



Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG 8.11

Title: Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Ready-To-Eat Siluriformes (Fish) and Egg Products, and Environmental Samples

Effective Date: 1/02/19

Description and purpose of change(s):

The rapid molecular screening assay was revised in the equipment section and additional rapid screen instructions were added to the procedure.

The MOX plating, examination, and interpretation procedure in Section 8.5.5 was revised for clarification.

Procedures not used by FSIS laboratories were removed from the method including references to Fraser Broth and optional tests such as tumbling motility testing and the CAMP test.

MLG 8A “FSIS Procedure for the Use of a *Listeria monocytogenes* Polymerase Chain Reaction (PCR) Screening Test” will be archived as the rapid molecular screen procedure is now included in MLG 8.

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Procedure Outline

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8.1 Introduction

The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use. FSIS utilizes the following performance criteria when evaluating the suitability of an alternative laboratory method or product for a given analyte and sample matrix pair:

- Sensitivity of 90% or greater
- Specificity of 90% or greater
- Accuracy of 90% or greater
- Positive predictive value of 90% or greater
- Negative predictive value of 90% or greater

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Performance criteria are relative to the reference cultural method for that analyte and sample matrix as outlined in the corresponding MLG chapter. Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled “FSIS Guidance for Evaluating Test Kit Performance.”

Listeria monocytogenes is a gram-positive rod-shaped bacterium associated with a variety of environments including soils, water, sewage, silage, as well as plant and animal food products. Although reported cases of human foodborne listeriosis are rare, the incidence of serious illness and death in affected individuals is high. Immunocompromised individuals, pregnant women, neonates and the elderly are particularly vulnerable.

Among all species in the genus *Listeria*, only *Listeria monocytogenes* is typically implicated in human foodborne illness. The method described below employs well-established media and tests for the isolation and specific identification of β -hemolytic *Listeria monocytogenes*. The method is broadly applicable to raw or cooked ready-to-eat (RTE) red meat, poultry products, egg products, RTE Siluriformes (fish) and environmental sponge samples.

8.2 Safety Precautions

CDC guidelines for the handling of BioSafety Level 2 organisms should be followed whenever live cultures of *Listeria* are used. The Safety Data Sheet (SDS) may be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the analysis. The personnel who will handle the material should read the SDS prior to startup.

Laboratories should develop a policy and inform pregnant women and potentially immunocompromised individuals of the risk from working in laboratory rooms or areas where *Listeria monocytogenes* isolation or identification procedures are in progress.

8.3 Quality Control Procedures

The correct performance of all stages of the analysis must be verified through the use of appropriate controls. One *Listeria monocytogenes* positive control and one uninoculated media control are required for each set of concurrently analyzed samples. In the absence of a positive test sample, control cultures may be terminated at the same point as the sample analyses.

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- a. The positive control culture should be inoculated into an appropriate matrix at a low inoculum level, e.g., by preparing a test organism suspension in broth or saline equivalent in turbidity to a 0.5 McFarland standard. Using a 1ul loop, inoculate the broth or streak the plates to be tested. Alternatively, commercially prepared bacterial pellets may be used. The laboratory may use tagged cultures such as those that visibly fluoresce under ultraviolet (UV) light to differentiate QC strains from true contaminants if available. Once the control cultures are started, incubate the controls along with the samples, and analyze them in the same manner as the samples.
- b. For the uninoculated control, use an aliquot of UVM broth. For all subsequent uninoculated control tests, use one unit of the medium at the volume specified for the test. Investigate the source of any contaminating organisms.
- c. Specific control requirements for each confirmatory test are addressed in the appropriate sections of this protocol.
- d. Additional control cultures may be employed for individual tests or the entire sequence of analysis at the discretion of the laboratory.

8.4 Equipment, Supplies, Media, Reagents, Test Systems and Cultures

8.4.1 Equipment

- a. Electronic top-loading balance capable of weighing a minimum of 25 ± 0.1 g (500 g capability recommended)
- b. Paddle blender or blade type blender with sterilized blender cutting assemblies and jars
- c. Incubator, $30 \pm 2^\circ\text{C}$
- d. Incubator, $35 \pm 2^\circ\text{C}$
- e. Vortex mixer
- f. UV lamp, blue light excitation 475-495 nm light
- g. 3M™ Molecular Detection System
 - 3M™ Molecular Detection Heat Block Insert MD SHBIN
 - 3M™ Molecular Detection Speed Loader Tray MD SSLT
 - 3M™ Molecular Detection Cap/ Decap Tool-Lysis
 - 3M™ Molecular Detection Chill Block Insert MD SCBIN held at $20\text{-}25^\circ\text{C}$
 - VWR Digital 2 Block Heater or equivalent set at $100 \pm 1^\circ\text{C}$

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- Pipettor to deliver 20 µL and sterile disposable filter tips
- Eight channel Pipettor to deliver 20 µL and sterile disposable filter tips

8.4.2 Supplies

- a. Supplies for all samples:
 - i. Sterile tablespoons, scissors, forceps, knives, glass stirring rods, petri dishes, test tubes, bent glass rods ("hockey sticks") as needed
 - ii. Sterile, filter or non-filter bags
 - iii. Non-filter plastic bags for double-bagging sample homogenates (optional)
 - iv. Pipettor and sterile disposable tips for dispensing 100 µl and 1 ml
 - v. Disposable plastic and/or platinum inoculating needles and loops
 - vi. Sterile cotton-tipped applicators (i.e. swabs)
- b. Additional supplies necessary for environmental sponge samples:
Non-bactericidal sampling sponges, polyurethane or cellulose
- c. Additional supplies necessary for environmental aqueous chilling solutions:
 - i. 0.45 µm hydrophobic grid membrane filter system
 - ii. Vacuum flask/hose and vacuum source
 - iii. Sterile disposable scalpels
 - iv. Sterile forceps
 - v. Whirl-pak filter bag
 - vi. 2.7µm Glass fiber filter
 - vii. 500 ml graduated polypropylene beaker with handle
 - viii. Sterile 500 - 1000 ml non-polystyrene sample container

8.4.3 Media

Refer to the MLG Media Appendix for formulations and preparation instructions.

- a. Media required for enrichment, plating and preliminary confirmation tests:
 - i. Modified University of Vermont broth (UVM, also known as UVM1)
 - ii. Morpholinepropanesulfonic acid-buffered *Listeria* enrichment broth (MOPS-BLEB)

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- iii. Modified Oxford agar (MOX)
- iv. Horse blood overlay agar (HL, also known as HBO)
- v. Trypticase soy agar with 5% sheep blood (TSA-SBA)
- vi. Brain Heart Infusion agar slants (BHI)

8.4.4 Reagents and Test Systems

- a. Biochemical test panel (VITEK® 2 Compact System or equivalent)
- b. Ribosomal RNA-based test system (GenProbe Accuprobe® *L. monocytogenes*-specific test) or equivalent *Listeria monocytogenes*-specific genetic test system
- c. 3M™ Molecular Detection Assay 2 – *Listeria monocytogenes*

8.4.5 Cultures

- a. At least one *Listeria monocytogenes* positive control strain is required. Appropriate cultures include *L. monocytogenes* Microbiologics 01248UV-V (requires blue light excitation 475-495 nm light), ATCC® 19111, NCTC 7973 or other *Listeria monocytogenes* cultures validated to perform in an equivalent manner.
- b. *Listeria innocua* negative control strain is required for GenProbe.

8.5 Detection and Isolation Procedures

8.5.1 Sample preparation

- a. Meat, poultry, and RTE Siluriformes products: A 25 ± 1 g portion or a 125 ± 5 g portion is analyzed for raw and processed red meat and poultry product testing. A 25 ± 1 g portion is used for pasteurized egg products testing.
 - i. Intact retail packages must be disinfected at the incision sites immediately prior to incision for sampling. Appropriate disinfectants include but are not limited to ca. 3% hydrogen peroxide, ca. 70% ethanol or ca. 70% isopropanol. If the package does not appear to be clean, scrub gently using soapy water and rinse thoroughly prior to disinfection. A sterile scalpel may be

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helpful for cutting the packaging. Aseptically pull the packaging away to expose the product for sampling.

Note: For RTE sausages in casing, the shell/casing is an integral part of the sample and should be free of pathogens and toxins. The casing is not to be disinfected since some casings are permeable and the disinfectant may be introduced into the core of the product. In addition consumers often slice through an inedible casing and then remove it thus any contamination on the surface of the casing could be transferred to the edible core of the product.

- ii. Using a sterile scalpel, knife, spoon, chisel or other tool, cut small pieces from representative sites on the sample to prepare a composite sample. While multiple packages of a product are usually submitted, for large products a single package may be submitted.

For multi-component ready-to-eat products, follow the appropriate sample preparation instructions listed below:

If the meat, poultry or Siluriformes component is separate and distinct from other non-meat ingredients, analyze only the representative meat/poultry portion of the RTE product. Examples include products with the meat/poultry/Siluriformes portion separate from any vegetable/dessert component, or fajita kits with meat/poultry/Siluriformes, onions/peppers and tortillas in three separate internal packages/bags within an outer package.

When meat/poultry/Siluriformes is combined with other ingredients to form the product (e.g., beef stew containing vegetables, potatoes, etc.), analyze representative meat/poultry/Siluriformes portions in combination with other ingredients.

- iii. Place the test portion in a sterile bag (i.e. filter bag recommended). If necessary, double-bag with a non-filter bag prior to stomaching (e.g. zip-lock bag).

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- iv. If analysis of the test portion is not to be initiated within 1 h, store at $\leq -10^{\circ}\text{C}$. Do not dilute the sample until ready to initiate analysis.
 - v. For analysis, proceed to Section 8.5.2.
- b. Outbreak/recall samples: Some samples or sample lots, particularly those implicated in foodborne illness outbreaks, may require analysis of up to thirteen 25 g test portions. The test portion should be a composite representative of the entire sample or available samples common to a specific lot. The need for multiple subsample analyses must be determined on a case-by-case basis, or by client requirements.
- c. Most Probable Numbers (MPN) Determination: Follow MPN instructions given in the specific program protocol or see MLG Appendix 2, Most Probable Number Procedure and Tables.

8.5.2 Primary Enrichment in UVM broth

Table 1. Sample Preparation and Enrichment Guide

Product	Sample Preparation		Incubation
	Portion Size	Enrichment Amount Determined by volume or weight	Cultural or rapid screen
Meat, poultry, Siluriformes, and egg products	25 ± 1 g	225 ± 5 ml UVM broth.	$30 \pm 2^{\circ}\text{C}$ for 20 - 26 h
For programs allowing compositing of five product subsamples	125 ± 5 g	1125 ± 25 ml UVM broth	$30 \pm 2^{\circ}\text{C}$ for 23 - 26 h
Environmental Sponge samples	1 single sponge	225 ± 5 ml UVM broth	$30 \pm 2^{\circ}\text{C}$ for 20 -26 h
	multiple sponges	100 ± 2 ml of UVM <u>per sponge</u>	$30 \pm 2^{\circ}\text{C}$ for 20 -26 h
Environmental aqueous chilling solutions and surface rinse solutions	500 ± 2 ml of sample solution filtered	225 ± 5 ml UVM broth	$30 \pm 2^{\circ}\text{C}$ for 20 - 24 h

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- a. For all meat, poultry, Siluriformes and egg product samples (including outbreak/recall samples):
To a 25 ± 1 g test portion, dispense 225 ± 5 ml (or 225 ± 5 g) of UVM broth. To a 125 ± 5 g test portion, dispense 1125 ± 25 ml (or 1125 ± 25 g) of UVM broth. Stomach or blend for approximately 2 minutes. If blended, aseptically transfer the homogenate to an appropriate sterile container. Incubate 25 ± 1 g test portions at $30 \pm 2^\circ\text{C}$ for 20 - 26 h. Incubate 125 ± 5 g test portions at $30 \pm 2^\circ\text{C}$ for 23 - 26 h. Proceed to Section 8.5.3.

Note: Dried products such as soup mixes may require a sample/broth dilution greater than 1:10 because of physical difficulties encountered by absorption of broth by dehydrated product.

- b. For environmental sponge samples:

Add 225 ± 5 ml (or 225 ± 5 g) of UVM broth to each bagged single sponge sample or add 100 ± 2 ml of UVM per sponge to each bagged composite sponge sample that contains up to five sponges. For example, a composite of five sponge samples would require 500 ± 10 ml of UVM. Stomach for 2 ± 0.2 min. Hand mixing is an acceptable alternative for stomaching. To hand mix, briefly massage each sponge to expel the collection broth into the UVM broth.

Incubate at $30 \pm 2^\circ\text{C}$ for 20 -26 h. Proceed to Section 8.5.3.

- c. For environmental aqueous chilling solutions and surface rinse solutions:

Aqueous chilling solutions may include water, brine and propylene glycol solutions.
- i. Pour 500 ± 2 ml of sample solution into a filter bag. Filter the solution by pouring it through a glass fiber filter and a $0.45 \mu\text{m}$ hydrophobic grid membrane filter in a vacuum filter system. These filters can be easily clogged by particulates. Therefore, more than one filter may be necessary to filter the entire test portion. When the sample has

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been filtered, aseptically remove the glass filter and transfer it back to the filter bag for enrichment.

- ii. Aseptically remove the membrane from the membrane filter plastic housing and transfer to the same filter bag as above. A sterile scalpel may be used to cut around the circumference of the hydrophobic grid membrane to excise it from the filter housing. All membranes associated with a given sample are combined in the single enrichment bag.
- iii. Add 225 ± 5 ml (or 225 ± 5 g) of UVM broth. Stomach 2 ± 0.2 minutes. Ensure that the filters are submerged. Incubate the homogenate at $30 \pm 2^\circ\text{C}$ for 20 - 24 h. Proceed to Section 8.5.3.

8.5.3 Secondary Enrichment in MOPS-BLEB and Direct MOX Plating of UVM

- a. Transfer 0.1 ± 0.02 ml of the UVM enrichment to 10 ± 0.5 ml of MOPS-BLEB. Incubate inoculated MOPS-BLEB tubes at $35 \pm 2^\circ\text{C}$ for 18-24 h. Proceed to Section 8.5.4 for use of the rapid screen assay on MOPS-BLEB tubes or refer to Section 8.5.5.b to continue the cultural analysis.
- b. Streak a MOX plate directly from the primary UVM enrichment. Streak a loopful or a drop approximating 0.1 ml of the UVM over the surface of the plate for isolation. Alternatively, dip a sterile cotton-tipped applicator or equivalent into the UVM and swab 25-50% of the surface of a MOX plate. Use a loop to streak for isolation from the swabbed area onto the remainder of the plate. Incubate the direct MOX at $35 \pm 2^\circ\text{C}$ for 26 ± 2 h. Proceed to Section 8.5.5.a for examination of direct MOX plates.

8.5.4 Rapid Screening *Listeria monocytogenes* Test Procedure

Following incubation of MOPS-BLEB, perform the rapid screen on the secondary enrichment using the current 3M™ Molecular Detection System User guide for preparing reagents, performing the remainder of the assay, and reading the results.

- a. Samples that are rapid screen-negative will be reported as negative for *Listeria monocytogenes* if the concurrent 26-hour direct (UVM-streaked) MOX plate is negative. Cultural analysis will continue on samples that are

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rapid screen-negative but have typical colonies on the 26-hour direct MOX plates or have a rapid screen positive or inconclusive result.

- b. Alternatively, for samples with a rapid screen-inconclusive or an invalid result, the laboratory may investigate. Based on the findings, the laboratory may analyze the inconclusive result samples by:
 - repeating the rapid screen analysis from the lysate step or
 - preparing new rapid screen lysate tubes and repeating the analysis
- c. In analytical runs where the positive control results do not show as positive, all samples are affected and an investigation shall be performed. Based on the findings the laboratory may analyze the samples by:
 - repeating the rapid screen analysis from the lysate step
 - preparing new rapid screen lysate tubes and repeating the analysis or
 - analyzing all of the samples culturally.
- d. If reanalysis of an inconclusive rapid screen sample is unsuccessful, repeat sample preparation from the sample reserve or discard the sample. If circumstances (e.g. a power outage or equipment failure) do not allow testing using the rapid screen system, the laboratory shall complete the cultural method by streaking all samples and controls from MOPS-BLEB medium onto MOX plates per MLG 8 Section 8.5.5.b then proceed with all isolation and purification procedures.

8.5.5 Examination of Direct MOX and MOX Plating of MOPS-BLEB

- a. Examine the direct (UVM-streaked) MOX for colonies typical of *Listeria* spp. At 26 ± 2 h, suspect colonies are typically small (ca. 1 mm) and are surrounded by a zone of darkening due to esculin hydrolysis.
 - i. If suspect colonies are present on direct MOX, transfer suspect colonies to HL agar as described in Section 8.5.6 to proceed with isolation and purification procedure.
 - ii. If there are no typical colonies on 26 hour-incubated direct MOX plate and the rapid screen result is negative, then the direct branch result is negative.

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- iii. If there are no typical colonies on the direct MOX plate but the rapid screen result is positive/inconclusive or no rapid screen was performed, then re-incubate the MOX plate for an additional 26 ± 2 hour.

After re-incubation, suspect colonies are transferred to HL agar as described in Section 8.5.6. The direct branch result is negative if there are no typical colonies on MOX after re-incubation.

- b. After 18-24 h of secondary enrichment in MOPS-BLEB at $35 \pm 2^\circ\text{C}$, rapid screen positive samples are streaked from MOPS-BLEB onto MOX. Inoculate a MOX plate for isolation using a loopful of the MOPS-BLEB, or by streaking a drop approximating 0.1 ml MOPS-BLEB, or aseptically dip a sterile cotton-tipped applicator or equivalent into the MOPS-BLEB and swab 25-50% of the surface of a MOX plate.

After inoculating the plate, use a loop to streak for isolation from the swabbed area onto the remainder of the plate. Then, incubate the secondary enrichment MOX plate at $35 \pm 2^\circ\text{C}$ for 26 ± 2 hours.

- i. If typical colonies are present on the secondary enrichment MOX (small colonies surrounded by a zone of darkening due to esculin hydrolysis), transfer suspect colonies to HL agar as described in Section 8.5.6 to proceed with isolation and purification procedure.
- ii. If no suspect colonies are evident on secondary enrichment MOX plate, then re-incubate the MOX plate for an additional 26 ± 2 hour. After re-incubation, suspect colonies are transferred to HL agar as described in Section 8.5.6. The secondary enrichment result is negative if there are no typical colonies on MOX after re-incubation.
- c. If no suspect MOX and/or HL colonies have been demonstrated on the direct (UVM streaked) MOX plate or the secondary enrichment (MOPS-BLEB streaked) MOX plate, the sample is considered negative for *Listeria monocytogenes*.

8.5.6 Isolation and Purification Procedures

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- a. If suspect colonies are present on MOX from any source, use a loop or equivalent sterile device to contact a minimum of 20 (if available) suspect colonies and collectively streak for isolation on one or more HL agar plates. Alternatively, a swipe of suspect growth representing at least 20 colonies may be used. Incubate the streaked HL at $35 \pm 2^{\circ}\text{C}$ for 22 ± 4 h.
- b. After incubation, examine the HL plate(s) against backlight for translucent colonies surrounded by a small zone of β -hemolysis.
 - i. If at least one suspect colony is clearly isolated, proceed to confirmatory testing (Section 8.6 below). Hold all HL plates containing suspect colonies (room temperature or refrigeration) until confirmatory testing is complete.
 - ii. If suspect colonies or β -hemolytic growth are present on HL but not clearly isolated, re-streak representative suspect colonies/growth onto one or more fresh HL plates and incubate per section 8.5.6.a.
 - iii. If no suspect isolates are present on HL, pursue follow-up of MOX and/or HL isolates from other branches of analysis (e.g. Rapid screen follow-up vs. UVM Primary Enrichment streak follow-up). If no branch of the analysis produces suspect β -hemolytic colonies on HL, the sample may be reported as negative for *L. monocytogenes*.

Note: Removal of a few colonies may assist the analyst in observing medium clearing for weakly hemolytic strains.

8.6 Confirmation and Identification Procedures

Confirmatory identification of *L. monocytogenes* consists of preliminary confirmation tests (Section 8.6.1) followed by biochemical tests (Section 8.6.2). Genetic tests (Section 8.6.3) may be required in certain circumstances. All confirmatory identification tests require a pure culture. A *L. innocua* negative control must also be analyzed concurrently with the *L. monocytogenes* positive control during GenProbe identification.

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8.6.1 Preliminary Confirmation Tests for *Listeria* spp.

a. Inoculation of preliminary confirmation test media:

Pick one typical isolated colony from HL and streak onto TSA-SB or equivalent for inoculation of biochemical test systems. Incubate TSA-SB at $35 \pm 2^\circ\text{C}$ for 16-24 hr. If typical isolated colonies are not obtained on HL, restreak on HL for isolation. In addition, media required for inoculation of biochemical test systems (e.g. HL, TSA-SB or equivalent) must be inoculated from the same colony or growth subcultured from that colony. A minimum of one colony must be confirmed. If the first selected suspect HL colony does not confirm as *L. monocytogenes*, confirmation must be attempted for additional suspect HL colonies, if available, until at least three isolates from the test portion have failed confirmation.

Incubate the optional HL purity streak plate at $35 \pm 2^\circ\text{C}$ for 22 ± 4 h. Proceed to Section 8.6.2.

b. Purity streak test (optional):

Examine the re-streaked HL plate for consistent morphological characteristics. The colonies on this second HL plate should represent a single clone. If the culture appears to be mixed, repeat Steps 8.5.6.b.ii. A pure culture is required for all confirmatory tests.

8.6.2 Biochemical Tests

Using a pure culture, perform confirmatory biochemical tests. Commercially available test systems (e.g. VITEK[®] 2 Compact System) or validated equivalent systems, including well-established schemes involving traditional tube biochemical media (e.g. Compendium of Methods for the Microbiological Examination of Foods, Bacteriological Analytical Manual), may be employed. However, exercise caution in interpreting the identification of atypical *Listeria* spp. isolates when using biochemical systems. Cultures identified as “*L. monocytogenes/innocua*” or any beta-hemolytic *Listeria* spp. that is biochemically indeterminate or identified as *L. innocua* must be further characterized using a ribosomal RNA-based test system as described in Section 8.6.3.

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To use the VITEK[®] 2 Compact System for biochemical testing:

- a. If using UV positive controls, perform a fluorescence check on the HL or TSA-SB plate for the positive control and presumptive positive sample following incubation. Use long wave UV light to examine plates for purity and evidence of cross contamination with the positive control. Only the positive control culture should fluoresce. If the presumptive positive sample TSA-SB plates are pure and uncontaminated, perform the biochemical test.
- b. Follow the instructions provided by the manufacturer for inoculation and loading the VITEK[®] 2 Compact System.
- c. TSA-SBA or HL agar may be used for preparing the inoculum suspension. All growth on the agar of choice must represent the same clone.
- d. At a minimum, one *L. monocytogenes* positive control must be analyzed concurrently with sample isolates.

8.6.3 Genetic Identification Tests

Ribosomal RNA-based tests or equivalent *L. monocytogenes* specific genetic tests

Ribosomal RNA-based tests (e.g. GenProbe) may be performed as a confirmatory test for all biochemically identified *L. monocytogenes* strains or on typical HL colonies (see Section 8.5.6) prior to conducting confirmatory procedures on positive colonies. A ribosomal RNA-based test is required to resolve the identity of any atypical suspected *L. monocytogenes* strain. In some circumstances, available phenotypic tests cannot clearly distinguish strains of *L. monocytogenes* from *L. innocua*. In particular, rhamnase-negative β -hemolytic *L. monocytogenes* and phospholipase C-negative, weakly hemolytic *L. monocytogenes* can be misidentified as *L. innocua* on biochemical testing. Some β -hemolytic *L. innocua* strains exist. Several *L. monocytogenes*-specific ribosomal RNA-based tests are commercially available.

Follow the test kit instructions provided by the manufacturer for performing and interpreting these tests.

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A *L. monocytogenes* positive control, a *L. innocua* negative control and an uninoculated sterility control must be analyzed concurrently with sample isolates.

A positive ribosomal RNA-based test result from either of the above test systems indicates that atypical phenotypes are confirmed *L. monocytogenes*. A negative result indicates that atypical phenotypes are not *L. monocytogenes* and reported as negative.

8.7 Culture Storage and Shipment

- a. BHI slants may be used for short-term storage of *Listeria* spp. The culture should be stabbed into the agar using an inoculating needle. Tubes should be sealed with Parafilm® or equivalent to prevent desiccation and stored at 2-8°C. Under these conditions, *Listeria* spp. can remain viable for many months.
- b. For long-term storage (i.e. for more than one year) or to assure that the genetic character of the strain does not change over time (e.g. lose plasmids or other unstable genetic elements), cultures should be lyophilized and/or frozen at -20 to -80° C. Fetal calf serum or commercially available cryobead products are appropriate media for frozen storage of *Listeria* spp.

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