HiCrome™ L. mono Rapid Differential Agar Base

Recommended for the rapid identification and differentiation of *Listeria monocytogenes* from other *Listeria species* based on rhamnose fermentation and PIPLC activity.

M1924

Composition **

Ingredients	Grams/Litre
Peptone special	23.00
Tryptone	10.00
Soya peptone	2.00
Sodium chloride	4.00
Lithium chloride	5.00
Chromogenic mixture	1.16
Rhamnose	10.00
Phenol red	0.12
Agar	15.00

Final pH (at 25°C) 7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.14 grams in 470 ml 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 L. mono Enrichment Supplement I (FD214) and sterile rehydrated contents of 1 vial of HiCrome™ Listeria Selective Supplement (FD181). 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 (5). Since *L. monocytogenes* and *L.innocua* have similar biochemical properties, they cannot be differentiated on traditional media (PALCAM, Oxford). This medium is based on the specific chromogenic detection of β-glucosidase activity, rhamnose fermentation and PIPLC activity. *Listeria* species hydrolyse the purified chromogenic substrate in the medium giving blue coloured colonies. Since β-glucosidase activity is specific for *Listeria* species, other organisms cannot utilize the chromogenic substrate and therefore give white colonies. Differentiation between *Listeria* species is based on the property of rhamnose fermentation and PIPLC activity. The colonies of *L. monocytogenes* appear bluish green with a yellow halo (rhamnose positive) while the colonies of *L.ivanovii* appear bluish green without a yellow halo (Rhamnose negative) (1, 2). The differentiation of *L.mono* and *L.innocua* is based on PIPLC (phosphatidylinositol-specific phospholipase C) activity. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies. *L.ivanovii* also demonstrates PIPLC activity however since it does not ferment rhamnose it can be easily distinguished from *L. monocytogenes* (3, 4).

Peptone special, tryptone and soya peptone provide nitrogenous compounds, carbon, long chain amino acids vitamin B complex and other essential growth nutrients. Rhamnose is the fermentable carbohydrate with phenol red as an indicator. Sodium chloride maintains the osmotic equilibrium. The added lithium chloride and HiCrome™ Listeria Selective Supplement (FD181) inhibit growth of most gram- positive bacteria, gram-negative bacteria, yeasts and moulds. Phospholipase C enzyme hydrolyses the purified substrate (FD214) added to the medium resulting in an opaque halo around *Listeria monocytogenes* colonies demonstrating PIPLC activity.

Type of specimen

Food samples

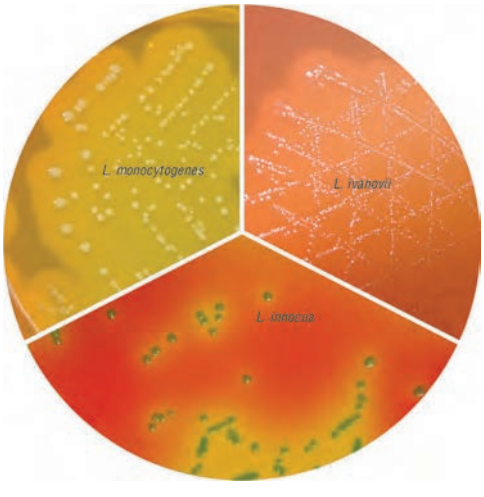
Specimen Collection and Handling

For Food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6).

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



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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 recommended temperature.

Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Red coloured, opalescent gel forms in Petri plates

Reaction : Reaction of 7.03% w/v aqueous solution at 25°C. pH : 7.4±0.2.

Cultural Response : Cultural characteristics observed w/added HiCrome™ Listeria Selective Supplement (FD181)and L.mono Enrichment supplement I (FD214), after an incubation at 35-37°C for 24-48 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony	Rhamnose fermentation	PIPLC Activity
Bacillus subtilis subsp. spizizenii (6633) (00003*)	≥10^3	inhibited	0%			
Candida albicans (10231) (00054*)	≥10^3	inhibited	0%			
Escherichia coli (25922) (00013*)	≥10^3	inhibited	0%			
Listeria innocua (33090) (00017*)	50-100	luxuriant	≥50%	bluish green (yellow back-ground)	positive reaction,	negative reaction
Listeria ivanovii (19119) (00018*)	50-100	luxuriant	≥50%	bluish green	negative reaction	**positive
Listeria monocytogenes (19118)	50-100	luxuriant	≥50%	bluish green (yellow back-ground)	positive reaction,	**positive,
Pseudomonas aeruginosa (27853) (00025*)	≥10^3	inhibited	0%			

Key : * : Corresponds to WDCM number

** : opaque halo around the colony exhibiting phosphatidyl inositol specific phospholipase activity.

Storage and Shelf-life

Store between 2-8°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 inorder 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (7, 8).

References

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