Technical Data

L. mono Differential Agar Base

M1540

Intended use

Recommended for the selective and differential isolation of *Listeria monocytogenes*. The composition and performance criteria of this media is as per the specification laid down in ISO 11290-1:2017 and ISO 11290-2:2017

Composition**

Ingredients	Gms / Litre
HM Peptone #	18.000
Tryptone ##	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Dextrose (Glucose)	2.000
Magnesium glycerophosphate	1.000
Magnesium sulphate	0.500
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate anhydrous	2.500
5-Bromo-4 chloro-3-indolyl-β–D-glucopyranoside	0.050
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Key : # - Equivalent to Enzymatic digest of animal tissues, ## - Equivalent to Enzymatic digest of casein

Directions

Suspend 36.02 grams in 460 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile contents of 1 vial of L. mono Enrichment Supplement I (FD214) and sterile rehydrated contents of L .mono Selective Supplement I (FD212), L .mono Selective Supplement II (FD213). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Listeria monocytogenes is a gram-positive foodborne human pathogen responsible for serious infections in pregnant women that may ultimately result in abortion, stillbirth, birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles. The pathogenicity of *Listeria ivanovii* for humans is uncertain. Since *L. monocytogenes* and *L.innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford). L. mono Differential Agar Base is based on the formulation of Ottoviani and Agosti (3, 4) for the selective and differential isolation of *Listeria monocytogenes* from food and animal feeds which is adopted by ISO Committee (5,6). HM peptone, tryptone and yeast extract supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Sodium pyruvate provide essential growth nutrients. Glucose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplements (FD212 and FD213) inhibit accompanying microflora and allow the growth of *Listeria* species. *Listeria* species hydrolyse the chromogenic substrate (5-Bromo-4 chloro-3-indolyl-β-D-glucopyranoside) which produces green coloured colonies. Differentiation of *Listeria monocytogenes* from other *Listeria* species is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies.

Type of specimen

Clinical samples; Food and animal feeds, environmental samples in the area of food manufacturing and handling.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and animal feeds, environmental samples follow appropriate techniques for handling specimens as per established guidelines (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. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Further biochemical tests must be carried out to differentiate between *L.monocytogenes* and *L. ivanovii*.

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

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, opalescent gel forms in Petri plates

Reaction

Reaction of 7.2% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed with added sterile L. mono Selective supplement I (FD212), L. mono Selective Supplement II (FD213) and L.mono Enrichment supplement I (FD214) after an incubation for 24 h ± 2 hours and an additional 24 h ± 2 hours at 37° ± 1°C .

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	PIPLC activity
Cultural Response					
Candida albicans ATCC 10231 (00054)*	>=10^4	inhibited	0%		
Enterococcus faecalis ATCC 29212 (00087)*	>=10^4	inhibited	0%		
Enterococcus faecalis ATCC 19433 (00009)*	>=10^4	inhibited	0%		
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	0%		
Escherichia coli ATCC 8739 (00012)*	>=10^4	inhibited	0%		
Pseudomonas aeruginosa ATCC 27853 (00025)*	>=10^4	inhibited	0%		
Listeria innocua ATCC 33090 (00017)*	>=10^4	luxuriant	>=50%	greenish-blue	negative
Listeria grayi ATCC 19120	50-100	luxuriant	>=50%	greenish-blue	negative

Listeria ivanovii ATCC 19119	50-100	luxuriant	>=50%	greenish-blue	positive, opaque halo around the colony exhibiting phophatidyl -inositol specific phospholipase acivity
Listeria monocytogenes ATCC 35152 (00109)*	50-100	luxuriant	>=50%	greenish-blue	positive, opaque halo around the colony exhibiting phophatidylinositol specific phospholipase acivity
Listeria monocytogenes ATCC 13932 (00021)*	50-100	luxuriant	>=50%	greenish-blue	positive, opaque halo around the colony exhibiting phophatidylinositol specific phospholipase acivity
Listeria monocytogenes ATCC 19112	50-100	luxuriant	>=50%	greenish-blue	positive opaque halo around the colony exhibiting phophatidylinositol specific phospholipase acivity
Listeria seeligeri ATCC 35967	50-100	luxuriant	>=50%	greenish-blue	negative
Listeria welshimeri ATCC 43549	50-100	luxuriant	>=50%	greenish-blue	negative

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated powder and the prepared medium at 2-8º C in 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. Use before expiry date on the label.
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 (1,2).

Reference

1.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
2. 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.
3.Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3
4. Ottaviani F., Ottaviani M., and Agosti M. (1997 b), Quimper Froid Symposium Proceedings p. 6, A.D.R.I.A. Quimper, France, 16-18 June 1997.
5. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1 , Detection method ; ISO 11290-1:2017

6.Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 2 , Enumeration method ; ISO 11290-2:2017

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