Technical Data

Listeria Enrichment Broth, Modified

M2100

Intended Use:

Recommended for selective enrichment of *Listeria* species.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Dextrose	2.500
Yeast extract	6.000
Acriflavin hydrochloride	0.015
Nalidixic acid	0.040
Actidione	0.050

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.10 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45 - 50°C. Mix well and aseptically dispense into sterile tubes or flasks as desired.

Principle And Interpretation

Listeria monocytogene 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* (2).

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 (7). *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 (6). To recover low numbers of *L. monocytogenes* from food samples, initial enrichment is required.

Tryptone and soya peptone provide amino acids and other complex nitrogenous substances. Dextrose is the energy source. Potassium 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. Actidione inhibits the growth of saprophytic fungi. Nalidixic acid inhibits the growth of gram-negative organisms, whereas acriflavin suppresses growth ofgram-positive microorganisms.

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 *Listeria* species.

Type of specimen

Food samples : meat and meat products; Dairy samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Limitations

1. Due to variable nutritional requirements, some strains show poor growth on this medium.
2. After 24 - 48 hours of enrichment, recovery on selective media is required.
3. Presumptive *Listeria* colonies are selected and subjected to variety of biochemical tests, for complete identification of species.

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 homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent solution with slight precipitate

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited
Listeria monocytogenes ATCC 13932 (00021*)	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 19117	50-100	good-luxuriant
Listeria monocytogenes ATCC 19118	50-100	good-luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	none-poor
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°C in a tightly closed container. 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 (4,5).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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3. Hitchens, 1995, FDA Bacteriological Analytical Manual, 8th Ed. AOAC International, Gaithersburg, Md.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.
6. Murray, Webb and Swann, 1926, J. Pathol. Bacteriol., 29:407.
7. Patel, Hwang, Beuchat, Doyle and Brackett, 1995, J. Food Prot., 58:244
8. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

Revision : 00 / 2020