HiCrome™ Listeria Ottaviani Agosti HiCynth™ Agar Base MCD1540A

Intended use

Recommended for the selective and differential isolation of *Listeria monocytogenes.*

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.1*	24.000
HiCynth™ Peptone No.5*	10.000
Sodium pyruvate	2.000
Glucose (Dextrose)	2.000
Magnesium glycerophosphate	1.000
Magnesium sulphate	0.500
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate	2.500
Chromogenic substrate	0.050
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

*Chemically defined peptones

Directions

Suspend 36.02 grams in 460 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 contents of 1 vial of LP Enrichment Supplement 1 (FD214) and sterile rehydrated contents of OA Selective Supplement (FD212A). 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 (1,2) for the selective and differential isolation of *Listeria monocytogenes* from food and animal feeds which is adopted by ISO Committee (3). HiCrome™ Listeria Ottaviani Agosti HiCynth™ Agar Base is prepared by completely replacing animal or vegetable based peptones with chemically defined peptones to avoid BSE /TSE risks associated with animal peptones.

HiCynth™ peptone No.1 and HiCynth™ peptone No.5 provides nitrogenous and carbonaceous substances, long chain amio acids, vitamins and essential growth nutrients. Sodium pyruvate support good growth. Glucose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplements (FD212A) inhibit accompanying microflora and allow the growth of *Listeria* species. *Listeria* species hydrolyze the chromogenic substrate 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

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

Specimen Collection and Handling

For food and animal feeds, environmental samples follow appropriate techniques for handling specimens as per established 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. 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 LP Enrichment Supplement 1 (FD214) and OA Selective Supplement (FD212A).after an incubation at 35 - 37°C for 24 - 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	PIPLC activity
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*)	50-100	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 between 15-25°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. 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 and - Part 2, Enumeration method; ISO 11290-2:2017.
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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