Technical Information

Listeria Selective Broth Base

Product Code: DM1889

Application: Recommended for selective isolation and cultivation of *Listeria monocytogenes* from clinical specimens.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Yeast extract	6.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Dextrose (Glucose)	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Methodology

Suspend 36 grams in 1000 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 and aseptically add rehydrated contents of 1 vial of Listeria Selective Supplement II (MS2063) or 2 vials of Listeria Selective Supplement II (MS2063I) as desired. Mixwell and dispense into sterile tubes or flasks or as desried

Principles and Interpretation

Listeria monocytogenes is a short, gram-positive, non spore-forming rod shaped bacterium that appears coccoidal in older cultures. *Listeria* multiplies over a wide range of temperatures from 3°C to 45°C with optimum temperature range of 30°C to 37°C. *L.monocytogenes* has been isolated from numerous environmental sources such as silage, soil, decaying vegetation, sewage, damp earth, straw and faeces (2,9). Detection of *L.monocytogenes* in foods is not difficult. Low numbers of organisms are commonly isolated from raw milk, meat, vegetables, seafood and the food-processing environment. Enrichmentprocedures are used to isolate low numbers of *L.monocytogenes*. Injured *L. monocytogenes* are sublethally stressed as a result of exposure to heat, freezing or acidic conditions. Sublethally stressed *L. monocytogenes* require resuscitation in anon-selective medium at a temperature favouring repair of the sublethal injury.

Listeria Selective Broth is formulated as per Lovett et al (7) for the selective enrichment of *Listeria* species from milk andmilk products and other foods. Listeria Selective Broth is recommended by ISO Committee (3) with a slight modification in the supplement (MS2063I).

Tryptone, soya peptone and yeast extract provide carbon and nitrogen compounds, long chain amino acids, vitamins essentialfor bacterial metabolism. Dextrose is the energy source. The medium is rendered selective by addition of selectivesupplement. Cycloheximide inhibits the growth of saprophytic fungi. Nalidixic acid inhibits growth of gram-negative organisms and acriflavin suppresses gram-positive microorganisms (6,8). Acriflavin is an acridinic derivative with bacteriostatic properties towards many gram-positive bacteria and a weak fungicidal activity.

For enrichment, 25 grams or 25 ml sample is added to 225 ml medium in a stomacher bag. Homogenize the material if required. Incubation is carried out at 30°C for upto 7 days. Ajello et al (1) showed that incubation period of 7 days allows betterrecovery of environmentally stressed *Listeria* from milk and milk products. The enrichment broth is further subcultured on Listeria Selective Agar (DM1567) after 1, 2 and 7 days.

Listeria monocytogenes is a highly pathogenic organism and therefore proper precautions should be taken while handling them.

Type of specimen

Clinical samples - Body tissues or body fluids

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :
In Vitro diagnostic Use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :
1. Further biochemical tests are needed for a final identification of the isolated organisms.
2. *Listeria monocytogenes* is a highly pathogenic organism and therefore proper precautions should be taken whilehandling them.

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
Fluorescent yellow coloured, clear solution in tubes
Reaction
Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.3±0.2
pH
7.10-7.50

Cultural Response
Cultural characteristics observed with added Listeria Selective Supplement II (MS2063 / MS2063I) after an incubation at 30-35°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Candida albicans ATCC 10231 (00054*)	>=10^4	inhibited
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited
Listeria monocytogenes ATCC 19111 (00020*)	50-100	luxuriant
Listeria monocytogenes ATCC 19112	50-100	luxuriant
Listeria monocytogenes ATCC 19118	50-100	luxuriant
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	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 dueto 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Further Reading

1. Ajello G., Hayes P. and Fuley J., 1986, Abstracts of the Annual Meeting, ASM, Washington, D.C
2. 2.Gray M. L., 1960, Science, 132:1767.
3. International Organization for Standardization (ISO), 1993, 10560 Ind. Technical, Corrigendum Cor. 1:1994.
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. Lee W. K. and McClain D., 1986, Appl. Environ, Microbiol., 52:1215.7.
7. Lovette J., Francis D. W. and Hunt J. M., 1987, J. Food Prot., 50:188.
8. .McClain D. and Lee W. H., 1988, J. Assoc. off. Anal. Chem., 71:660.
9. Weis J., and Seeliger H. P. R., 1975, Appl. Microbiol. 30:29.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate
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