HIMEDIA

Technical Data

Listeria Identification Agar Base (PALCAM) M1064I

Intended use

Recommended for the detection and enumeration of *Listeria monocytogenes* from food and animal feeds. The composition and performance criteria of this medium are as per the specifications laid down in ISO 11290-2:1998.

Composition**

ISO 11290-2:1998 Specification-Listeria Identification Agar Base (PALCAM)

Ingredients	g / L
Peptones	23.000
Starch	1.000
Sodium chloride	5.000
Yeast extract	3.000
D-Mannitol	10.000
D-glucose	0.500
Aesculin	0.800
Ammonium iron(III) citrate	0.500
Lithium chloride	15.000
Phenol red	0.080
Agar	9.000-18.000
Final pH (at 25°C)	7.2±0.2

M1064I-Listeria Identification Agar Base (PALCAM)

Ingredients	g / L
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Yeast extract	3.000
D-Mannitol	10.000
Dextrose, anhydrous	0.500
Esculin	0.800
Ferric ammonium citrate	0.500
Lithium chloride	15.000
Phenol red	0.080
Agar	10.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Supplements to be added after autoclaving

Ingredients	Concentration
Polymyxin B sulphate	0.1g
Sodium Ceftazidime pentahydrate	0.116g
Acriflavine hydrochloride	0.05g

FD061-PALCAM Selective Supplement **1 Vial**

Ingredients	Concentration
Polymyxin B sulphate	5000IU
Ceftazidime	10mg
Acriflavine hydrochloride	2.500mg

Directions

Suspend 34.44 grams in 500 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 and aseptically add rehydrated contents of 1 vial of PALCAM Selective Supplement (FD061). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Listeria Identification Agar also known as Polymyxin Acriflavine Lithium Chloride Ceftazidime Esculin Mannitol (PALCAM) Agar was originally formulated by Van Netten et al (1). ISO Committee has recommended this medium with a slight modification for the detection and enumeration of *Listeria monocytogenes* from food and animal feeds (2). Peptone special and yeast extract provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. This is highly selective medium due to the presence of lithium chloride, acriflavine hydrochloride, ceftazidime and polymyxin B. This medium employs two indicator systems, esculin-ferric ammonium citrate and mannitol-phenol red. *Listeria monocytogenes* hydrolyzes esculin to esculetin and dextrose. Esculetin reacts with ferric ammonium citrate to form a brown-black complex seen as a black halo around colonies. Dextrose and starch serve as energy source. Contaminants such as Staphylococci ferment mannitol and is indicated by colour change from red to yellow rendering easy differentiation. Incubation in microaerophilic condition inhibits strict aerobes such as *Bacillus* and *Pseudomonas* species.

Depending upon the sample type, selective enrichment is done prior to inoculation onto PALCAM Agar. Generally Listeria Selective Enrichment Medium is used for dairy products while Listeria Selective Enrichment Medium UVM (M890), Fraser Secondary Enrichment Broth (M1083) are used for meat and poultry products. After 24 hours incubation *Listeria* species show grey-greenish or olive coloured, 1.5-2 mm diameter colonies with black halo, usually with black center. After 48 hours grey-green coloured, about 2 mm diameter colonies are observed with black halo and black sunken center.

Type of specimen

Food and dairy samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,3). 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. Further biochemical identification of organisms is required 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 1.3% Agar gel.

Colour and Clarity of prepared Medium

Red coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 6.9% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed under microaerophilic condition, with added PALCAM Selective Supplement (FD061), after an incubation at 35-37°C for 48 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics
*Enterococcus faecalis ATCC 29212 (00087)**	50-100	none - poor	<=10%	grey colonies with a brown-green halo
*Enterococcus faecalis ATCC 19433 (00009)**	50-100	none - poor	<=10%	grey colonies with a brown-green halo
*Listeria monocytogenes ATCC 19111 (00020)**	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19117	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19118	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
Staphylococcus aureus subsp. *aureus ATCC 25923 (00034)**	50-100	none - poor	<=10%	yellow colonies with yellow halo

Listeria monocytogenes ATCC 19112	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
*Listeria monocytogenes ATCC 13932 (00021)**	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
*Listeria monocytogenes ATCC 35152 (00109)**	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
*Listeria innocua ATCC 33090 (00017)**	50-100	good-luxuriant	>=50%	grey-green with black center and a black halo
*Eschrichia coli ATCC 25922 (00013)**	50-100	none - poor	<=10%	
*Escherichia coli ATCC 8739 (00012)**	50-100	none - poor	<=10%	

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°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 (4,5).

Reference

1.Van Netten P. et al, 1989, Int. J. Food Microbiol., 8(4):299.

2. International Organisation for Standardization (ISO), ISO/DIS11290-2 : 2017. Microbiology of food and other animal feeding stuffs- Horizontal method for the detection and enumeration of *L. monocytogenes* and other *Listeria* species.

3.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.

4.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

5.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.

Revision : 04/ 2024