TECHNICAL DATA SHEET

PALCAM AGAR

DETECTION OF *LISTERIA*

1 INTENDED USE

PALCAM Agar is a selective medium used for the differentiation and isolation of *Listeria monocytogenes* and other *Listeria*, from milk and cheese, as well as in other food products, even highly contaminated.

The media can be used as a second media of choice in the context of the directives concerning the detection of *Listeria monocytogenes* in food microbiology (NF EN ISO 11290-1).

PALCAM agar can also be used as a confirmation test for *Listeria* spp using the COMPASS® *Listeria* method.

2 HISTORY

The medium was formulated by van Netten *et al.* in 1989 to overcome the insufficient selectivity of media used in the past for the detection and enumeration of *Listeria.* The studies were based on the work of Rodriguez (1984), who was the first to use esculin and iron salts to visualize *Listeria monocytogenes* by its esculinase-positive character. Many selective media for *Listeria* containing esculin, however, also enable the growth of several group D streptococci so that the use of esculin was only of limited value. Based on the work of Rocourt (1987), van Netten supplemented the medium with D-mannitol in order to differentiate mannitol-positive enterococci from mannitol-negative *Listeria*. Based on these two principles, and using the ecometric evaluation method, the authors showed that the combined action of ceftazidim and lithium chloride was more effective in terms of selectivity than that obtained by using 2-phenylethanol. PALCAM Agar is a combination of ALPAMY medium (van Netten *et al*., 1988 b) and Oxford Medium (Curtis *et al*., 1989). The authors showed that among the 13 selective media tested, PALCAM Agar gave satisfactory results, producing highly typical *Listeria* colonies at the same time as inhibiting almost all other contaminating bacteria.

3 PRINCIPLES

Peptones and yeast extract favor the excellent growth of *Listeria*.
Glucose and starch are the energy sources for microbial development.
Sodium chloride maintains osmotic balance.
Listeria hydrolyze esculin to glucose and esculetin, the latter compound forming a black complex with ferric ions supplied by ferric citrate.
Accompanying microflora are inhibited by lithium chloride, ceftazidim, polymyxin and acriflavin.
The fermentation of mannitol by contaminating bacteria that may grow causes phenol red to turn yellow, thereby orienting the diagnosis.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media:

- Peptones ..... 23,00 g
- Yeast extract..... 3,00 g
- Glucose ..... 0,50 g
- Starch ..... 1,00 g
- D-mannitol ..... 10,00 g
- Esculin. ..... 0,80 g
- Ferric ammonium citrate ..... 0,50 g
- Sodium chloride ..... 5,00 g
- Lithium chloride..... 15,00 g
- Polymyxin B sulfate ..... 10,0 mg
- Ceftazidim..... 20,0 mg
- Acriflavin ..... 5,0 mg
- Phenol red ..... 0,08 g
- Bacteriological agar..... 10,00 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

For 68,9 g od dehydrated base media BK145

- Peptones	23,00 g
- Yeast extract	3,00 g
- Glucose	0,50 g
- Starch	1,00 g
- D-mannitol	10,00 g
- Esculin	0,80 g
- Ferric ammonium citrate	0,50 g
- Sodium chloride	5,00 g
- Lithium chloride	15,00 g
- Phenol red	0,08 g
- Bacteriological agar	10,00 g

For one vial of supplement BS004 Qsp 500 mL

- Polymyxin B (sulfate)	5,0 mg
- Ceftazidim	10,0 mg
- Acriflavin	2,5 mg

For one vial of supplement BS049 Qsp 2,5 L

- Polymyxin B (sulfate)	25,0 mg
- Ceftazidim	50,0 mg
- Acriflavin	12,5 mg

5 PREPARATION

- Dissolve 68,9 g of dehydrated base media (BK145) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense into vials or flasks, at 100 mL per vial.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain in a molten state at 44-47 °C.
- Rehydrate the supplement with:
  - 5 mL sterile distilled water for supplement Qsp 500 mL (BS004)
  - 25 mL sterile distilled water for supplement Qsp 2.5 L (BS049)
- Mix or vortex well to insure complete dissolution, avoiding the formation of foam.
- Aseptically add 1 mL of reconstituted supplement per 100 mL volume of base.
- Mix well.
- Pour into sterile Petri plates.
- Let solidify on a cold, flat surface.
- Dry the plates in an incubator, covers partially removed.

✓ **Reconstitution: 68,9 g/L**

✓ **Sterilization: 15 min at 121°C**

✓ **Supplement rehydration**
5 mL sterile distilled water (BS004)
25 mL sterile distilled water (BS049)

✓ **Add to base :**
1 mL / 100 mL

6 INSTRUCTIONS FOR USE

- Using the plates prepared as above or by using the pre-poured plates (BM020), isolate by streaking on the surface a loop of selective enrichment broth.
- Incubate at 30, 35 or 37 ± 1 °C.
- Examine the plates after 24 hours, and if necessary, after 48 hours in order to detect the presence of characteristic colonies of presumptive *Listeria monocytogenes*.

✓ **Inoculation**
1 loop of enrichment broth

✓ **Incubation**
24 to 48 h at 30, 35 or 37°C

7 LECTURE

After 24 or 48 hours of incubation, *Listeria monocytogenes* forms olive-green colonies with a hollow black center and surrounded by black zones. When the colonies reach confluence, the medium becomes brown-black. PALCAM Agar is highly selective, but it is sometimes possible to observe colonies of *staphylococci* or *enterococci* (which ferment mannitol and produce yellow colonies with a yellow halo, thereby being easily distinguished from *Listeria*). Suspected colonies are subjected to biochemical identification tests.

See ANNEX 1: PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated base media: cream to pinkish colored powder, free-flowing and homogeneous.
Supplement appearance: yellow, giving rise to a limpid, yellow solution after reconstitution.
Prepared (complete) media: red agar.

Typical culture response after 48 hours of incubation at 37 °C (NF EN ISO 11133):

Microorganisms		Growth (Productivity Ratio : P_R)
Listeria monocytogenes	WDCM 00021	$P_R \geq 50\%$, characteristic colonies
Listeria monocytogenes	WDCM 00109	$P_R \geq 50\%$, characteristic colonies
Escherichia coli	WDCM 00013	Inhibited, score 0
Enterococcus faecalis	WDCM 00087	Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated base media: 2-30 °C.
Pre-poured complete media in Petri plates: 2-8 °C.
PALCAM Selective Supplement for agar: 2-8 °C.
The expiration dates are indicated on the labels.

Prepared base media in vials (*): 180 days at 2-8 °C.
Prepared (complete) media in plates, with supplement (*): 30 days at 2-8 °C.
Rehydrated Supplement (*): 30 days at 2-8°C, shielded from light.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media:
500 g bottle..... BK145HA

PALCAM Selective Supplement for agar:
10 vials qsp 500 mL BS00408
8 vials qsp 2,5 L BS04908

Pre-poured (complete) media in Petri plates (Ø 90 mm):
20 plates..... BM02008

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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Updated : 07-2022
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ANNEX 1 : PHOTO SUPPORT

PALCAM Agar

Detection of *Listeria*.

Results :

Growth obtained by surface inoculation after 48 hours of incubation at 37 °C.

***Listeria monocytogenes* & other *Listeria* spp.**

Characteristic colony : Green-olive color with a typical, concave center, surrounded by a black halo.

Confirmation of *Listeria* spp or *L. monocytogenes* : Growth obtained by inoculation by picking colonies after 24 hours of incubation at 37 °C.

***Listeria monocytogenes* & other *Listeria* spp.**

Presence of a black halo