Technical Information

Listeria Identification Agar Base (PALCAM)

Product Code: DM 2064

Application: - Listeria Identification Agar Base (PALCAM) with added supplement is used for selective isolation and identification of *Listeria* species from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	23.000
Starch	1.000
Sodium chloride	5.000
Mannitol	10.000
Ammonium ferric citrate	0.500
Esculin	0.800
Dextrose (Glucose)	0.500
Lithium chloride	15.000
Phenol red	0.080
Agar	13.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The genus *Listeria* constitutes *Listeria monocytogenes, Listeria ivanovii, Listeria seeligeri, Listeria welshimerii, Listeria innocua, Listeria grayi, Listeria murrayi* and *Listeria denitrificans*. Among these, *L. monocytogenes* and *L. ivanovii* are associated with diseases in humans. The pathogenicity of *L. ivanovii* is uncertain. *L. monocytogenes* is found in a wide variety of habitats, including the normal microflora of healthy ruminants, gastrointestinal tract of asymptomatic humans and environmental sources including river water, sewage, soil, silage, fertilizers and decaying vegetation (4).

Listeria Identification Agar also known as Polymyxin-Acriflavin-Lithium chloride-Ceftazidime-Aesculin-Mannitol (PALCAM) Agar was formulated by Van Netten et al (1) and is recommended for the isolation of *L. monocytogenes* from foods. PALCAM medium is highly selective due to the presence of lithium chloride, ceftazidime, polymyxin B and acriflavin hydrochloride. PALCAM medium is a differential diagnostic medium utilizing two indicator systems, as esculin and ferric citrate and mannitol and phenol red.

Peptone acts as carbon, nitogen substances, long chain amino acids, vitamns and essential growth nutrients for the organisms. Dextrose (Glucose), starch and mannitol are the carbohydrate and energy sources. Sodium chloride helps to maintain the osmotic equilibrium of the medium. Phenol red acts as pH indicator dye that exhibits changes in the pH of the medium. *L. monocytogenes* hydrolyzes esculin to form esculetin and dextrose. Esculetin reacts with ammonium ferric citrate and forms a brown-black complex seen as a black halo around colonies. *L. monocytogenes* does not ferment mannitol but contaminants such as Enterococci and Staphylococci ferment mannitol and is indicated by colour change from red to yellow. Under microaerophilic conditions, strict aerobes such as *Bacillus* species and *Pseudomonas* species are inhibited. The addition of egg yolk (2.5% v/v) to PALCAM Agar has been reported to aid repair of damaged cells (2). Medium containing blood when overlaid on PALCAM Agar enables to differentiate and enumerate haemolytic

Depending upon the type of sample used, selective enrichment broth should be used prior to inoculation onto PALCAM Agar. Generally Listeria Selective Enrichment Medium is used for dairy products and Listeria Selective Enrichment Medium UVM (DM2890A), Fraser Secondary Enrichment Broth (DM2083) are used for meats and poultry. On PALCAM Agar, colonies of *Listeria* appear as grey-green with a black precipitate, following inoculation and incubation at 35°C for 24-48 hours under aerobic or microaerophillic conditions.

Type of specimen
Food samples; Water samples

Specimen Collection and Handling:
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
For water 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

Limitations :
1. The medium is not differential, so further biochemical testing is reuired for identification between *Listeria* species.

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.

Methodology

Suspend 34.44 grams of dehydrated powder media in 500 ml distilled water. Mix thoroughly & heat to boil 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 Listeria Selective Supplement (PALCAM) (MS2061). Shake well before pour into sterile Petri plates.

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.3% Agar gel.

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

Reaction
Reaction of 6.9% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH Range
6.80-7.20

Cultural Response
DM2064: Cultural characteristics observed under microaerophilic condition, with added Listeria Selective Supplement (MS2061), after an incubation at 35-37°C for 24-48 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
Listeria monocytogenes ATCC 19111 (00020)*	50-100	luxuriant	>=50%	grey-green with black center and a black halo

Listeria monocytogenes ATCC 19112	50-100	luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19117	50-100	luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19118	50-100	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

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life

Dried Media: 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 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).

Further Reading

1. Van Netten P., Peralse I, Van de Mosdik A., Curtis G.D.W., Mossel D. A.A., 1989, Int. J. Food Microbiol., 8(4):299.
2. int Veld P.H. and de Boer E., 1991, Int. J. Food Microbiol., 13:295.
3. Van Netten P., van Gaal B. and Mossel D. A. A., 1991, Lett. Appl.Microbiol, 12:20.
4. Watkin J., Sleath K. P., J. Appl. Bacteriol., 50: 1-9, 1981.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
8. 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.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate
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