

Palcam/Agar

356-3674
356-4752
356-4754

DEFINITION

Selective medium for the isolation of *Listeria* spp. in food samples.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 11290-1/A1 (February 2005):** Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Detection method.
- **Standard FIL 143A (1995):** Milk and dairy products - Detection of *Listeria monocytogenes*.
- **Method for the enumeration of *Listeria monocytogenes* in vegetable products and products of vegetable origin** (BOCCRF of 30 May 1992, amended 5 September 1992).

PRINCIPLE

The Palcam medium is used as a selective isolation medium for the detection of *Listeria* spp. in food products. Due to the combined action of lithium chloride and the selective supplement, this medium inhibits non-*Listeria* flora. On this medium, at 37°C, in 24 hours *Listeria* spp. form shiny gray or gray-green colonies of about 1 mm diameter, surrounded by a brown-black halo (esculin hydrolysis). After 48 hours incubation, typical colonies with a diameter of approximately 2 mm are encrusted in the agar and present a central depression.

Palcam medium also contains a mixture of mannitol and Phenol red which makes it possible to distinguish between colonies formed by *Listeria* spp. and colonies formed by noninhibited esculin-positive *Enterococcus*. Unlike *Listeria* (other than *L. Grayi* and *L. Murrayi*), *Enterococcus* metabolizes mannitol. The acidification resulting from this metabolization causes the pH indicator to change (yellow haloes around the colonies).

PRESENTATION

- **Pre-poured**
20 dishes x 90 mm **code 356-3674**
- **Dehydrated**
500g **code 356-4754**
- **PALCAM supplement**

Freeze-dried **code 356-4752**
(Packs of 10 bottles q.s.p. 500 ml of base agar)

STORAGE

- Pre-poured: +2°C to 8°C in a dark place.
- Dehydrated: +15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Freeze-dried selective supplement: +2°C to 8°C in a dark place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA (COMPLETE MEDIUM)

Base medium

| | |
|-------------------------------|----------|
| Peptone | 23 g |
| Starch | 1 g |
| Sodium chloride | 5 g |
| Yeast extract | 3 g |
| D-Mannitol | 10 g |
| Ferric ammonium citrate (III) | 500 mg |
| Esculin | 800 mg |
| D-Glucose | 500 mg |
| Lithium chloride | 15 g |
| Phenol red | 80 mg |
| Agar | 12 g |
| Distilled water | 1,000 ml |
| Final pH (25°C) = 7.2 ± 0.2 | |

Selective supplement (per bottle)

| | |
|---------------------|-----------|
| Polymyxin B sulfate | 50.000 UI |
| Ceftazidime | 10 mg |
| Acriflavine HCl | 2.5 mg |

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water
- Sterile distilled water for the preparation of the selective supplement.

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Vortex-type shaker
- Sterile Petri dishes (Ø = 90 mm)
- Sterile disposable platinum loop or inoculating loop
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment.

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PREPARATION OF DEHYDRATED MEDIUM

• Dehydrated.

Always shake before use

Dissolve 70.9 g of powder in 1 liter of distilled water, and mix until a homogenous suspension is obtained.

Heat gently, swirling frequently, then bring to the boil until completely dissolved; Dispense in 100 ml bottles. This medium must be sterilized in an autoclave at 121°C for 15 minutes. Cool to 44°C - 47°C.

Reconstitution ratio: 70.9 g/l.

500 g of powder makes 7 liters of base PALCAM.

• Freeze-dried selective supplement

Under aseptic conditions, reconstitute a bottle of PALCAM selective supplement in 5 ml of sterile water.

• Complete medium

Add 1 ml of reconstituted supplement to 100 ml of autoclaved base agar cooled to 44°C - 47°C. Mix well. Dispense in Petri dishes (90 mm).

PROTOCOL

• Preparation of samples and enrichment phase

To be carried out in compliance with the standards applicable to the product concerned.

• Inoculation and incubation

Using a sterile inoculating loop, collect a drop of enrichment broth at the end of incubation and isolate on the surface of the PALCAM agar. Incubate at 37°C ± 1°C for 18-24 hours, and if necessary prolong incubation to 48 hours.

READING AND INTERPRETATION

After incubation, *Listeria* spp. form shiny gray or gray-green colonies surrounded by a brown-black halo, of about 1 mm diameter (24 hours incubation) or of 2 mm diameter (48 hours incubation).

To verify membership of the genus *Listeria* and determine the species, select 5 typical colonies (if there are fewer, select them all) and proceed with the identification tests summarized in Tables I and II following:

| | GRAM | CATALASE | MOBILITY |
|--------------------------|------|----------|----------|
| GENUS <i>Listeria</i> | + | + | + |

Table I: Test of membership of the genus *Listeria*.

| STRAINS | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-------------------------|---|---|---|---|---|---|---|
| <i>L. monocytogenes</i> | + | - | + | - | + | - | - |
| <i>L. innocua</i> | - | - | - | - | V | - | - |
| <i>L. ivanovii</i> | + | + | - | + | - | - | - |
| <i>L. welshimeri</i> | - | - | - | + | V | - | - |
| <i>L. seeligeri</i> | + | - | + | + | - | - | - |
| <i>L. grayi</i> | - | - | - | - | - | + | - |
| <i>L. murrayi</i> | - | - | - | - | - | + | + |

Table II: Identification of *Listeria* species.

Legend:

- 1: HEMOLYSIS
- 2: C.A.M.P. TEST *R. equi*
- 3: C.A.M.P. TEST *S. aureus*
- 4: D-XYLOSE
- 5: L-RHAMNOSE
- 6: MANNITOL
- 7: REDUCTION OF NITRATES

V: variable reaction
+: over 90% positive reactions
-: no reaction

PRECAUTIONS

- The selective supplement contains toxic products. It is recommended that protective clothing be worn during its preparation (gloves and safety glasses).
- Comply with Good Laboratory Practice.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with different strains: Please refer you to the Quality control certificate of the product available on the website

KEY WORDS

PALCAM / *Listeria* / Food products / Detection / Medium.

BIBLIOGRAPHY

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