BD™ PALCAM Listeria Agar

INTENDED USE

BD PALCAM Listeria Agar is a selective differential medium for the isolation and detection of *Listeria monocytogenes* and other *Listeria* species from foods and clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

PALCAM Agar is based on the formulation of van Netten et al., who developed this selective and differential medium for use in the isolation and enumeration of *Listeria* spp. from food samples.[1] PALCAM medium is recommended by APHA and by AFNOR for use in the detection of *Listeria monocytogenes* in foods, and by the International Dairy Federation as an additional plating medium for the detection of *Listeria* spp. in milk and milk products.[2-4] PALCAM medium is also recommended by Health Canada for the detection of *L. monocytogenes* in food and environmental samples and by the OADC for detection of the organism from foods.[5,6] It may also be used for the isolation of *L. monocytogenes* from clinical specimens.[7,8]

In **BD PALCAM Listeria Agar**, Columbia Blood Agar Base provides the nutrients and cofactors required for growth of *Listeria*. Selectivity of the medium is achieved through the presence of lithium chloride, polymyxin B sulfate, acriflavine-HCl, and ceftazidime, which suppress growth of most non-*Listeria* spp. present in foods and clinical specimens. The ceftazidime concentration is reduced from 20 mg/l to 8 mg/l for improved growth and recovery of *Listeria*. Differentiation on PALCAM Medium is based on esculin hydrolysis and mannitol fermentation. All *Listeria* spp. hydrolyze esculin as evidenced by a blackening of the medium. The product of the hydrolysis, esculetin (=6,7-dihydroxycoumarin), reacts with ferric ions to a brown to black complex. On occasion, organisms other than *Listeria*, such as staphylococci or enterococci, may grow on this medium. Mannitol and the pH indicator, phenol red, have been added to differentiate mannitol-fermenting strains of these species from *Listeria.* Mannitol fermentation is demonstrated by a color change of the medium from red to yellow due to the production of acids.

REAGENTS

BD PALCAM Listeria Agar

Formula* Per Liter Purified Water

Bacto™ Columbia Blood Agar Base	39.0 g
Mannitol	10.0
Glucose	0.5
Esculin	1.0
Ferric Ammonium Citrate	0.5
Lithium Chloride	15.0
Phenol Red	0.08
Acriflavine HCl	0.005
Polymyxin B Sulfate	0.01
Ceftazidime	0.008
Bacto Agar	2.0

pH 7.2 ± 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate medium. Incubate aerobically for 42 to 48 hours at 35 to 37° C and read after 18 to 24 and after 42 to 48 hours.

Strain	Growth Result
Listeria monocytogenes ATCC™ 19112	Fair to excellent growth; gray-green colonies surrounded by dark brown to black halos in the medium
Listeria monocytogenes ATCC 19115	Good to excellent growth; gray-green colonies surrounded by dark brown to black halos in the medium
Escherichia coli ATCC 25922	Inhibition (partial to) complete
Enterococcus faecalis ATCC 29212	Inhibition complete
Pseudomonas aeruginosa ATCC 27853	Inhibition (partial to) complete
Staphylococcus aureus ATCC 25923	Inhibition partial; yellowish colonies, surrounded by yellow halos
Uninoculated	Red, clear to trace hazy

PROCEDURE

Materials Provided

BD PALCAM Listeria Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types and Transport

This medium is especially used for the isolation of *Listeria* from foods and dairy materials. It may also be used for the isolation from clinical specimens such as stools in epidemiologic studies of carriage rates of *Listeria* or for other clinical specimens strongly contaminated with normal flora (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). For specimens from primarily sterile body sites, see **Test Procedure**. Specimens and samples should be sent to the laboratory directly or in transport media. Liquid enrichment media for *Listeria* may be used as a transport medium.[2-7] For transport exceeding 24 hours, specimens may be kept frozen to avoid overgrowth by contaminants.[7]

Test Procedure

Streak the specimen or sample directly or from an enrichment broth onto the surface of the medium. **BD PALCAM Listeria Agar** should be inoculated with clinical specimens if heavy contamination is suspected. Clinical specimens from primarily sterile body sites, e.g., cerebrospinal fluid, amniotic fluid, placenta, or fetal specimens, must be primarily streaked onto nonselective media such as a **BD Columbia Agar with 5% Sheep Blood** or **BD Chocolate Agar (GC Agar with IsoVitaleX)** plate. Selective media for Gram positive bacteria, e.g., **BD Columbia CNA Agar with 5% Sheep Blood** may also be included.[7,8] Incubate the inoculated **BD PALCAM Listeria Agar** medium aerobically (and all media containing blood in an aerobic atmosphere enriched with CO_2) for 18 to 24 hours at 35 – 37° C. If negative, reincubate for additional 24 hours.

Results
On **BD PALCAM Listeria Agar**, colonies of *Listeria* appear gray-green with a black halo. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/serological identification.[2,3,7] Colonies of mannitol-fermenting organisms such as staphylococci, which may grow on this medium, appear yellow with a yellow halo.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD PALCAM Listeria Agar is one of the approved media used for selective isolation of *Listeria monocytogenes* and other *Listeria* species from foods and clinical specimens.[2-8]
On this medium, all *Listeria* species will grow and will have the same colony appearance. Therefore, all isolates must be identified with biochemical and/or serological procedures to the species level.
Incubation of this medium in an aerobic atmosphere enriched with carbon dioxide or in a microaerobic atmosphere may result in a color change of the phenol red pH indicator from red to yellow or orange, mimicking a false positive mannitol fermentation. Therefore, an aerobic atmosphere is preferred.
Certain strains of *Listeria* may grow slowly on this medium. Therefore, the inoculated plates must be re-incubated if negative after 18 to 24 hours.
Listeria may be present in very low numbers in certain specimens or samples that are below the level of detection of this medium. Pre-enrichment may be necessary in these cases.[3-7]

REFERENCES

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PACKAGING/AVAILABILITY

BD PALCAM Listeria Agar
Cat. No. 254539 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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