

PALCAM Medium Base

PALCAM Antimicrobial Supplement

Intended Use

PALCAM Medium Base is used with PALCAM Antimicrobial Supplement in isolating and cultivating *Listeria*, particularly from foods and milk products.

Summary and Explanation

PALCAM Medium Base and PALCAM Antimicrobial Supplement are based on the PALCAM agar formulation of van Nerren et al.,¹ who developed this selective and differential medium for use in the isolation and enumeration of *Listeria* spp. from food samples. PALCAM medium is widely recommended for use in the detection of *L. monocytogenes* in foods,²⁻⁷ milk and milk products,⁸ and environmental samples.⁴

Principles of the Procedure

Good growth of *Listeria* spp. is obtained by including Columbia Agar Base in PALCAM Medium Base. Columbia Agar Base provides the nutrients and cofactors required for good to excellent growth of *Listeria*. 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 Antimicrobial Supplement. These agents effectively suppress growth of most commonly occurring non-*Listeria* spp. of bacteria present in foods. The ceftazidime concentration is reduced from 20 mg/L to 8 mg/L for improved growth and recovery of *Listeria*.

Differentiation on PALCAM Medium is based on esculin hydrolysis and mannitol fermentation. 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.

User Quality Control

Identity Specifications

Difco™ PALCAM Medium Base

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 6.8% solution, soluble in purified water upon boiling. Solution is dark red, slightly opalescent.

Prepared Appearance: Medium red, very slightly to slightly opalescent with slight precipitate.

Reaction of 6.8%
Solution at 25°C: pH 7.2 ± 0.2

Difco™ PALCAM Antimicrobial Supplement

Lyophilized Appearance: White, free-flowing, homogeneous powder.

Rehydrated Appearance: Colorless solution.

Cultural Response

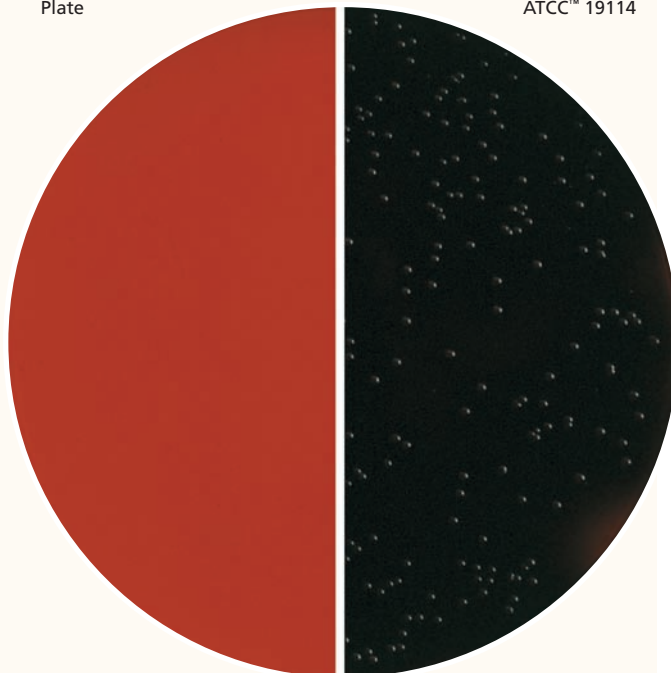
Difco™ PALCAM Medium Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours in a microaerophilic environment.

ORGANISM	ATCC™	INOCULUM CFU		RECOVERY	ESCULIN REACTION
<i>Enterococcus faecalis</i>	29212	10 ³ -2	10 ³	Inhibition	–
<i>Escherichia coli</i>	25922	10 ³ -2	10 ³	Inhibition	–
<i>Listeria monocytogenes</i>	19114	100-300		Good	+
<i>Staphylococcus aureus</i>	25923	10 ³ -2	10 ³	Inhibition	–

Uninoculated
Plate

Listeria monocytogenes
ATCC™ 19114



Formulae

Difco™ PALCAM Medium Base

Approximate Formula* Per Liter

Columbia Blood Agar Base.....	39.0	g
Pancreatic Digest of Casein.....	10.0	g
Proteose Peptone No. 3.....	5.0	g
Yeast Extract.....	5.0	g
Beef Heart, Infusion from 500 g.....	3.0	g
Corn Starch.....	1.0	g
Sodium Chloride.....	5.0	g
Agar.....	15.0	g
Mannitol.....	10.0	g
Dextrose.....	0.5	g
Esculin.....	1.0	g
Ferric Ammonium Citrate.....	0.5	g
Lithium Chloride.....	15.0	g
Phenol Red.....	0.08	g
Acridine HCl.....	5.0	mg
Polymyxin B Sulfate.....	0.01	g
Agar.....	2.0	g

Difco™ PALCAM Antimicrobial Supplement

Formula Per 10 mL Vial

Ceftazidime.....	40.0	mg
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*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

Difco™ PALCAM Medium Base

1. Suspend 68 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 2 mL rehydrated PALCAM Antimicrobial Supplement. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.
6. Store the prepared medium at 2-8°C.

Difco™ PALCAM Antimicrobial Supplement

1. Aseptically add 10 mL sterile purified water to the vial.
2. Shake to dissolve the contents.
3. Upon rehydration, Difco PALCAM Antimicrobial Supplement is stable for 1 month when stored at 2-8°C.

Sample Collection and Handling

Follow appropriate standard methods for details on sample preparation and processing according to sample type and geographic location.²⁻⁸

Procedure

Consult appropriate references²⁻⁸ and follow applicable standard methods. Inoculate incubated enrichment broth or screened food sample particle onto PALCAM Medium and streak for isolation. Incubate plates at 35°C for 24-48 hours under aerobic or microaerophilic conditions in an inverted position (agar side up).

Expected Results

On PALCAM Medium, colonies of *Listeria* appear gray-green with a black precipitate. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/serological identification.²⁻⁸ Colonies of mannitol-fermenting organisms such as staphylococci, which may grow on this medium, appear yellow with a yellow halo.

References

1. Van Netten, Perales, Van de Moosalijs, Curtis, and Mossel. 1989. Int. J. Food Microbiol. 8:299.
2. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. Chapter 10: Detection and enumeration of *Listeria monocytogenes* in foods (January 2003). AOAC International, Gaithersburg, Md.
3. Downes and Ito (eds.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. 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>.
5. Pagotto, Daley and Farber. 2002. Enumeration of *Listeria monocytogenes* in foods. Health Products and Food Branch Ottawa, MFLP-74. Published on the Food Directorate (Health Canada's) website at <www.hc-sc.gc.ca/food-aliment>.
6. 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.
7. International Organization for Standardization. 2004. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes*; Part 1: Detection method. Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data. ISO 11290-1, Amendment 1. International Organization for Standardization, Geneva, Switzerland.
8. Henning, Flowers, Reiser, and Ryser. 2004. Pathogens in milk and milk products. In Wehr and Frank (eds.), Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ PALCAM Medium Base

BAM CCAM COMPF ISO SMD

Cat. No.	263620	Dehydrated – 500 g
	263610	Dehydrated – 2 kg

Difco™ PALCAM Antimicrobial Supplement

BAM CCAM COMPF ISO SMD

Cat. No.	263710	Vial – 3	10 mL*
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Europe

Cat. No.	254539	Prepared Plates (complete) – Pkg. of 20*
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*Store at 2-8°C.