HOLOGIC®
AccuProbe®

LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST

INTENDED USE

The ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Listeria monocytogenes* isolated from culture.

SUMMARY AND EXPLANATION OF THE TEST

Listeria monocytogenes (L. monocytogenes) is primarily a soil-borne microorganism dispersed throughout the environment. It has been found in such sources as water, agricultural products, and in animals. Recognized as a human pathogen for more than 50 years, *L. monocytogenes* has been identified as the etiologic agent of listeriosis, causing meningitis, encephalitis, septicemia, endocarditis, abortion, abscesses and local purulent lesions in humans (1,3). Four major outbreaks of listeriosis within the last decade have been connected to consumption of contaminated food. Pregnant women, neonates, immunocompromized patients and the elderly are at greatest risk of acquiring listeriosis (4).

Current methods for identification of *L. monocytogenes* rely on physiological and biochemical methods. These include Gram stain morphology, catalase, motility, beta hemolysis on blood agar and oblique illumination of colonies on blood free agar (6).

The ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST identifies *Listeria monocytogenes* isolated from culture within 35 minutes of sample preparation.

PRINCIPLES OF THE PROCEDURE

Nucleic acid hybridization tests are based on the ability of complementary nucleic acid strands to specifically align and associate to form stable double-stranded complexes (5). The AccuProbe system uses a single-stranded DNA probe with a chemiluminescent label that is complementary to the ribosomal RNA of the target organism. After the ribosomal RNA is released from the target organism, the labeled DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. The Selection Reagent allows for the differentiation of non-hybridized and hybridized probes. The labeled DNA:RNA hybrids are measured in a Hologic luminometer. A positive result is a luminometer reading equal to or greater than the cut-off. A value below this cut-off is the negative result.

REAGENTS

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Reagents used for the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE LISTERIA MONOCYTOGENES KIT

Probe Reagent (4 X 5 tubes).
Listeria monocytogenes.

ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT

Reagent 1 (Lysis Reagent). 1 X 10 mL
buffered solution containing 0.04% sodium azide.

Reagent 2 (Hybridization Buffer). 1 X 10 mL
buffered solution.

Reagent 3 (Selection Reagent). 1 X 60 mL
buffered solution.

HOLOGIC DETECTION REAGENT KIT

Detection Reagent I. 1 X 240 mL
0.1% hydrogen peroxide in 0.001 N nitric acid.

Detection Reagent II. 1 X 240 mL
1 N sodium hydroxide.

WARNINGS AND PRECAUTIONS

A. For *in vitro* diagnostic use.

B. Use universal safety precautions when performing this assay (2).

C. Use only for the identification of *L. monocytogenes* isolated from culture.

D. Use only supplied or specified disposable laboratory ware.

E. Reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. Upon disposal of these reagents, always dilute the material with a large volume of water to prevent azide buildup in the plumbing.

F. Avoid contact of Detection Reagents I and II with skin and mucous membranes. Wash with water if these reagents come into contact with skin. If spills of these reagents occur, dilute with water before wiping dry.

G. To ensure optimal performance we recommend that prior to testing you inspect the tubes for dislodged material. If dislodged material is present, tap tube on the counter top in order to settle contents to the bottom of the tube.

STORAGE AND HANDLING REQUIREMENTS

Probe Reagent Tubes must be stored in their foil pouches at 2° to 8°C. The Probe Reagent Tubes are stable in the unopened pouches until the expiration date indicated. Once opened, the pouch should be resealed and the tubes should be used within two months and prior to the expiration date.

Other reagents used in the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST may be stored between 2° and 25°C and are stable until the expiration date indicated.

DO NOT FREEZE THE REAGENTS

SAMPLE COLLECTION AND PREPARATION

The ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST is designed to determine the identity of *L. monocytogenes* isolated from culture.

A. **Solid Media Method.** Growth isolated from appropriate solid media, such as 5% Sheep Blood, Brain Heart Infusion, or Chocolate Agar may be tested. In addition, growth from selective media such as McBride Agar and LPM Agar (Remel) is also acceptable. Samples may be tested as soon as growth is visible but should be less than 72 hours old.

1. Growth can be removed with a 1 µL disposable plastic loop, a wire loop, a disposable plastic needle or an applicator stick. Swabs should not be used due to the small volume of liquid in which the cells are subsequently resuspended.
2. If a single colony is to be tested, it should be at least 1 mm in diameter. A 1 µL loopful of cells or several (3-4) smaller colonies can be tested.
3. Avoid taking any of the solid media with the cells.
4. The operator may elect to inoculate another culture plate at this time to confirm the purity of the isolate.

B. **Broth Culture Method.** Growth in broths, such as Trypticase Soy, Brain Heart Infusion, or Listeria Enrichment Broth with turbidity equivalent to or greater than a McFarland I Nephelometer standard may be tested with the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST. Pipette

a 50 µL sample from the well-mixed broth suspension into the Probe Reagent Tubes as described below.

MATERIALS PROVIDED

The ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST

Cat. No. 102920	**20 tests**
Probe Reagent	4 X 5 tubes

MATERIALS REQUIRED BUT NOT PROVIDED

1 µL plastic sterile inoculating loops, wire loops, plastic needles, or applicator sticks for collecting colonies
Control culture strains
Incubator or water bath (35° to 37°C)
Water bath or heating block (60° ± 1°C)
Micropipettes (50 µL, 300 µL)
Re-pipettors (50 µL, 300 µL)
VORTEX mixer

AVAILABLE FROM HOLOGIC:

Hologic Leader® Luminometer
ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT (Cat. No. 102800)
HOLOGIC DETECTION REAGENT KIT (Cat. No. 201791)
Hologic Heating Block (Cat. No. 102775)

TEST PROCEDURE

A. EQUIPMENT PREPARATION

1. Adjust the incubator or water bath to 35° to 37°C.
2. Adjust the water bath or heating block to 60° ± 1°C.
3. Prepare the Hologic luminometer for operation. Make sure there is sufficient volume of Detection Reagents I and II to complete the tests.

B. CONTROLS

Positive and negative control strains should be tested routinely in each laboratory, according to local regulations. A culture of *L. monocytogenes* (e.g., American Type Culture Collection, ATCC #35152) may be used as the positive control while a culture of *Listeria grayi* (e.g., ATCC #19120) may be used as the negative control.

C. SAMPLE PREPARATION

1. Open the foil pouch by cutting evenly across the top of the pouch. Remove enough Probe Reagent tubes to test the culture isolates and/or controls. Reseal the pouch by folding the opened edge over several times and securing with adhesive tape or a clip. **Leave the desiccant pillow in the pouch.**
2. Label a sufficient number of Probe Reagent Tubes to test the culture isolates and/or controls. Remove and retain the caps.
3. Pipette 50 µL of Reagent 1 (Lysis Reagent) into all Probe Reagent Tubes. **If broth cultures are to be tested, do not add Reagent 1 to the Probe Reagent Tubes.**
4. Transfer the sample from the solid media or 50 µL of a well-mixed broth culture into the labeled Probe Reagent Tubes, as described in the SAMPLE COLLECTION AND PREPARATION Section. Twirl the loop, needle or stick in Reagent 1 (Lysis Reagent) to remove the cells if testing growth from solid media and mix thoroughly.
5. Recap the Probe Reagent Tubes and incubate at 35° to 37°C for 5 minutes in a water bath or 10 minutes at 35° to 37°C in an incubator.

D. HYBRIDIZATION

1. Remove the Probe Reagent Tubes from the water bath or incubator. Remove and retain the caps. Pipette 50 µL of Reagent 2 (Hybridization Buffer) into all Probe Reagent Tubes.
2. Recap the Probe Reagent Tubes and incubate for 15 minutes at 60° ± 1°C in a water bath or heating block.

E. SELECTION

1. Remove the Probe Reagent Tubes from the water bath or heating block. Remove and retain the caps. Pipette 300 µL of Reagent 3 (Selection Reagent) into each tube. Recap the tubes and vortex them to mix completely.
2. Incubate the Probe Reagent Tubes for at least 5 minutes at 60° ± 1°C in a water bath or heating block.
3. Remove the Probe Reagent Tubes from the water bath or heating block and leave them at room temperature for at least 5 minutes. Remove and discard the caps. **Read the results in the luminometer within 1 hour after removing from the water bath or heating block.**

F. DETECTION

1. Select the appropriate protocol from the menu of the luminometer software.
2. Using a damp tissue or paper towel, wipe each tube to ensure that no residue is present on the outside of the tube and insert the tube into the luminometer according to the instrument directions.
3. When the analysis is completed, remove the tube(s) from the luminometer.

PROCEDURAL NOTES

A. REAGENTS: Reagent 2 (Hybridization Buffer) may precipitate. Warming and mixing the solution at 35° to 60°C will dissolve the precipitate.

B. TEMPERATURE: The Sample Preparation, Hybridization and Selection reactions are temperature-dependent. Therefore, it is imperative that the incubator, water bath, or heating block is maintained within the specified temperature range.

C. TIME:

1. The Hybridization Reaction should be started within 1 hour of adding the cells and Reagent 1 to the Probe Reagent Tubes.
2. The Hybridization and Selection reactions are time-dependent. Hybridize for at least 15 minutes but no more than 20 minutes. Incubate the Probe Reagent Tubes during the SELECTION Step for at least 5 minutes, but no more than 6 minutes.

D. WATER BATH: The level of water in the water bath should be maintained to ensure that the entire liquid reaction volume in the Probe Reagent Tubes is submerged.

E. VORTEXING: It is critical to obtain a homogeneous mixture during the SELECTION Step, specifically after the addition of Reagent 3.

F. TROUBLE-SHOOTING

1. Elevated negative control values (*Listeria grayi* ATCC #19120) greater than 20,000 RLU (Relative Light Units) in the Leader luminometer or 600 PLU (Photometric Light Units) in the AccuLDR (formerly PAL) luminometer can be caused by insufficient mixing after adding Reagent 3 (Selection Reagent), or by testing mixed cultures. Because mixed cultures can occur, a portion of the growth may be streaked onto the appropriate agar medium and incubated to check for multiple colony types.
2. Low positive control values (*Listeria monocytogenes*, ATCC #35152) less than 50,000 RLU in the Leader luminometer or 1,500 PLU in the AccuLDR (formerly PAL) luminometer can be caused by insufficient call numbers or by testing mixed or aged cultures. Because mixed cultures can occur, a portion of the growth may be streaked onto the appropriate agar medium and incubated to check for multiple colony types.

RESULTS

A. INTERPRETATION OF RESULTS

The results of the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST are based on the following cut-off values. Samples producing signals greater than or equal to these cut-off values are considered positive. Signals less than these cut-off values are considered negative. Results in repeat ranges should be repeated.

	AccuLDR (formerly PAL)	Leader
Cut-off value	1,500 PLU	50,000 RLU
Repeat range	1,200-1,499 PLU	40,000-49,999 RLU

B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Negative controls (e.g. *Listeria grayi*, ATCC #19120) and positive controls (e.g. *Listeria monocytogenes*, ATCC #35152) should satisfy the following values:

	AccuLDR (formerly PAL)	Leader
Negative control	<600 PLU	<20,000 RLU
Positive control	>1,500 PLU	>50,000 RLU

LIMITATIONS

This method has been tested using fresh growth from solid media and from broth cultures listed in the SAMPLE COLLECTION AND PREPARATION section. The efficacy of this test has not been demonstrated on direct clinical specimens (e.g., cerobrospinal fluid or blood).

Results from the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

EXPECTED VALUES

The ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST was compared to standard culture biochemical identification methods using 175 isolates of *L. monocytogenes* species and 102 additional isolates representing 21 genera. A second evaluation was undertaken with 296 strains isolated from suspected contaminated food materials. Standard culture identification is dependent on Gram stain, catalase reaction, motility and the RAPID STREP TEST (API System, Plainview, New York). Isolates were categorized as either positive (≥ 50,000 RLU) or negative (< 50,000 RLU). The range of observations for negative cultures was 520 to 27,990 RLU and 77,088 to 1,283,789 RLU for positive cultures. A comparison of these results to standard culture identification methods is shown below:

ACCUPROBE / CULTURE IDENTIFICATION

AccuProbe Culture	Pos Pos	Pos Neg	Neg Pos	Neg Neg	Sensitivity/ Specificity	Percent Agreement
Site 1	175	0	0	102	100%/100%	100%
Site 2	81	1	0	110	100%/99.5%	99.7%
Total	**256**	**1**	**0**	**316**	**100%/99.7%**	**99.8%**

One AccuProbe positive isolate, culture negative, from Site 2 was retested with the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST and gave a negative result.

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST was calculated by assaying three concentrations of ribosomal RNA isolated from *L. monocytogenes* using 10 replicates in a single assay.

Sample	A	B	C
Number of replicates	10	10	10
Mean Response	132,370	72,720	41,074
Standard Deviation	9,981	3,126	3,837
Coefficient of variation	7.5%	4.3%	9.3%

B. BETWEEN-RUN PRECISION

The between-run precision was calculated by assaying the same three concentrations of *L. monocytogenes* ribosomal RNA using single determinations in 12 consecutive runs.

Sample	A	B	C
Number of replicates	12	12	12
Mean Response	146,469	77,240	40,306
Standard Deviation	19,793	8,634	4,343
Coefficient of variation	13.5%	11.2%	10.8%

C. SPECIFICITY

A total of 97 culture isolates were evaluated using the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST. These isolates represented a total of 87 species from 53 genera. Fifteen isolates from 7 species of *Listeria* were tested (*L. grayi, L. innocua, L. ivanovii, L. monocytogenes, L. murrayi, L. seeligeri, L. welshimen)*. Only the isolates of *Listeria monocytogenes* produced positive results in the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICAITON TEST.

D. RECOVERY

Five serial dilutions of *L. monocytogenes* ranging from 0 to 30 million cells per test were assayed in the presence of 30 million cells of the following selected non-target species: *Listeria grayi, Listeria ivanovii, Erysipelothrix rhusiopathiae, Brochothrix thermosphacta.* The presence of these non-target species did not interfere with the positive signal of the *L. monocytogenes* rRNA dilutions, nor did they generate a positive reaction with the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST.

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103051 Rev. 001
2016-03