HiCrome™ Listeria Ottaviani-Agosti Agar Base

M1540I

Intended use

Recommended for the selective and differential isolation of *Listeria monocytogenes*. The composition and performance criteria of this media is as per the specification laid down in ISO 11290-1:2017 and ISO 11290-2:2017.

Composition**

ISO 11290 Specification / FDA BAM/ APHA - Agar Listeria according to Ottaviani and Agosti

Ingredients	g / L
Enzymatic digest of animal tissues	18.000
Enzymatic digest of Casein	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Glucose	2.000
Magnesium glycerophosphate	1.000
Magnesium sulphate (anhydrous)	0.500
Sodium chloride	5.000
Lithium chloride	10.000
Disodium hydrogen phosphate (anhydrous)	2.500
5-Bromo-4 chloro-3-indolyl-β−D-glucopyranoside	0.050
Agar	12.00 - 18.00
Final pH (after sterilization)	7.2±0.2

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Ingredients	g / L
HM Peptone #	18.000
Tryptone ##	6.000
Yeast extract	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
5-Bromo-4 chloro-3-indolyl-β−D-glucopyranoside	0.050
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Key : # - Equivalent to Enzymatic digest of animal tissues, ## - Equivalent to Enzymatic digest of casein

Supplements to be added after autoclaving	g / L
I	
Nalidixic acid sodium salt	0.020
Ceftazidime	0.020
Polymyxin B sulfate	76 700 IU
Cycloheximide OR	0.050
Amphotericin B	0.010
II	
L-α- phosphatidylinositol	2.00

FD212A - 2 vials OA Selective Supplement	mg / vial
Nalidixic acid sodium salt	10.000
Ceftazidime	10.000
Polymyxin B sulfate	38350 IU
Amphotericin B	5.000
(FD214) - 2 vials	
LP Enrichment Supplement 1	1.000g

Directions

Suspend 36.02 gram in 465 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at15 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). The media is based on the formulation of Ottoviani and Agosti (1,2) for the selective and differential isolation of *L.monocytogenes* from food and animal feeds which is adopted by ISO Committee (3,4,5). It is also recommended by APHA (6) & FDA-BAM (7). HM peptone, tryptone and yeast extract supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Sodium pyruvate provide essential growth nutrients. Glucose (Dextrose) 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 hydrolyse the chromogenic substrate (5-Bromo-4 chloro-3-indolyl-β−D-glucopyranoside) which produces blue to green coloured colonies. Differentiation of *L.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 *L.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 (3-7). 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. Some strains of *L.monocytogenes* exposed to stress condition particularly acid stress may show a very weak halo (or even no halo).
2. Further biochemical tests must be carried out to differentiate between *L.monocytogenes* and *L.ivanovii*, sine both shows opaque halo of PIPLC activity.
3. Some organisms other than *Listeria* spp. may also produce blue colonies on this medium, so biochemical characterization is required for differentiation.
4. Further biochemical and serological test are need to 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
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
7.00-7.40
Cultural Response
Productivity : Cultural characteristics observed with added sterile OA Selective Supplement (FD212A) and LP Enrichment Supplement 1 (FD214) after an incubation at 37°±1°C for 48±4 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar). The characteristic reaction are compared with previously approved lot.
Specificity : Cultural characteristics observed with added sterile OA Selective Supplement (FD212A) and LP Enrichment Supplement 1 (FD214) after an incubation at 37°±1°C for 48±4 hours. The characteristic reaction are compared with previously approved lot.
Selectivity : Cultural characteristics observed with added sterile OA Selective Supplement (FD212A) and LP Enrichment Supplement 1 (FD214) after an incubation at 37° ± 1°C for 48 ± 4 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	PIPLC activity
Productivity					
Listeria monocytogenes ATCC 13932 (00021*)	50-100	luxuriant	>=50%	Blue-green	positive,opaque halo around the colony exhibiting phophatidylinositol specific phospholipase activity

Listeria monocytogenes ATCC 35152 (00109*)	50-100	luxuriant	>=50%	Blue-green	positive,opaque halo around the colony exhibiting phophatidylinositol specific phospholipase activity
Specificity					
Listeria innocua ATCC 33090 (00017*)	10^3-10^4	luxuriant		Blue-green	negative
Selectivity					
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited			
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited			
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited			
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited			

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 (8,9).

Reference

1.Ottaviani F., Ottaviani M., and Agosti M. (1997 a), Industrie Alimentari 36, 1-3.
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4.Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 2 , Enumeration method ; ISO 11290-2:2017.
5.Microbiology of food, animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014 (E) /Amd.2020.
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7.BAM Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods, 2022.
8.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9.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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