PALCAM AGAR BASE (7669)

Intended Use

Palcam Agar Base is used with supplements as a selective and differential medium for the detection and isolation of *Listeria monocytogenes* from foods and environmental samples.

Product Summary and Explanation

Listeria monocytogenes, described in 1926 by Murray, Webb, and Swann, is a widespread problem in public health and food industries.[1] This organism has the ability to cause human illness and death, particularly in immunocompromised individuals.[2] Evidence from outbreaks of listeriosis indicate the principle route of transmission is via the consumption of foods contaminated with *Listeria monocytogenes*.[3]

PALCAM Agar Base is based on the formulation of Van Netten et al.,[4] while he was investigating the isolation of *Listeria* spp. from food samples. PALCAM Agar Base is recommended by AFNOR for the detection of *Listeria monocytogenes* in foods,[5] Health Canada in food and environmental samples,[6] and the International Dairy Federation in milk and milk products.[7]

Principles of the Procedure

Peptone, Yeast Extract, and Starch provide nitrogen, vitamins, minerals, and cofactors required for growth of *Listeria* spp. Sodium Chloride maintain the osmotic balance of the medium. Dextrose is a carbon source. Differentiation on PALCAM Agar Base is based on Esculin hydrolysis and Mannitol fermentation. *Listeria* spp. hydrolyze esculin, which appears as blackening in the medium. The 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. Mannitol and the pH indicator, Phenol Red, have been added to differentiate mannitol-fermenting strains of possible contaminants, including enterococci and staphylococci. *Listeria* spp do not ferment Mannitol, which is demonstrated by a color change in the colony and/or the surrounding medium from red or gray to yellow based on the production of acidic end products. Polymyixin B, Acriflavin, Ceftazidime, and Lithium Chloride are selective agents used to suppress Gram-negative and certain Gram-positive bacteria.

Formula / Liter

Peptone	23 g
Starch	1 g
Sodium Chloride	5 g
Yeast Extract	3 g
Mannitol	10 g
Ferric Ammonium Citrate	0.5 g
Esculin	0.8 g
Dextrose	0.5 g
Lithium Chloride	15 g
Phenol Red	0.08 g
Agar	13 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / 10 mL

Polymixin B, 5 mg
Acriflavin, 2.5 mg
Ceftazidime, 10 mg
Filtered sterilized aqueous solution

Precautions

1. For Laboratory Use.
2. Harmful. Harmful if swallowed, inhaled, or absorbed through the skin. Irritating to eyes, respiratory system, and skin. Skin irritation may be severe. May cause central nervous system effects.

Directions

1. Suspend 72 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C.
4. Aseptically add 10 mL of a filtered sterilized solution containing 5 mg Polymixin B, 2.5 mg Acriflavin, and 10 mg Ceftazidime.
5. Dispense into sterile petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a faint pink tint.
Prepared Appearance: Prepared medium is trace to slight hazy and pinkish-red.
Expected Cultural Response: Cultural response on PALCAM Agar Base prepared with PALCAM Supplement at 35 ± 2°C after 24 - 48 hours incubation.

Microorganism	Results
Escherichia coli ATCC® 25922	inhibited
Enterococcus faecalis ATCC® 29212	inhibited
Listeria monocytogenes ATCC® 7644	grey-green w/ black ppt
Listeria monocytogenes ATCC® 19114	grey-green w/ black ppt
Listeria monocytogenes ATCC® 19116	grey-green w/ black ppt
Staphylococcus aureus ATCC® 25923	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Several procedures may be used to isolate *Listeria monocytogenes* and *Listeria* spp. on PALCAM Agar Base. Refer to the appropriate references for specific guidelines.[5,6,7,8]

Results

Listeria is presumptively indicated by grey-green colonies with a black precipitate following incubation for 24 - 48 hours at 35°C on PALCAM Agar Base. Consult references for complete identification and confirmation of *Listeria* spp.[5,6,7,8] Rapid slide and macroscopic tube tests can be used for definitive serological identification. Colonies of mannitol-fermenting organisms such as staphylococci, appear yellow with a yellow halo.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitations of the Procedure

Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

Packaging

PALCAM Agar Base	**Code No.**	**7669A**	**500 g**
		7669B	**2 kg**
		7669C	**10 kg**

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Path. Bacteriol. **29:**407-439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat, frozen and refrigerated ground beef. J. Food Prot. **57:**969-974.
3. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels prepared for hot smoking. J. Food Prot. **58:**604-608.
4. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55:**4-7.
5. **Van Netten, P., I. Perales, A. Van de moosalijk, G. D. W. Curtis, and D. A. A. Mossel.** 1989. Liquid and solid selective differential media for the detection and enumeration of *L. monocytogenes* and other *Listeria* spp. Int. J. of Food Microbiol. **8:**299-317.
6. **L'association francaise de normalisation (AFNOR).** 1993. Food Microbiology-Detection of *Listeria monocytogenes*-Routine Method, V 08-055. AFNOR, Paris, France.
7. **Farber, J. M., D. W. Warburton, and T. Babiuk.** 1994. Isolation of *Listeria monocytogenes* from all food and environmental samples. Heath Protection Branch Ottawa, MFHPB-30. Polyscience Publications, Quebec, Canada.
8. **International Dairy Federation.** 1990. Milk and milk products – Detection of *Listeria monocytogenes*. IDF Provisional International Standard no. 143. International Dairy Federation, Brussels.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

NEOGEN CORPORATION
620 Lesher Place, Lansing MI 48912
517/372-9200 • 800/783-3212 • fax: 800/875-8563
neogen-info@neogen.com • www.neogen.com