PALCAM Medium Base PALCAM Antimicrobic Supplement

Intended Use

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

Summary and Explanation

PALCAM Medium Base and PALCAM Antimicrobic 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 Antimicrobic 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 Specifica	tions					
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:	рН 7.2 ± 0.2					
Difco™ PALCAM Antimicrobic 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 $35 \pm 2^{\circ}$ C for 48 hours in a microaerophilic environment.

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





Formulae

Difco[™] PALCAM Medium Base

Columbia Blood Agar Base	39.0	g
Pancreatic Digest of Casein	g	5
Proteose Peptone No. 3	g	
Yeast Extract 5.0	g	
Beef Heart, Infusion from 500 g	g	
Corn Starch 1.0	g	
Sodium Chloride5.0	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
Acriflavine HCI	5.0 r	ng
Polymyxin B Sulfate	0.01	g
Agar		α

Difco[™] PALCAM Antimicrobic Supplement

Formula Per 10 mL Vial

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 Antimicrobic 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 Antimicrobic Supplement

- 1. Aseptically add 10 mL sterile purified water to the vial.
- 2. Shake to dissolve the contents.
- 3. Upon rehydration, Difco PALCAM Antimicrobic 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 Moosalijk, Curtis, and Mossel. 1989. Int. J. Food Microbiol. 8:299.
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Availability

Difco[™] PALCAM Medium Base

BAM CCAM COMPF ISO SMD

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

Difco[™] PALCAM Antimicrobic Supplement

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BAM CCAM COMPF ISO SMD
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Cat. No. 263710 Vial – 3 10 mL*

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

