



HiCrome™ L. mono Differential Agar Base

M2009

Intended use:

This media is recommended for the selective and differential isolation, enumeration and identification of *Listeria monocytogenes* and *Listeria species* based on PCPLC activity from food samples.

Composition**

Ingredients	Gms / Litre
Peptone	15.000
Tryptone	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Maltose	4.000
Magnesium glycerophosphate	1.000
Magnesium sulphate	0.500
Sodium chloride	5.000
Lithium chloride	5.000
Disodium hydrogen phosphate anhydrous	2.500
Chromogenic substrate	2.200
Agar	14.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.60 grams in 480 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile contents of 1 vial of Lecithin solution (FD332) and sterile rehydrated contents of Modified L.mono Selective Supplement (FD333). 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, and birth of a child with neonatal listeriosis and meningitis or primary bacteremia in adults and juveniles (4). 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). HiCrome™ L.mono Differential Agar Base is based on for the selective and differential isolation of *Listeria species* on the basis of utilization of chromogenic substrate and lecithinase activity [Phosphatidylcholine phospholipase C (PCPLC)] (6). PI-PLC and PC-PLC, the major virulence factors, are only produced by pathogenic *L. monocytogenes* and *Listeria ivanovii* (3). Peptone, tryptone, yeast extract and sodium pyruvate provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and essential growth nutrients. Maltose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Phosphate buffers the medium. Lithium chloride and added selective supplement (FD333) inhibit accompanying microflora and allow the growth of *Listeria species*. *Listeria species* hydrolyse the chromogenic substrate and produces green coloured colonies. Lecithin solution (FD332) helps in detecting PCPLC activity. Differentiation of *Listeria species* is based on phosphatidylcholine phospholipase C (PCPLC) activity. *L.monocytogenes* and *L.ivanovii* exhibits PCPLC activity which is seen as opaque halo around the colony.

Type of specimen

Clinical samples; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1, 2). For food samples, follow appropriate techniques for sample collection and processing as per 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. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.

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 1.4% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, opalescent gel forms in Petri plates

Reaction

Reaction of 6.72% 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 Modified L.mono Selective Supplement (FD333) and Lecithin solution (FD332) after an incubation at 35 - 37°C for 24 - 48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	PCPLC activity
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited	0%		
<i>Escherichia coli</i> ATCC 8739 (00012*)	≥10 ⁴	inhibited	0%		
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 ⁴	inhibited	0%		
<i>Listeria innocua</i> ATCC 33090 (00017*)	≥10 ⁴	luxuriant	≥50%	greenish-blue	negative
<i>Listeria ivanovii</i> subsp. <i>ivanovii</i> serovar 5 ATCC 19119 (00018*)	50-100	luxuriant	≥50%	greenish-blue	positive, opaque halo around the colony exhibiting phosphatidylcholine phospholipase activity
<i>Listeria monocytogenes</i> serovar 1 ATCC 19112 (00020*)	50-100	luxuriant	≥50%	greenish-blue	positive, opaque halo around the colony exhibiting phosphatidylcholine specific phospholipase activity
<i>Listeria monocytogenes</i> serovar 4b ATCC 13932 (00021*)	50-100	luxuriant	≥50%	greenish-blue	positive, opaque halo around the colony exhibiting phosphatidylcholine specific phospholipase activity
<i>Listeria monocytogenes</i> serovar 1/2a ATCC 35152 (00109*)	50-100	luxuriant	≥50%	greenish-blue	positive, opaque halo around the colony exhibiting phosphatidylcholine specific phospholipase activity

Key : (*) - Corresponding WDCM Numbers

Storage and Shelf Life

Store dehydrated powder and prepared medium at 2-8° 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. 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. Mengaud J, Braun-Breton C, Cossart P 1991. Identification of phosphatidylinositol-specific phospholipase C activity in *Listeria monocytogenes*: a novel type of virulence factor. Mol. Microbiol. 5:367–372. doi:10.1111/j.1365-2958.1991
4. Painter J, Slutsker L. 2007. Listeriosis in humans, p 85–109. In Ryser ET, Marth EH (ed), *Listeria*, listeriosis, and food safety. Marcel Dekker, New York, NY.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Sang-Hyun Park, Pahn-Shick Chang, Sangryeol Ryu and Dong-Hyun Kang. Development of a Novel Selective and Differential Medium for the Isolation of *Listeria monocytogenes*. Applied and Environmental Microbiology 2014.



In vitro diagnostic medical device

Revision 01/2019



CE Marking



Storage temperature



Do not use if package is damaged



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