

Instructions for Use

PALCAM AGAR

Cat. no. G149	PALCAM Agar, 15x100mm Plate, 19ml.	10 plates/bag
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INTENDED USE

Hardy Diagnostics PALCAM Agar is recommended for use as a selective and differential growth medium for the cultivation, isolation and differentiation of *Listeria* species from food and dairy products.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Listeria spp. are microaerophilic, gram-positive, short motile rods or coccobacilli that are asporogenous, non-encapsulated, and non-branching. Motility is best observed at 20-25°C. *Listeria monocytogenes* is a pathogenic organism that affects humans and a large number of animal species. Members of the population most at risk are neonates, the elderly and those compromised by pregnancy or an underlying illness such as malignancy, alcoholism or some condition requiring immunosuppressive procedures. There are some reports of nosocomial infections of *Listeria monocytogenes*, usually among infants or immunosuppressed adults. Intrauterine infection of the fetus results in death, or an acutely ill infant with a septic disseminated form of listeriosis.⁽¹⁾ Papular lesions of the skin may be found in listeriosis of the newborn. A similar cutaneous form has been reported in veterinarians working with infected animals.⁽¹⁾

Pasteurized milk is common vehicle for *Listeria monocytogenes*. Since the induction of the Pasteurized Milk Ordinance in 1924, there have been fewer reported cases of milk contaminants other than *Listeria* spp. The ability of *L. monocytogenes* to grow between 4 and 10°C. and over a wide pH range (4.4-9.6) complicates the issue. The most effective prevention method still involves post-pasteurization pathogen detection.⁽²⁾

Other types of food that have been found to contain *Listeria* species are raw milk, raw vegetables, fish, poultry, and both fresh and processed meats. The CDC recommends that immunocompromised, pregnant or elderly individuals avoid soft cheeses, cold cuts and salami.

Listeria monocytogenes is ubiquitous in nature and has been isolated from soil, mud, sewage, decaying vegetation, silage, feces, and river water. Many animal species are vulnerable to infection by *Listeria* species. Some lactating mammals can function as asymptomatic carriers while still excreting the organisms in their milk. Sheep, cattle and goats have been found to shed *Listeria monocytogenes* in their feces.

Van Netten et al. described a modification of Columbia Agar that provides a selective and differential medium for culturing *Listeria* species.⁽⁴⁾ Columbia Agar base provides the essential nutrients required for growth. The addition of lithium chloride, acriflavine, polymyxin b and ceftazidime makes the medium selective for *Listeria* species. The selective agents suppress the growth of most common bacteria found in food. The addition of esculin, ferric ammonium citrate, phenol red, and mannitol make the medium differential. *Listeria* species hydrolyze esculin to dextrose and esculentin, which forms a dark brown to black precipitate with ferric citrate. Mannitol fermenting organisms, such as *Staphylococcus*, will produce acid products from mannitol. The acidic end products will turn the colonies yellow and/or

produce a yellow halo around the colonies.

FORMULA

Ingredients per liter of deionized water:*

Columbia Agar Base	39.0gm
Mannitol	10.0gm
Dextrose	0.5gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm
Lithium Chloride	15.0gm
Phenol Red	0.08gm
Acriflavine HCL	5.0mg
Polymyxin B Sulfate	10.0mg
Ceftazidime	40.0mg
Agar	2.0gm

Final pH 7.2 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Sample collection and preparation should be performed following appropriate standards and guidelines.⁽²⁻⁶⁾

Enrich and culture sample according to US Food and Drug Administration (FDA), US Department of Agriculture (USDA), or ISO Standards appropriate for the sample type.^(2,3,5)

Method of Use: Allow plates to warm to room temperature. The agar surface should be dry before inoculating. Inoculate and streak the specimen for isolation. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35-37 °C. for 24-48 hours. Examine plates for colonial morphology.

INTERPRETATION OF RESULTS

Listeria colonies are gray-green with a black precipitate. Mannitol fermenting organisms have yellow colonies and/or a yellow halo around their colonies. Other organisms that develop on the media may appear as gray colonies with a brown-green halo.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Listeria monocytogenes</i> ATCC® 7644	A	24-48hr	35°C	Aerobic	Growth. Gray-green with black precipitate
<i>Escherichia coli</i> ATCC® 25922	B	24-48hr	35°C	Aerobic	Partial to complete inhibition
<i>Staphylococcus aureus</i> ATCC® 25923	B	24-48hr	35°C	Aerobic	Partial to complete inhibition
<i>Enterococcus faecalis</i> ATCC® 29212	B	24-48hr	35°C	Aerobic	Partial to complete inhibition

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

PALCAM Agar should appear slightly opalescent, and dark red in color with a slight precipitate.

REFERENCES

1. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
2. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.
3. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.
4. Van Netten, Perales, Van de Moosalijk, Curtis, and Mossel. 1989. *Int. J. Food Microbiol.* 8:299.
5. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC, Arlington, VA.
www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
6. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

ATCC is a registered trademark of the American Type Culture Collection.

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