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Listeria Identification Agar Base (PALCAM) (DM932)

Intended Use

Listeria Identification Agar Base (PALCAM) (DM932) is recommended for selective isolation and identification of *Listeria* species.

Product Summary and Explanation

Listeria genus 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.[1]

Listeria Identification Agar also known as Polymyxin-Acriflavin-Lithium chloride-Ceftazidime-Aesculin-Mannitol (PALCAM) Agar was formulated by Van Netten et al[2] and is recommended for the isolation of *L. monocytogenes* from food samples. PALCAM medium is widely recommended for use in the detection of *L.monocytogenes* in foods,[3-8] milk and milk products,[9] and environmental samples.[5] 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.

Principles of the Procedure

Listeria Identification Agar Base (PALCAM) contains 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.

Selectivity of the complete medium is achieved through the presence of lithium chloride, polymyxin B sulfate and acriflavine HCl, present in PALCAM Medium Base, and ceftazidime, provided by PALCAM Antimicrobic Supplement. These agents effectively suppress growth of most commonly occurring non-*Listeria* spp. of bacteria present in foods. Differentiation on PALCAM Medium is based on esculin hydrolysis and mannitol ermentation. All *Listeria* spp. Hydrolyze esculin as evidenced by a blackening of the medium. This blackening by esculin-hydrolyzing bacteria results from the formation of 6,7-dihydroxycoumarin, which reacts with ferric ions that are present in the medium as ferric ammonium citrate. On occasion, organisms other than *Listeria*, such as staphylococci or enterococci, may grow on this medium. Mannitol and the pH indicator, phenol red, have been added to differentiate mannitol-fermenting strains of these species from *Listeria* based on mannitol fermentation. Mannitol fermentation is demonstrated by a color change in the colony and/or the surrounding medium from red or gray to yellow due to the production of acidic end products.

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 / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	23.00
Starch	1.00
Sodium chloride	5.00
Mannitol	10.00
Ammonium ferric citrate	0.50
Esculin	0.80
Dextrose	0.50
Lithium chloride	15.00
Phenol red	0.08
Agar	13.00
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Directions

1. Suspend 34.44 grams of the medium in 500 ml of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to around 50°C and aseptically add rehydrated contents of 1 vial of Listeria Selective Supplement (PALCAM) (MS070).
5. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Red coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 6.9% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.3% Agar gel

Expected Cultural Response: Cultural characteristics observed under microaerophilic condition, with added Listeria Selective Supplement (MS070), after an incubation at 35-37°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colony Characteristics
1.	*Enterococcus faecalis ATCC 29212*	50 -100	none-poor	>=10%	grey colonies with a brown green halo
2.	*Listeria monocytogenes ATCC 19111*	50 -100	good-luxuriant	>=50%	grey-green with black center and a black halo
3.	*Listeria monocytogenes ATCC 19112*	50 -100	good-luxuriant	>=50%	grey-green with black center and a black halo
4.	*Listeria monocytogenes ATCC 19117*	50 -100	good-luxuriant	>=50%	grey-green with black center and a black halo
5.	*Listeria monocytogenes ATCC 19118*	50 -100	good-luxuriant	>=50%	grey-green with black center and a black halo
6.	*Staphylococcus aureusATCC 25923*	50 -100	none-poor	>=10%	yellow colonies with yellow halo

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Refer to appropriate references and follow applicable standard methods.
2. Inoculate incubated enrichment broth or screened food sample particle onto PALCAM Medium and streak for isolation.
3. Depending upon the type of sample used, selective enrichment broth should be used prior to inoculation onto PALCAM Agar.
4. Generally Listeria Selective Enrichment Medium is used for dairy products and Listeria Selective Enrichment Medium UVM (DM521), Fraser Secondary Enrichment Broth (DM1293) are used for meats and poultry.
5. Incubate plates at 35°C for 24-48 hours under aerobic or microaerophilic conditions in an inverted position (agar side up).

Results

1. *Listeria* colonies appear gray-green with a black centre and black halo is observed when *L. monocytogenes* hydrolyzes esculin to form esculetin, which reacts with ammonium ferric citrate to form a brown-black complex seen as a black halo around colonies.
2. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/ serological identification.
3. Colonies of mannitol-fermenting organisms such as staphylococci, which may grow on this medium, appear yellow with a yellow halo.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Listeria Identification Agar Base (PALCAM)
Product Code : DM932
Available Pack sizes : 100 gm/ 500gm

References

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4. Downes and Ito (eds.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. Pagotto, Daley, Farber, and Warburton. 2001. Isolation of *Listeria monocytogenes* from all food and environmental samples. Health Products and Food Branch Ottawa, MFHPB-30. Published on the Food Directorate (Health Canada's) website at <www.hc-sc.gc.ca/food-aliment>.
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7. International Organization for Standardization. 1996. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes*; Part 1: Detection method. ISO 11290-1. International Organization for Standardization, Geneva, Switzerland.
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Further Information

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