PALCAM BROTH (7670)

Intended Use

PALCAM Broth is used with supplements for the selective enrichment of *Listeria* spp. 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 and susceptible pregnant women.[2] Epidemiological evidence from outbreaks of listeriosis indicate the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[3] Implicated vehicles of transmission include turkey frankfurters, coleslaw, pasteurized milk, Mexican style cheese, and pate′.[4]

PALCAM Broth is based on the formulation of Van Netten et al.,[5] while he was investigating the isolation of *Listeria* spp. from food samples. PALCAM Broth has been used for testing *Listeria* from raw milk.[6]

Principles of the Procedure

Peptone and Yeast Extract provide nitrogen, vitamins, minerals, and cofactors required for growth of *Listeria* spp. The addition of Lithium Chloride, Polymixin B, Acriflavine, and Ceftazidime suppress the growth of Gram-negative bacteria and most Gram-positive bacteria. Lithium Chloride provides selectivity to PALCAM Broth due to the high salt tolerance of *Listeria spp*. Lecithin inactivates quaternary ammonium. Polysorbate 80 neutralizes phenols, hexachlorophene, and formalin. *L. monocytogenes* hydrolyses Esculin to esculetin and glucose. Esculetin form an olive-green to black complex with iron (III) ions, and PALCAM Broth turns brown-black if *Listeria* is present. Mannitol fermenting species of *Listeria* give the broth a yellow color due to the pH indicator Phenol red.

Formula / Liter

Peptone	23 g
Yeast Extract	5 g
Lithium Chloride	10 g
Esculin	0.8 g
Ferric Ammonium Citrate	0.5 g
Mannitol	5 g
Phenol Red	0.08 g
Lecithin	1.0 g
Polysorbate 80	2 g

Final pH: 7.4 ± 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. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 47 g of the medium in one liter of purified water.
2. Heat with frequent agitation 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 tubes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, soft lumpy, and beige with a faint pink tint.
Prepared Appearance: Prepared medium contains a slight haze to haze and red.

Expected Cultural Response: Cultural response in PALCAM Broth prepared with PALCAM Supplement at 30 ± 2°C after 24 - 48 hours incubation.

Microorganism	Results
Escherichia coli ATCC® 25922	inhibited
Enterococcus faecalis ATCC® 29212	marked to complete inhibition
Staphylococcus aureus ATCC® 25923	marked to complete inhibition
Listeria monocytogenes ATCC® 7644	brown-black
Listeria monocytogenes ATCC® 19112	brown-black
Listeria monocytogenes ATCC® 15313	brown-black

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

Test Procedure

To isolate *Listeria* from food and environmental samples using PALCAM Broth, the following procedure is recommended by the Canadian Health Protection Branch:[7]

1. Add 25 g of food into 225 ml of PALCAM Broth. Stomach, or Blend. For environmental samples, add the sample to 100 ml of PALCAM Broth. Stomach or blend.
2. Incubate the pre-enrichment for 26 hrs at 35°C.
3. Transfer 1 ml of PALCAM Broth to 9 ml of UVM 2. Incubate 26-48 hrs at 30°C.
4. After vortexing, streak the UVM 2 onto two different plating media. Use Oxford and PALCAM Agar Base. Incubated the plates at 35°C for 24-48 hrs.
5. In PALCAM Agar Base, *Listeria* spp form grey-green colonies with a black halo. Some *Enterococcus* and *Staphylococcus* strains form grey colonies with a brown-green halo or yellow colonies with a yellow halo.
6. Confirm the identity of each presumptive *Listeria* spp. through biochemical and / or serological testing.

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 by keeping container tightly closed.

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 when stored as directed.

Limitations of the Procedure

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

Packaging

PALCAM Broth	**Code No.**	**7670A**	**500 g**
		7670B	**2 kg**
		7670C	**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. **Lund, A.M.** 1991. Comparison of methods for isolation of *Listeria* from raw milk. J. Food Prot. 54:602-608.
7. **Canadian Food Directorate.** 2001. The Compendium of anallytical methods. Polyscience Publications, Laval, Quebec, Canada.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.