HiCrome L.mono Rapid Differential Agar Base

M1924

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

Composition**

Ingredients	Gms / Litre
Peptone special	23.000
Tryptone	10.000
Soya peptone	2.000
Sodium chloride	4.000
Lithium chloride	5.000
Chromogenic mixture	1.160
Rhamnose	10.000
Phenol red	0.120
Agar	15.000
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.

Warning : Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin wash with plenty of water immediately .

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 (1). 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 ß-glucosidase activity, rhamnose fermentation and PIPLC activity. *Listeria* species hydrolyse the purified chromogenic substrate in the medium giving blue coloured colonies. Since ß-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) (2,3).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* (4,5) .

Peptone special, tryptone and soya peptone provide nitrogenous substances, 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.

Quality Control

Appearance
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

pH
7.20-7.60

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.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Rhamnose fermentation	PIPLC Activity
Cultural Response						
Bacillus subtilis ATCC 6633	>=10^3	inhibited	0%			
Candida albicans ATCC 10231	>=10^3	inhibited	0%			
Escherichia coli ATCC 25922	>=10^3	inhibited	0%			
Listeria innocua ATCC 33090	50-100	luxuriant	>=50%	bluish green	positive reaction, (yellow background)	negative reaction
Listeria ivanovii ATCC 19119	50-100	luxuriant	>=50%	bluish green	negative reaction	positive, opaque halo around the colony exhibiting phosphatidyl inositol specific phospholipase activity.
Listeria monocytogenes ATCC 19118	50-100	luxuriant	>=50%	bluish green	positive reaction, (yellow background)	positive, opaque halo around the colony exhibiting phosphatidyl inositol specific phospholipase activity.
Pseudomonas aeruginosa ATCC 27853	>=10^3	inhibited	0%			

Storage and Shelf Life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1.Schlech WF, Lavigne PM, Bortolussi RA, et al.(January 1983). "Epidemic listeriosis-evidence for transmission by food". N. Engl. J.Med. 308(4): 203–6. doi:10.1056.
2.Notermans S.H. and Dufrenne J., (1991), Applied and Environmental Microbiology, 57(09): 2666-70.

3.Mengaud J., Braun-Breton C. and Cossart P., (1991), Molecular Microbiology, 5(2): 367-372.P
4.Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3.
5.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.

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