HiCrome™ L.mono Rapid Differential Agar Base

M1924

Intended Use:

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

Composition**

Ingredients	g / L
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 gram 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 contents of 1 vial of LP Enrichment Supplement 1 (FD214) and sterile rehydrated contents of 1 vial of CA 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 (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. monocytogenes* 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*(1,4).

Peptone special, tryptone and soya peptone provide nitrogenous, carbonaceous substances, 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 CA 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 and processing as per guidelines (5,6).
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.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organisms.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
4. Further biochemical tests must be carried out for confirmation.

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

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 CA Selective Supplement (FD181) and LP Enrichment Supplement 1(FD214), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Rhamnose fermentation	PIPLC Activity
Listeria innocua ATCC 33090 (00017*)	50-100	luxuriant	>=50%	bluish green	positive reaction, (yellow background)	negative reaction
Listeria ivanovii subsp. *ivanovii serovar* 5 ATCC 19119 (00018*)	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.
Listeria monocytogenes ATCC 13932 (00021*)	50-100	luxuriant	>=50%	bluish green	positive reaction, (yellow background)	positive, opaque halo around the colony exhibiting phosphatidyl inositol specific phospholipase activity.

Listeria monocytogenes ATCC 35152 (00109*)	50-100	luxuriant	>=50%	bluish green	positive reaction, (yellow background)	positive, opaque halo around the colony exhibiting phosphatidyl inositol specific phospholipase activity.
**Bacillus spizizenii* ATCC 6633 (00003*)	>=10^4	Inhibited	0%			
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%			
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%			
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0%			
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%			

Key : (*) Corresponding WDCM numbers and **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

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 (7,8).

Reference

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