Listeria Identification Agar Base (PALCAM)

Intended Use

Listeria Identification Agar Base (PALCAM) with added supplement is recommended for selective isolation and identification of *Listeria* species.

Summary

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. Listeria Identification Agar also known as Polymyxin Acriflavin Lithium-chloride Ceftazidime Aesculin Mannitol (PALCAM) Agar was formulated by Van Netten *et al.*, 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.

Principle

Peptic digest of animal tissue serves as the main source of nutrients for the organisms. Dextrose, starch and mannitol are the carbohydrate and energy sources. Sodium chloride maintains the osmotic equilibrium of the medium. Phenol red is the 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. Medium containing blood when overlaid on PALCAM Agar enables to differentiate and enumerate haemolytic *Listeria* species.

Formula*

Ingredients	g/L
Peptone	23.0
Lithium Chloride	15.0
Mannitol	10.0
Sodium Chloride	5.0
Starch	1.0
Phenol Red	0.08
Esculin	0.80
Ammonium Ferric Citrate	0.50
Dextrose	0.50
Agar	13.0
Final pH (at 25°C)	7.0 ± 0.2
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*Adjusted to suit performance parameters.

Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen

Food and Dairy samples

Specimen Collection and Handling

Ensure that all samples are properly labelled.

Follow appropriate techniques for handling samples as per established guidelines.

Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.

The samples must be stored and tested within the permissible time duration.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

- 1. Suspend 68.88 g of the powder in 1000 mL purified / distilled water.
- 2. Mix thoroughly.
- 3. Boil with frequent agitation to dissolve the powder completely.
- 4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
- 5. Cool to 45°C-50°C and aseptically add rehydrated contents of 2 vials of Listeria Selective Supplement (PALCAM) (204120550005).
- 6. Mix well and pour into sterile petridishes.

Quality Control

Dehydrated Appearance: Light pink coloured coloured, homogenous, free flowing powder.

Prepared Appearance: Red coloured, slightly opalescent gel forms in petridishes.

Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 24 to 48 hours in microaerophilic condition.

Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating \leq 100 cfu of appropriate microorganism at 30-35°C for 24 hours in microaerophilic condition.

Indicative Properties: The test results observed are within the specified temperature and time, inoculating \leq 100 cfu of appropriate microorganism.

Organism (ATCC)	Growth	Colour of Colony
Listeria monocytogenes strain	Good	Grey-green with black centre and black halo
Li 23 (19114)		
Listeria monocytogenes serotype	Good	Grey-green with black centre and black halo
4b (19115)		
Staphylococcus aureus subsp.	Partial Inhibition	Yellow colonies with yellow halo
aureus (25923)		
Enterococcus faecalis (29212)	Partial Inhibition	Grey colonies with a brown-green halo
		orey colonics with a brown green halo

Interpretation of Results

Typical *Listeria* species form colonies that are approximately 2mm in diameter, green-grey in colour with black sunken centre and a black halo against a red background.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

- 1. Directions
- 2. Storage
- 3. Expiry

Precautions / Limitations

- 1. Acriflavin hydrochloride in the Listeria Selective Supplement should be protected from light because photooxidation causes it to become inhibitory to *Listeria* growth.
- 2. Occasionally, *Enterococcus* or *Staphylococcus* strains develop on Listeria Identification Agar Base, PALCAM to form grey colonies with a brown green halo or yellow colonies with a yellow halo.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

- 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. Data on file: Microxpress[®], A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201120220100	Dehydrated Culture Media	100 g
201120220500	Dehydrated Culture Media	500 g

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.