal route of transmission is via the consumption of foodstuffs contaminated with *L. monocytogenes* (1).

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 (2). *Listeria* species grow over a pH range of 4.4-9.6, and survive in food products with pH levels outside these parameters (3). *Listeria* species are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C. Food samples are often contaminated with organisms other than *Listeria,* which makes its isolation difficult (4). To recover low numbers of *L. monocytogenes* from food samples, initial enrichment is required. Listeria Enrichment Broth was modified by adding buffering strength thereby making it possible for the medium to be used successfully in conjunction with DNA probe and other methods that are more sensitive than conventional culture procedure. This medium is also recommended by APHA for the selective enrichment of *L.monocytogenes*.

Tryptone and soya peptone 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.

Type of specimen

Food and Dairy samples

Specimen Collection and Handling

According to FDAs enrichment procedure (5) 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 the 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 (M1145) and LPM Agar (M1228) / Listeria Identification Agar Base, PALCAM (M1064) 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 (M1214). Purified isolates are then subjected to a variety of biochemical tests to confirm the presence of *L. monocytogenes*. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:

1. Further biochemical tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow coloured granular medium

Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent solution with slight precipitate

Reaction

Reaction 4.8% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added ANC Selective Supplement (FD063I) or ANA Selective Supplement, Modified (FD163I) after an incubation at 30°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited
Listeria innocua ATCC 33090 (00017*)	50-100	good to luxuriant
Listeria monocytogenes ATCC 19111 (00020*)	50-100	good to luxuriant
Listeria monocytogenes ATCC 19112	50-100	good-luxuriant
Listeria monocytogenes ATCC 19118	50-100	good-luxuriant
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	none-poor
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	none-poor

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10- 30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1.Bremer and Osborne, 1995, J. Food Prot., 58:604.

2.Patel, Hwang, Beuchat, Doyle and Brackett, 1995, J. Food Prot., 58:244

3.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

4.Murray, Webb and Swann, 1926, J. Pathol. Bacteriol., 29:407.

5.Hitchins, 1995, FDA Bacteriological Analytical Manual, 8th Ed. AOAC International, Gaithersburg, Md.

6.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

7.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 02 / 2025