# PALCAM Selective Supplement

## PRODUCT INFORMATION

P007-1MU - Polymyxin B Sulfate, Powder, 1MU

P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

C059-1g - Ceftazidime Pentahydrate, Powder, 1g

C059-5g - Ceftazidime Pentahydrate, Powder, 5g

## DESCRIPTION

PALCAM Agar Base with PALCAM Selective Supplement is a selective and differential diagnostic medium for the detection of *Listeria monocytogenes*.

## BACKGROUND

Polymyxin B is an antibiotic primarily used for resistant gram-negative infections. It is derived from the bacterium Bacillus polymyxa. Polymyxin B is a mixture of two closely related compounds, polymyxin B1 and polymyxin B2. It has a bactericidal action against almost all gram-negative bacilli except the Proteus group.

Ceftazidime is a third-generation cephalosporin antibiotic. Like other third-generation cephalosporins, it has broad spectrum activity against Gram-positive and Gram-negative bacteria. Unlike most third-generation agents, it is active against *Pseudomonas aeruginosa*.

### Mechanism of action

Polymyxins bind to the cell membrane and alter its structure, making it more permeable. The resulting water uptake leads to cell death.

## APPLICATION IN PALCAM AGAR BASE

PALCAM Medium is based on the formulation described by Van Netten et al. and is recommended for the isolation of *Listeria monocytogenes* from foods.

The heightened awareness and concern surrounding the presence of *Listeria monocytogenes* in food has resulted in the development of many media for its isolation. However, Cassiday and Brackett6 conclude that no single method currently available at the time was suitable for use with all types of food.

PALCAM Medium is highly selective due to the presence of lithium chloride, ceftazidime, polymyxin B and acriflavine hydrochloride. It allows the easy differential diagnosis of *Listeria monocytogenes* by utilising the double indicator system. 1. aesculin and ferrous iron. 2. mannitol and phenol red. *Listeria monocytogenes* hydrolyses aesculin resulting in the formation of a black halo around colonies. *Listeria monocytogenes* does not ferment mannitol so easy differentiation from contaminants, such as enterococci and staphylococci, can be made as these will ferment mannitol. This formulation produces a change from red to yellow in the pH indicator phenol red. The addition of egg yolk to PALCAM medium has been reported to aid repair of damaged cells.

Incubation under microaerophilic conditions serves to inhibit strict aerobes such as *Bacillus* spp. and *Pseudomonas* spp. that might otherwise appear on the medium. A modification to PALCAM medium in which incubated plates are overlaid with medium containing blood enables haemolytic *Listeria* spp. to be differentiated and enumerated. Incubation under microaerobic conditions serves to inhibit strict aerobes such as *Bacillus* spp. and *Pseudomonas* spp. that might otherwise appear on the medium.

### Content concentrations

| Typical Formula* | mg/litre |
|---|---|
| **PALCAM Agar Base** | |
| Columbia Blood Agar Base | 39 |
| Yeast extract | 3 |
| Glucose | 0.5 |
| Aesculin | 0.8 |
| Ferric ammonium citrate | 0.5 |
| Mannitol | 10 |
| Phenol red | 0.08 |
| Lithium chloride | 15 |
| Final pH 7.2 ± 0.2 @ 25°C | |
| **PALCAM Selective Supplement** | |
| Polymyxin B | 10 |
| Acriflavine hydrochloride | 5 |
| Ceftazidime | 20 |
| * Adjusted as required to meet performance standards | |

**Table 1 - Typical Formula for PALCAM Agar Base and PALCAM Selective Supplement**

# METHOD

## Preparation

Suspend appropriate amount of PALCAM Agar Base in distilled water. Bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, and aseptically add PALCAM Selective Supplement, reconstitutes as directed. Mix well and pour into sterile Petri dishes.

The addition of 2.5% (v/v) Egg Yolk Emulsion to the medium may aid the recovery of damaged Listeria.

## Protocol

Protocols for the isolation of *Listeria monocytogenes* will depend on the material under test. It is usual for the test sample to be first inoculated into an enrichment broth to allow multiplication before isolation and identification. Depending on the type of sample used, the appropriate method and selective enrichment broth should be used prior to inoculation onto PALCAM Medium plates. As a general rule, use Listeria Selective Enrichment Medium for dairy products, and Listeria Selective Enrichment Media UVM and Fraser Broth for meats and poultry.

1. Inoculate one loopful of the selective enrichment broth onto the PALCAM Medium plates.

2. Incubate at 37°C for 48 hours under micro-aerophilic conditions.

3.Examine for typical colonies of *Listeria* after 48 hours incubation.

4.Colonies identified as presumptive *Listeria* spp. must be confirmed by biochemical and serological testing.

After 48 hours incubation, typical *Listeria* spp. form colonies that are approximately 2 mm in diameter, grey-green in colour with a black sunken centre and a black halo against a cherry-red medium background. Occasional *enterococci* or *staphylococci* develop on PALCAM Medium to forming grey colonies with a brown-green halo or yellow colonies with a yellow halo.

## Quality control

Positive control:

*Listeria monocytogenes* ATCC® 7644: Good growth; dimpled brown/black coloured colonies with black halo

Negative control:

*Enterococcus faecalis* ATCC® 29212: Inhibited

# REFERENCES

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**3. in't Veld P.H. and de Boer E. (1991) Int. J. Food Microbiol. 13. 295-300.**

**4. Gunasinghe C.P.G.L. Henderson C and Rutter M.A. (1994) Lett. Appl. Microbiol. 18. 156-158.**

**5. Lund A.M., Zottola E.A. and Pusch D.J. (1991) J. Food Prot. 54. 602-606.**

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**7. van Netten P., van Gaal B. and Mossel D.A.A. (1991) Lett. Appl. Microbiol. 12. 20-22.**

**8. Bille J. and Doyle M.P. (1991) ``Listeria and Erysipelothrix" 287-295 in Balows A., Hausler W.J. Jnr., Herrman K.L. Isenberg H.D. and Shadomy H.J. (Eds) Manual of Clinical Microbiology, 5th Edition, American Society for Microbiology, Washington D.C.**