

L. mono Confirmatory HiVeg™ Agar Base

MV1552

Intended Use:

Recommended for the selective and differential isolation of *Listeria monocytogenes* from food and other samples.

Composition**

Ingredients	g / L
HiVeg™ special peptone	30.000
Yeast extract	6.000
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate	2.500
B.C. indicator	8.600
α-Methyl D-mannoside	3.000
Agar	12.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.5 grams in 470 ml purified/distilled water Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile rehydrated contents of one vial of OA Selective Supplement (FD212A) and sterile contents of one vial of **LM Enrichment Supplement II (FD227). 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 Confirmatory Agar Base is a modification of the formulation of Ottoviani and Agosti (1,2) for the selective and differential isolation of *Listeria monocytogenes*. L. mono Confirmatory HiVeg™ Agar Base is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks.

HiVeg™ special peptone and yeast extract serve as nitrogen sources and provide essential nutrients required for the growth of *Listeria*. α-Methyl-D-mannoside is the fermentable carbohydrate. Lithium chloride and added selective supplements (FD212 and FD213) inhibit accompanying microflora and thus enhance the selectivity of the medium for *Listeria* species. Sodium chloride maintains the osmotic equilibrium and disodium hydrogen phosphate buffers the medium. Differentiation of *L. monocytogenes* from other *Listeria* species is based on phosphatidylinositol-specific phospholipase C (PIPLC) activity and fermentation of α-Methyl D-mannoside. Phospholipase C enzyme is an important virulence factor and is specific to only *L. monocytogenes* and *L.ivanovii*. Phospholipase C enzyme produced by *L. monocytogenes* and *L.ivanovii* hydrolyses the purified substrate (FD227) added to the medium and results in the formation of an opaque halo around the colonies.

Differentiation between *L.monocytogenes* and *L.ivanovii* is achieved on the basis of alpha-Methyl D-mannoside utilization and PIPLC activity. *L. monocytogenes* ferments alpha-Methyl D-mannoside forming yellow coloured colonies with halo whereas *L.ivanovii* fails to ferment alpha-Methyl D-mannoside and therefore forms purple coloured colonies with halo. Other *Listeria* species do not exhibit PIPLC activity and therefore they form either purple or yellow coloured colonies without halo.

Type of specimen

Food samples.

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3) 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. Other *Listeria* species do not exhibit PIPLC activity and therefore they form either purple or yellow coloured colonies without halo.

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

Beige to purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Purple coloured, opalescent gel forms in Petri plates

Reaction

Reaction of 7.7% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed with added supplements, OA Selective Supplement (FD212A) and **LM Enrichment Supplement II (FD227), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	PIPLC activity
<i>Candida albicans</i> ATCC 10231 (00054*)	≥10 ⁴	inhibited	0 %		
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 ⁴	inhibited	0 %		
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited	0 %		
<i>Listeria innocua</i> ATCC 33090 (00017*)	50-100	luxuriant	≥50%	yellow	negative
<i>Listeria grayi</i> ATCC 19120	50-100	luxuriant	≥50%	yellow	negative
<i>Listeria ivanovii</i> ATCC 19119	50-100	luxuriant	≥50%	light purple	positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity
<i>Listeria monocytogenes</i> ATCC 19112	50-100	luxuriant	≥50%	yellow	positive, opaque halo around the colony exhibiting phosphatidylinositol specific phospholipase activity
<i>Listeria seeligeri</i> ATCC 35967	50-100	luxuriant	≥50%	light purple	negative

<i>Listeria welshimeri</i> ATCC 43549	50-100	luxuriant	$\geq 50\%$	yellow	negative
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	$\geq 10^4$	inhibited	0%		

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 (4,5).

Reference

1. Ottaviani F., Ottaviani M., and Agosti M. (1997 a), *Industrie Alimentari* 36, 1-3.
2. 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.
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. 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.

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