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Instructions for Use

PATHFinder™

PATHFINDER™ LISTERIA BROTH

Cat. no. K321	Pathfinder™ Listeria Broth, 20x125mm Polycarbonate Tube, 15ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Pathfinder™ Listeria Broth is recommended for use with environmental samples from food processing environments or food contact surfaces as a selective and differential screening medium to detect and confirm the presumptive presence of *Listeria* spp. after cleaning.

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

SUMMARY

Listeria are microaerophilic, gram-positive, short motile rods or coccobacilli that are asporogenous, non-encapsulated, and non-branching. Motility is best observed at 20-25ºC. The genus *Listeria* contains *L. monocytogenes*, along with *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. selligeri*, and *L. grayi*. Of these, only two are considered pathogens: *L. monocytogenes*, which infects humans and animals, and *L. ivanovii*, which infects ruminants (although there have been rare reports of infected humans). There are thirteen known serotypes of *L. monocytogenes*: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7. The serotypes most often associated with human illness are 1/2a, 1/2b and 4b.

Listeria monocytogenes can cause listeriosis, a disease with severe consequences for particular groups. Humans most at risk are neonates, the elderly, and those compromised by pregnancy or an underlying illness such as malignancy, alcoholism, or a condition which requires immunosuppressive procedures. In healthy people, listeriosis generally only causes a mild form of illness.

L. monocytogenes can be found throughout the environment. It has been isolated from domestic and wild animals, birds, soil, vegetation, fodder, water, and from floors, drains, equipment, and wet areas of food processing factories. *L. monocytogenes* is capable of growth between 4 and 10ºC and over a wide pH range (4.4 to 9.6). The CDC recommends that immunocompromised, pregnant, or elderly individuals avoid foods such as soft cheeses, cold cuts, and salami. There are also some reports of nosocomial infections of *Listeria monocytogenes*, usually among infants or immunosuppressed adults.

Routine sampling and monitoring in the food processing plant or on food contact surfaces after cleaning is an effective means to reduce the risk of *Listeria* transmission via foods. Pathfinder™ Listeria Broth contains a custom targeted formulation which includes growth enhancers, buffers, and trace minerals which promote the growth of sub-lethally injured cells that may be more challenging to recover after cleaning procedures. These ingredients also provide an energy source, essential vitamins, and minerals needed to promote cell growth. The medium also contains selective agents to reduce the growth of competing flora. Differentiation of *Listeria* is aided by indicator compounds ferric ammonium citrate and esculin, as cells capable of hydrolyzing esculin will utilize ferric ammonium citrate and cause a blackening of the medium. Since esculin hydrolysis can also be found in *Enterococcus* spp., Pathfinder™ Listeria Broth differs from comparable detection broths in that the medium can be used to confirm the presence of *Listeria* by a

green fluorescence observed under longwave ultraviolet light at 365nm after incubation.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration, 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: Consult reference methods, such as USDA or BAM, or follow standard laboratory procedure for information on sample collection.[1-5]

1. Use an appropriate sterile sampling swab, pre-moistened with a neutralizing buffer, such as EnviroMax® Environmental Sampling Swab (Cat. no. 2588050PF), to collect the sample. It is highly recommended to use this validated sampling device.

2. Label the sample accordingly and follow standard laboratory procedures to swab the appropriate surface area (10cm x 10cm, 4"x4", 9"x9", or 12"x12"), or perform replicate sampling to ensure adequate testing of the environment is performed.

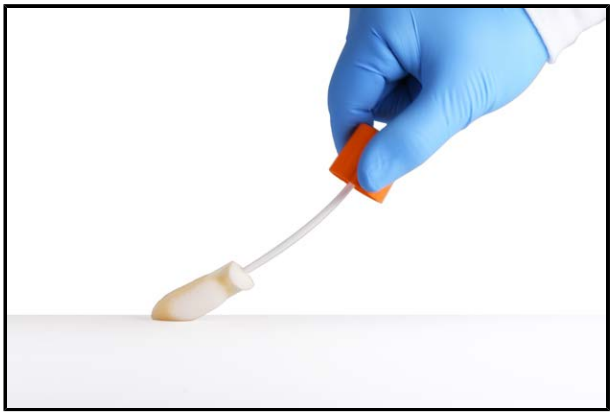
3. Return the swab to the container and return the sample to the laboratory for processing. Samples should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the sample should be refrigerated until incubation.

Method of Use: Allow the medium to warm to room temperature prior to use.

1. Aseptically add all the contents of the Pathfinder™ Listeria Broth (15ml) tube into the EnviroMax® Environmental Sampling Swab tube and close securely. Make sure the swab is completely submerged in the broth and cap is tight. **NOTE**: Alternative swabs can be used. However, incubation of the sample with Pathfinder™ Listeria Broth must be completed in a polypropylene tube. See Limitations section for more information.

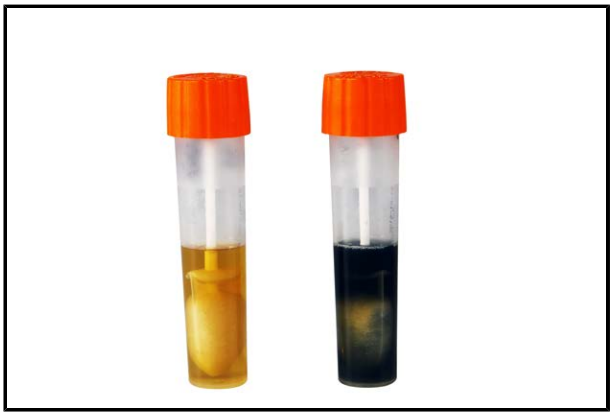
2. Incubate the tube upright with the sponge submerged in the Pathfinder™ Listeria Broth at 35-37ºC for 30-48hr.

3. Examine tubes for blackening of the medium, indicative of positive esculin hydrolysis. Make sure the cap is tight and invert the tube to examine the bottom of the tube for a green fluorescence using an ultraviolet lamp (Cat. no. UVL56) at 365nm. **NOTE**: The fluorescence reaction should be read from the walls of the polypropylene tube where the broth has incubated, **not** in the liquid medium.

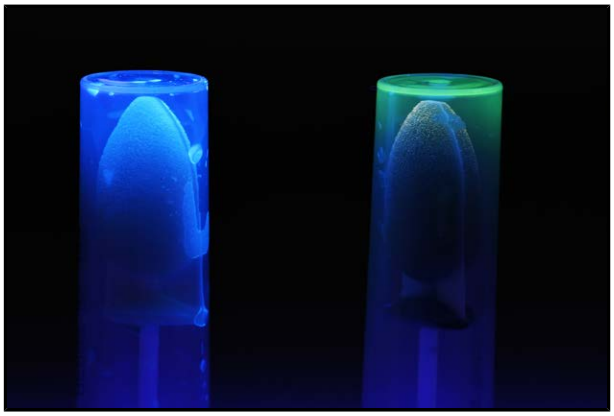


1. Aseptically remove the pre-moistened EnviroMax® Environmental Sampling Swab (Cat. no. 2588050PF) from the container and swab the surface according to the sampling plan.

2. Return the EnviroMax® Environmental Sampling Swab to its container and aseptically pour all the contents of the Pathfinder™ Listeria Broth tube into the swab container. Incubate tubes at 35-37ºC for 30-48hr with the sponge submerged.



3. Examine tubes for a blackening of the medium, indicative of positive esculin hydrolysis by Listeria.



4. For confirmation of *Listeria* spp., examine the bottom of an inverted polypropylene tube for a green fluorescence using a UV lamp at 365nm. Observe only the plastic tube for fluorescence, not the liquid.

INTERPRETATION OF RESULTS

A color change from amber to brown/black indicating a positive esculin hydrolysis reaction, in conjunction with a **green** fluorescence at the bottom of the inverted polypropylene tube examined using an ultraviolet lamp at 365nm, is confirmation for the presumptive presence of *Listeria* spp. The two pathogenic species of *Listeria*, *L. monocytogenes* and *L. ivanovii*, will be positive on this test. The non-pathogenic species will be negative (*L. innocua*, *L. welshimeri*, *L. selligeri*, and *L. grayi*). Further testing is required to confirm species and serotype. Corrective action in the event of a positive result should be consistent with HACCP plan guidance documents.

A color change from amber to brown/black indicating a positive esculin hydrolysis reaction, in conjunction with a blue (rather than green) fluorescence at the bottom of the tube when inverted and examined using an ultraviolet lamp at 365nm, indicates a negative result for *Listeria* spp. and will most likely be *Enterococcus* spp.

Growth and the absence of a brown/black coloration in the medium, regardless of fluorescence reaction at 365nm, is indicative of a negative result for *Listeria* spp

Use a 365nm wavelength handheld UV Lamp (Cat. no. UVL56 or LSS3) to detect fluorescence of the tube. These handheld lamps require that the room lights be turned off, since ambient light will interfere with fluorescence detection. Alternatively, a dark viewing box (Cat. no. CM10A) with its companion UV lamp (Cat. no. EA160) may be used so that the room lights will not need to be turned off.

CAUTION: Not all UV wavelengths are capable of producing sufficient fluorescence effects. It is important to use a UV light with a wavelength at or near 365nm, one with higher power (in watts, not lumens), and one that is high efficiency. Use of UV lights not meeting these criteria will fail to produce sufficient fluorescence. Most inexpensive battery operated LED UV lights produce light at multiple wavelengths, use less watts, and/or low power, and are thus **not acceptable** and will produce erroneous results. Cat. no. LSS3 is an exception and has been verified to work well. Please do not use cheaper versions.

Tips for using fluorescence

1. Use a 365nm handheld UV lamp (Cat. no. UVL56) or (Cat. no. LSS3) to detect fluorescence of the tube walls. See 'CAUTION' above regarding inexpensive handheld UV lights. Alternatively, a dark viewing box with its compatible UV lamp may be used as described above. Viewing must be done in the dark.
2. Hold the lamp directly over the tubes, approximately 3 to 4 inches (7 to 10cm) away.
3. The presence of *Listeria* in the broth will cause the tube walls to fluoresce a **green** glow. Negatives may fluoresce blue.
4. Fluorescence will fade over time.

Esculin Reaction	Fluorescence of tube under UV at 365nm	Interpretation
Brown/Black 	Green fluorescence 	*Listeria* spp.
Brown/Black 	Blue fluorescence 	*Enterococcus* spp.
No change 	No green fluorescence 	Not *Listeria* spp.

CONFIRMATION METHODS

In the event of a *Listeria* spp. positive result, confirmatory methods to the species level such as PCR, DNA fingerprinting, or biochemical analysis should be conducted on well isolated colonies obtained from a selective solid medium such as HardyCHROM™ Listeria (Cat. no. G317), Modified Oxford Agar (Cat. no. G46), or PALCAM Agar (Cat. no. G149). Additional biochemical analysis can be performed using Microgen® Listeria ID (Cat. no. MID67) or Microgen® Listeria Latex Test (Cat. no. F48).

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.

The positive fluorescence reaction is dependent upon incubation of the sample in a polypropylene tube. Use of the EnviroMax® Environmental Sampling Swab (Cat. no. 2588050PF) has been verified by Hardy Diagnostics. However, alternative polypropylene tubes can be used, but validation by the user is recommended prior to use in HACCP monitoring procedures.

Failure to incubate the sample in a polypropylene tube will result in a lack of fluorescence in positive samples and a false negative result. **DO NOT** use incubation tubes made from polystyrene, polycarbonate, or glass.

Positive confirmation of *Listeria* spp. in the sample requires both a blackening of the medium (positive esculin hydrolysis) and a green fluorescence using an ultraviolet lamp at 365nm.

Blue fluorescence at 365nm, regardless of esculin reaction, is indicative of a negative result.

Pathfinder™ Listeria Broth is an enrichment medium designed to amplify the concentration of *Listeria* cells in a sample. Use biohazard precautions when handling samples. Immunocompromised individuals or pregnant women should not handle enriched samples suspected of containing *Listeria*.

Samples obtained from unsanitary areas may contain *Enterococcus* spp. that will demonstrate a positive esculin reaction. However, these samples will not demonstrate the green fluorescence needed for confirmation of *Listeria* spp.

Fluorescence must be read in a darkened environment with a 365nm wavelength UV lamp of adequate power (see “Tips for Using Fluorescence” above).

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, other culture media, swabs, UV lamps, applicator sticks, incinerators, handheld UV lamp (Cat. no. UVL56 or LSS3) or dark viewing box (Cat. no. CM10A) with compatible UV lamp (Cat. no. EA160), 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	

Listeria monocytogenes ATCC® 15313	A	24-48hr	35°C	Aerobic**	Growth; slight blackening of media; green glow with UV light on inverted tube
Enterococcus faecalis ATCC® 29212	A	24-48hr	35°C	Aerobic**	Slight/no growth; slight/no blackening; blue/white glow from broth under UV light
Enterococcus faecium ATCC® 700221	A	24-48hr	35°C	Aerobic**	Slight/no growth; slight/no blackening; blue/white glow from broth under UV light
Staphylococcus aureus ATCC® 6538	B	24-48hr	35°C	Aerobic**	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Incubate tubes with tight caps and read UV light reactions by inverting tubes and observing for fluorescence at the bottom of the tube.

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

Hardy Diagnostics Pathfinder™ Listeria should appear clear, and amber to dark amber in color.

REFERENCES

1. United States Department of Agriculture - Food Safety Inspection Service. *Microbiology Laboratory Guidebook*. MLG 8.11. USDA-FSIS. Office of Public Health Science. Athens, GA.

2. U.S. Food and Drug Administration. FSIS. Department of Agriculture. *Code of Federal Regulations*. 9 CFR 430.1. Subchapter E. 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. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* Arlington, VA http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

5. Association of Official Analytical Chemists. *Official Methods of Analysis*, AOAC, Washington, D.C.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207D [D]