**PALCAM AGAR**

**INTENDED USE**

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

PALCAM Agar is also used in the context of the rapid alternative method for the detection of species belonging to the genus *Listeria* (*Listeria spp*) (COMPASS® *Listeria Agar*), in human food products and environmental samples. It is officially AFNOR-certified NF VALIDATION, under the reference number BKR 23/02 – 11/02, of which the validity runs until November 28, 2014.

**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.

**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.
PREPARATION

- Suspend 68.9 g of dehydrated base medium (BK145) in 1 liter of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense 100 mL in flasks.
- Sterilize in an autoclave at 121°C for 15 minutes.

NOTE:
Incomplete agar melting during preparation will invariably lead to significant inconsistency in the gel strength of the solidified agar, after sterilization and cooling.

INSTRUCTIONS FOR USE

- Melt the medium (if it was prepared in advance).
- Cool and maintain the medium at 44-47°C.
- Per 100 mL of base, aseptically add 1 mL of reconstituted PALCAM Selective Supplement (BS004 or BS049).
- Mix well.
- Pour into sterile Petri dishes.
- Let solidify on a cold surface.
- Dry in an incubator with the covers partially removed.
- For enumeration, transfer 0.1 mL of the sample to analyze (or of a selective enrichment broth) and its serial tenfold dilutions to the plates prepared as above or to ready-to-use plates (BM020) that have been brought to room temperature. Spread the inoculum on the surface of the agar with a sterile triangle.
- For detection, isolate individual colonies by inoculating a loop of selective enrichment broth.
- Incubate at 37°C for 24 and 48 hours.

When using COMPASS® Listeria Agar in the AFNOR-certified procedure, take an isolated characteristic colony on the surface of COMPASS® Listeria Agar (blue-green colonies with or without an opaque halo) and re-isolate by stabbing onto the surface of PALCAM Agar (up to 15 stabbings per agar plate). Incubate at (37 ± 1)°C for (24 ± 3) hours. The presence of a characteristic colony confirms the affiliation to the genus Listeria.

RESULTS

After 24 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.
TYPICAL COMPOSITION of the complete medium
(can be adjusted to obtain optimal performance)

For 1 liter of medium:
- 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) ................................................................. 10.00 mg
- Ceftazidime .............................................................................. 20.00 mg
- Acriflavin .................................................................................. 5.00 mg
- Phenol red .................................................................................. 0.08 g
- Bacteriological agar ................................................................. 10.00 g

pH of the ready-to-use medium at 25°C : 7.2 ± 0.2.

QUALITY CONTROL
- Dehydrated medium : pinkish powder, free-flowing and homogeneous.
- Prepared (complete) media : red agar.
- Typical culture response after 48 hours of incubation at 37°C :

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Growth (Productivity ratio: ( P_R ))</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria monocytogenes CIP 59.53</td>
<td>( P_R \geq 50% )</td>
<td>olive-green colonies surrounded by a black halo</td>
</tr>
<tr>
<td>Listeria monocytogenes CIP 78.31</td>
<td>( P_R \geq 50% )</td>
<td>olive-green colonies surrounded by a black halo</td>
</tr>
<tr>
<td>Escherichia coli ATCC® 25922</td>
<td>inhibited, score 0</td>
<td>olive-green colonies surrounded by a black halo</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212</td>
<td>inhibited, score 0</td>
<td></td>
</tr>
</tbody>
</table>

STORAGE / SHELF LIFE

Dehydrated medium : 2-30°C.
- The expiration date is indicated on the label.

Prepared medium (benchmark value*) :
- Base media in vials : 6 months at 2-8°C.
- Complete media in plates : 1 month at 2-8°C, shielded from light.

Pre-poured (complete) media in plates, PALCAM Selective Supplement :
- Store between 2-8°C, shielded from light.
- The expiration dates are indicated on the labels.
PHOTO SUPPORT

Product reference : [BK145HA, GC + BS00408 or BS04908], BM02008

Media used for : Selective isolation and enumeration of *Listeria monocytogenes* in foods.

*Listeria monocytogenes*

PALCAM agar
Ref : BM02008
Incubation : 48 hours / 37°C
Characteristics : olive-green colonies with distinctive concave center, surrounded by a black halo (indicative of esculin hydrolysis).
PACKAGING

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

Dehydrated base medium (without Polymyxin, Ceftazidime, Acriflavin):
- 500 g bottle BK145HA

PALCAM Selective Supplement (qs. 500 mL):
- 10 vial pack BS00408

PALCAM Selective Supplement (qs. 2.5 liters):
- 8 vial pack BS04908

BIBLIOGRAPHY


