United States Department of Agriculture

Food Safety and Inspection Service

MLG 8.15

Isolation and Identification of *Listeria monocytogenes* and *Listeria spp.* from Ready-to-Eat Meat, Poultry, Siluriformes (Catfish), Egg Products, and Environmental Samples

This method describes the laboratory procedure for performing the isolation and identification of *Listeria monocytogenes* and *Listeria spp.* from Ready-to-Eat Meat, Poultry, Siluriformes (Catfish), Egg Products, and Environmental Samples.

Notice of Change

This method has been revised to address new developments in laboratory procedures for performing the isolation and identification of *Listeria monocytogenes* and *Listeria spp.* from Ready-to-Eat Meat, Poultry, Siluriformes (Catfish), Egg Products, and Environmental Samples.

In a continued effort to modernize laboratory technologies, multiple components of this method have been updated.

- Horse Blood Overlay (HBO) agar, a differential media, and Modified Oxford (MOX) agar, a selective media, have been replaced with Harlequin® *Listeria* Chromogenic Agar (HLCA), a media that is both selective and differential for *L. monocytogenes.* HLCA differentiates *Listeria* from other organisms and differentiates *Listeria monocytogenes* from other *Listeria* species.
- Modified University of Vermont broth (UVM) and Morpholinepropanesulfonic acid-buffered *Listeria* enrichment broth (MOPS-BLEB) have been replaced with LPT broth. This change reduces the enrichment process to a single step instead of having a two-step primary and secondary enrichment process.

Combined, these changes to the enrichment broth and plating media have decreased the reporting time for most routine negative samples and increased laboratory efficiency through streamlined workflows and enhanced colony morphology determination. These changes will also provide a *Listeria* genus result to enhance sanitation verification in the post-lethality RTE processing environment.

This update will not affect the communication of presumptive positive or confirmed positive *Listeria monocytogenes* results to stakeholders.

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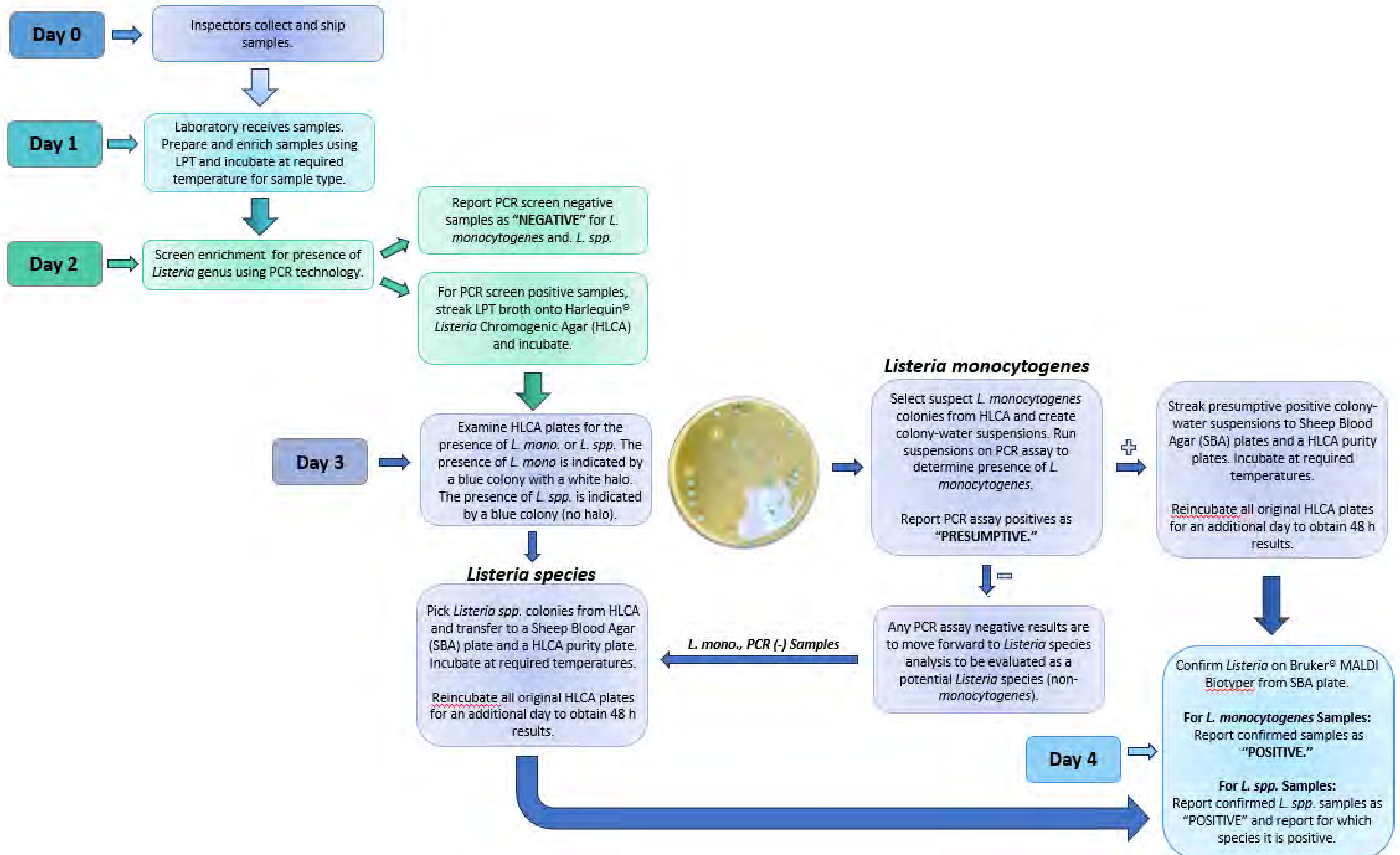
Isolation of *Listeria* Method Flow Chart



Introduction

Listeria spp. has an optimal growth temperature range of 30-37°C, but it can also withstand freezing. At refrigeration temperatures, *Listeria* spp. forms a biofilm to protect itself from environmental stress. This biofilm also protects the pathogenic organism from many common cleaners and disinfectants, making it difficult to fully eradicate from an environment, such as in a food processing plant. For this reason, the Food Safety and Inspection Service (FSIS) routinely tests environmental samples from food processing establishments to monitor for any instances of *Listeria* spp. in the establishments or on food contact surfaces.

Listeria monocytogenes is a member of the *Listeria* genus, which includes other *Listeria* species such as *L. ivanovii, L. seeligeri, L. innocua,* and *L. welshimeri.* Only *L. ivanovii* and *L. monocytogenes*, both virulent strains, cause disease in animals. However, only *L. monocytogenes* typically causes disease in humans, which can be life-threatening. *L. monocytogenes* contains four evolutionary lineages (I, II, III, and IV), although most clinical and foodborne isolates of *L. monocytogenes* come from lineages I and II. Lineages III and IV are most commonly isolated from animal origins due to a difference in phenotypic and genetic characteristics between lineages I/II and III/IV. These differences also directly impact each lineage's ability to cause foodborne human illness, a primary concern of the Food Safety and Inspection Service. Other *Listeria* species share similar growth characteristics with *Listeria monocytogenes*. The genus *Listeria* includes 28 species, and while only *L. monocytogenes and L. ivanovii* are pathogenic, the other species share common phenotypic characteristics such as the ability to grow at low temperatures and flagellar motility. These shared characteristics make *Listeria* spp. a useful indicator for the presence of *L. monocytogenes* in food safety testing.

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 among affected individuals is high. Immunocompromised individuals, pregnant women, neonates and the elderly are particularly vulnerable.

Among all 28 known 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 *Listeria monocytogenes*. The method is broadly applicable to ready-to-eat (RTE) red meat, poultry products, egg products, RTE Siluriformes (catfish), and environmental sponge samples. The method also targets other *Listeria* species, identifying them not as adulterants but as indicators of sanitation verification in post-lethality RTE processing environments.

Figure 1: Simple flowchart outlining the daily workflow of the *Listeria* detection method.

Safety Precautions

CDC guidelines for the handling of Biosafety Level 2 organisms must be followed whenever live cultures are used. The Safety Data Sheet (SDS) must be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the analysis. The personnel handling the material are to read the SDS prior to startup. See the Biosafety Chart at the end of the chapter for more information.

Laboratories are to 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.

QUALITY CONTROL

Lab Quality Control Guidelines

Include at least one positive *Listeria monocytogenes* control, (fluorescent strain of *Listeria monocytogenes*, commercially available), one *Listeria innocua* control (commercially available), and one uninoculated medium control. Incubate the controls along with the samples and analyze in the same manner as the samples.

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.

Each step of the analysis requires the use of appropriate controls to verify that the results are valid. Biochemical kit and rapid test manufacturers will specify control cultures for use with their products. If not specified, quality control procedures for biochemical tests and test media will include cultures that will demonstrate pertinent characteristics of the product.

Additional control cultures may be employed for individual tests or the entire sequence of analysis at the discretion of the laboratory.

Equipment, Reagents, Media, and Cultures

Table 1: Equipment for MLG 8

Equipment	Supplier	Purpose
Vacuum flask/hose and vacuum source	General lab supplier	Environmental aqueous chilling solutions preparation
Balance, sensitivity to at least 0.1 g	General lab supplier	Weigh samples
Blending/mixing equipment: Paddle blender or equivalent	General lab supplier	Homogenize samples
Vortex Mixer	General lab supplier	Vortex/mix samples
Incubators, 35 ± 1°C	General lab supplier	Incubation of enriched samples
Incubators, 35 ± 2°C	General lab supplier	Incubation of Sheep Blood Agar plates
Incubators, 37 ± 1°C	General lab supplier	Incubation of Harlequin® *Listeria* Chromogenic Agar plates
Refrigerator (2 - 8°C)	General lab supplier	Store media and sample reserves
Heating Block, (100 ± 1°C)	General lab supplier	Prepare sample DNA
Cooling Block (20 - 25°C)	General lab supplier	Prepare sample DNA
Neogen® Molecular Detection System	Neogen®, Model # MDS100	Screen enrichment
UV lamp, blue light excitation 475-495 nm light	General lab supplier	Detect fluorescence in blood plates
Bruker® MALDI Biotyper	Bruker® Inc.	Proteomic Confirmation

Table 2: Kits and Reagents for MLG 8

Kits and Reagents	Supplier	Purpose
Neogen® Molecular Detection Assay 2 - *Listeria monocytogenes*	Neogen®, Catalog # MDA2LMO96	Screen HLCA picks for *Listeria* analyses
Neogen® Molecular Detection Assay 2 - *Listeria*	Neogen®, Catalog # MDA2LIS96	Screen enrichment for *Listeria* analyses
Bruker® MALDI Biotyper reagents	Bruker® Inc. or General supplier	Proteomic Confirmation

Table 3: Supplies

Supplies	Supplier	Purpose
Sterile inoculating loops	General lab supplier	Spread/streak plates
Pipettor and sterile filter tips for 20 µL volume	General lab supplier	Add and mix reagents
Sterile disposable scalpels	General lab supplier	Sample preparation
Whirl-Pak® filter bags	General lab supplier	Sample preparation
2.7 µm Glass fiber filter	General lab supplier	Environmental aqueous chilling solutions preparation
0.45 um hydrophobic grid membrane filter system	General lab supplier	Environmental aqueous chilling solutions preparation
500 mL graduated polypropylene beaker with handle	General lab supplier	Environmental aqueous chilling solutions preparation
Sterile 500 - 1000 mL non-polystyrene sample container	General lab supplier	Environmental aqueous chilling solutions preparation
Sterile spoons, scissors, forceps, and knives	General lab supplier	Sample preparation
Stomacher bags	General lab supplier	Environmental aqueous chilling solutions preparation

Media required for enrichment, plating, and preliminary confirmation tests:

Media formulations are available in MLG Appendix 1, Media and Reagents.

1. bioMèrieux® LPT *Listeria* enrichment broth
2. Harlequin® *Listeria* Chromogenic Agar (HLCA)
3. Trypticase soy agar with 5% sheep blood (SBA)
4. Brain Heart Infusion agar slants (BHI)

Cultures and Controls

1. *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.
2. *Listeria innocua* control strain, such as ATCC® 33090, as a control for *Listeria spp*.

Method Overview

This procedure outlines the steps to screen and isolate *Listeria* from RTE meat, poultry, pasteurized egg products, siluriformes, and environmental sponges. Enriched samples are screened through Neogen® Molecular Detection System, a rapid screening technology. Samples that test positive are isolated through plating media. Isolates are then confirmed by Bruker® MALDI Biotyper.

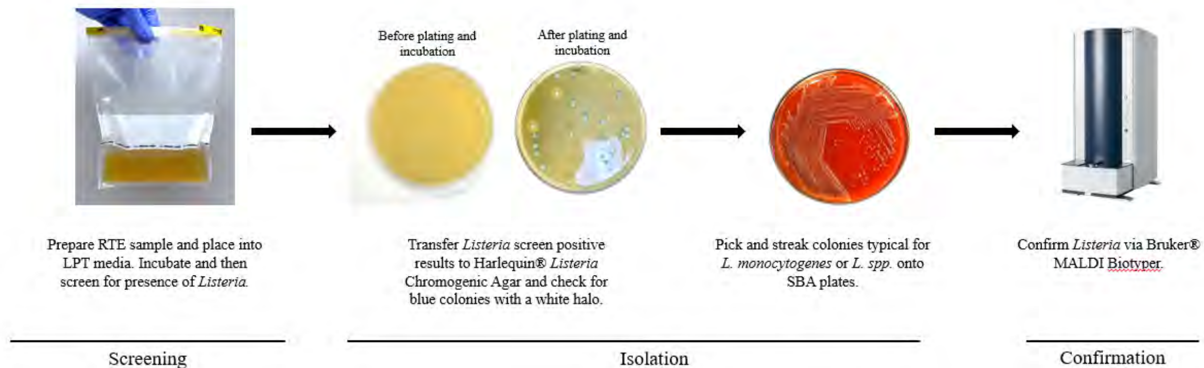


Figure 2: Overview of the steps for isolating *Listeria* from laboratory samples (Photo Credit: Leo Gude, Julie Lynn, Sherre Chambliss, and Makala Schulte).

Preparing Samples for Enrichment

QUALITY CONTROL

Preparation of Sample Enrichment Controls

Tagged *L. monocytogenes* and *L. innocua* reference strains that visibly fluoresce under UV light will differentiate QC culture control strains from true contaminants. Include controls with each sample batch for LPT broth control. Prepare a test organism suspension in broth or saline, equivalent in turbidity to a 0.5 McFarland standard. Using a 1 µL loop, inoculate the broth or streak the plates to be tested. Alternatively, use commercially prepared bacterial pellets. Confirm at least one isolate from each control sample. In the absence of a positive test sample, control cultures will be terminated at the same point as the sample analyses.

For the uninoculated control, use an aliquot of LPT 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.

Sterilize and Prepare the Package for Cutting

1. If the package does not appear to be clean, scrub gently using soapy water and rinse thoroughly prior to disinfection.
2. Disinfect retail packages at the incision sites immediately prior to incision for sampling using an appropriate disinfectant, e.g., 3% hydrogen peroxide, ca. 70% ethanol, or ca. 70% isopropanol.
3. Ready-to-eat (RTE) sausages in casing, such as kielbasas or other sausages that have plastic/paper casings, are to be prepared in a way that protects the integrity of the casing. The shell/casing is an integral part of the sample and must be free of pathogens and toxins. 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. Do not disinfect the casing since some casings are permeable and the disinfectant can be introduced into the core of the product.
4. Use a sterile scalpel or equivalent to cut the packaging. Aseptically pull the packaging away to expose the product for sampling and cut small pieces from representative sites on the sample to prepare a composite sample.

KEY FACT

Listeria monocytogenes is commonly found in the environment and can cross-contaminate food contact surfaces and foods. It is a particular hazard of concern in RTE products that are exposed to the environment after lethality treatment. Improper sanitation, product handling, and employee practices at processing establishments can lead to the transfer of *Listeria monocytogenes* from the environment to the surface of the product.

Cutting the Samples for Enrichment

1. Prepare samples for enrichment using Table 4. Follow additional program requirements for preparing sample and subsample composites. Outbreak samples will require different sample preparation. Follow customer specifications.
    a. *Listeria* enrichment is primarily used to verify for post lethality cross-contamination, hence why an analyst should maximize a product’s available surface area. Since the *L. monocytogenes* sample is prepared by maximizing the surface area of the product, it is to be prepared before the *Salmonella* sample, which uses the interior of the product.

b. Multi-component RTE Products:

i. **Non-Comminuted Samples**
If the meat, or poultry 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 portion separate from any vegetable/dessert component, or fajita kits with meat/poultry, onions/peppers, and tortillas in three separate internal packages/bags within an outer package.

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

2. Add ambient LPT broth and stomach approximately 2 minutes.
3. Incubate samples according to Table 4.
4. Proceed to the section entitled "Rapid Screening *Listeria* Test Procedure" for use of the rapid screen.

Table 4. 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 (including multi-component RTE products)	25 ± 1 g	225 ± 5 mL LPT broth	35 ± 1°C for 22 - 26h
For programs allowing compositing of five product subsamples	125 ± 1 g	375 ± 5 mL LPT broth	35 ± 1°C for 24 - 28h
Environmental Sponge samples	1 single sponge	225 ± 5 mL LPT broth	35 ± 1°C for 18 - 24h
	Multiple sponges	100 ± 2 mL of LPT per sponge	35 ± 1°C for 18 - 24h
Environmental aqueous chilling solution and surface rinse solutions	500 ± 2 mL of sample solution filtered	225 ± 5 mL LPT broth	35 ± 1°C for 18 - 24h

Meat, Poultry, RTE Siluriformes, and Egg Products

Figure 3: Example of 25 g of Lm product in a sterile bag (Photo Credit: Leo Gude and Sherre Chambliss).

Figure 4: Liquid whole egg products (Photo Credit: Leo Gude and Sherre Chambliss).

Weigh 25 ± 1 g of the product into a sterile polypropylene bag. Add a small portion of the ambient temperature sterile LPT and mix to obtain a homogeneous suspension. Add the remainder of the 225 ± 5 mL LPT. Mix until a lump-free suspension is obtained.

Incubate at 35 ± 1°C for 22 – 26 h.

Multi-Component RTE Products

Figure 5: A multi-component RTE product (Photo Credit: Leo Gude and Sherre Chambliss).

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.

Surface Sampling

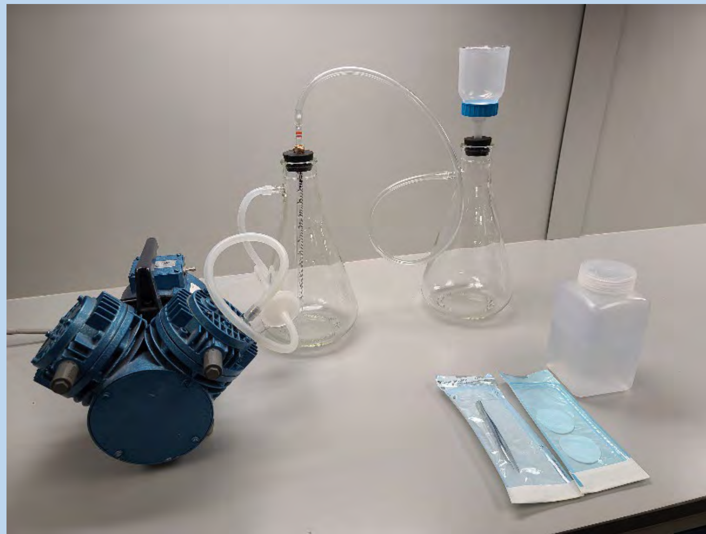
Figure 6: Examples of RTE products that require surface sampling (Photo Credit: FSIS Western Lab).

For intact RTE samples (examples: whole prosciutto, whole deli products, sausages, and hotdogs), cut thin slices from the surface of the product to maximize surface area sampled. Generally, for longer food products, slices cut longitudinally rather than horizontally will achieve maximum surface area. Do not cut deep into the tissue since *Listeria* is typically a post processing contamination event. Reserve the interior product for the *Salmonella* analysis which verifies under processing where interior product is more important.

Add 225 ± 5 mL (or 225 ± 5 g) of LPT broth to the bag.

Incubate at 35 ± 1°C for 22 – 26 h.

Environmental Aqueous Chilling Solutions Samples



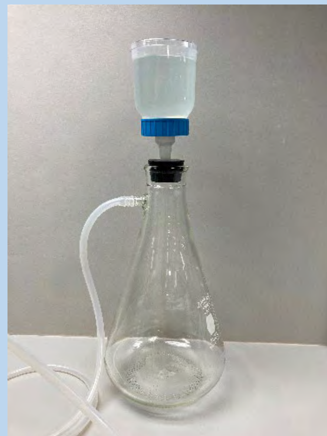


Figure 7: Example photos of how to set up for a brine sample (Photo Credit: Leo Gude and Sherre Chambliss).

For environmental aqueous chilling solutions and surface rinse solutions such as water, brine, and propylene glycol solutions, see below instructions.

Pour 500 ± 2 mL of sample solution into a sterile filter bag. Filter the solution by pouring it through a glass fiber filter and a 0.45 µm hydrophobic grid membrane filter in a vacuum filter system. When the sample has been completely filtered, aseptically remove both the glass fiber filter and the hydrophobic membrane filter and transfer them back to the used filter bag. These filters can be easily clogged by particulates. Therefore, it may be necessary to replace the filters during the filtration process. If more than one filtration is required, transfer all filters used into original filter bag.

Add 225 ± 5 mL (or 225 ± 5 g) of LPT broth to the bag containing the filters. Stomach 2 ± 0.2 minutes. Ensure that the filters are submerged.

Incubate the homogenate at 35 ± 1°C for 18 – 24 h.

Routine *Listeria* Sampling (RLm) & Intensified Verification Testing (IVT) Contact Sponges/IVT Environmental Sponges

Figure 8: An RLm contact sponge (Photo Credit: Leo Gude and Sherre Chambliss).

For RLm and IVT food contact sponge samples: Add 225 ± 5 mL (or 225 ± 5 g) of LPT broth to each bagged single sponge sample.

For IVT environmental sponge samples, the sponges are not composited. Each sponge is a separate sample. Add 225 ± 5 mL (or 225 ± 5 g) of LPT broth to each bagged single sponge sample.

Incubate at 35 ± 1°C for 18 – 24 h.

For RLm Products: Add 375 ± 5 mL (or 375 ± 5 g) of LPT broth and 125 g product sample in a sterile WhirlPak bag.

Incubate RLm Products at 35 ± 1°C for 24 – 28 h.

RLm Environmental Sponges

Figure 9: An environmental composite sample (Photo Credit: Leo Gude and Sherre Chambliss).

For environmental composite sponge samples: Add 100 ± 2 mL of LPT 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 LPT.

Incubate at 35 ± 1°C for 18 – 24 h.

Rapid Screening Procedure

Rapid Screening for *Listeria spp.*

1. Screen all enriched samples for *Listeria spp.* using the rapid screening kit, Neogen® Molecular Detection Assay 2 – *Listeria.* Follow the rapid screening technology Neogen® Molecular Detection System User Guide for preparing reagents, performing the test, and reading the results. Equivalent rapid screen technologies may be used.
   a. Samples that test negative for *L. monocytogenes* and *L. spp.* on the Neogen® MDS screen are reported as **NEGATIVE**.
   b. For samples that are positive on the Neogen® MDS screen, proceed with the Isolation Procedure.
   c. For samples with an inconclusive or invalid rapid screen result, the laboratory is to investigate. See the Troubleshooting Guide to determine appropriate actions to take.

TROUBLESHOOTING GUIDE FOR RAPID SCREEN ANALYSIS

For Inhibited Sample Screening Results:

- repeat the rapid screen analysis from the lysate step, or
- prepare new rapid screen lysate tubes and repeat the analysis, or
- analyze all inhibited samples culturally,

If reanalysis of an inconclusive rapid screen sample is unsuccessful, repeat sample preparation from the sample reserve or discard the sample.

For Control Issues:

- In analytical runs where the positive control results are not positive, perform an investigation as all samples are affected. Based on the findings the laboratory may:
  - repeat the rapid screen analysis from the lysate step, or
  - prepare new rapid screen lysate tubes from the enrichment broth and repeat the analysis, or
  - analyze all samples culturally.
- In analytical runs where the sterility control results are positive, perform an investigation as all samples are affected. Based on the finding the laboratory may:
  - identify the root cause, evaluate sterility of media, or
  - analyze sterility sample culturally and add an additional sterility sample to act as a process control.

Contact technical support from the manufacturer if experiencing equipment issues. If circumstances (e.g., a power outage or equipment failure) do not allow testing using the rapid screen system, the laboratory will, if possible, continue cultural analysis for all affected samples.

Isolation Procedure

Streak 100 µL of LPT broth from the screened positive sample onto Harlequin® *Listeria* Chromogenic Agar (HLCA) and incubate at 37 ± 1° C for 22 – 26 h.

Identification of *L. monocytogenes* and *Listeria spp.*

Samples may contain multiple species of *Listeria* including *L. monocytogenes*. Follow the procedure(s) applicable for each sample to ensure analysis continues for the available *Listeria* observed.

1. Examine the HLCA plates for colonies typical of *Listeria.* Re-incubate all original HLCA plates with typical, nontypical or no growth for an additional 22 – 26 h at 37 ± 1° C to obtain 48 h results. After 48-hr incubation, plates with no typical colonies are discarded as negative
   a. **For suspect *Listeria monocytogenes* colonies:**
      i At 24 ± 2 h, suspect colonies for *L. monocytogenes* are blue with a white halo.

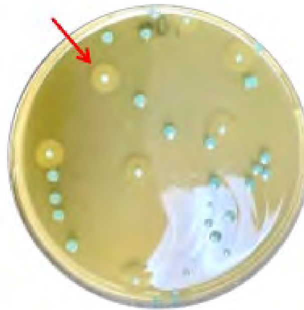


Figure 10: An HLCA plate featuring suspect colonies for *L. monocytogenes* (red arrow) that are blue with a white halo (Photo Credit: Sherre Chambliss and Julie Lynn).

1) If suspect *L. monocytogenes* colonies are present on HLCA, analyze up to five suspect colonies by adding an isolated colony to 200 µL of PCR water and run on Neogen® MDS using the Neogen® Molecular Detection Assay 2- *Listeria monocytogenes* kit.
   a. Report Neogen® MDS screen positives as **PRESUMPTIVE** for *L. monocytogenes*.
      i. Streak suspect *L. monocytogenes* samples to SBA plates from the undiluted cell suspension.
      ii. Concurrently, streak the same cell suspension to fresh HLCA plates for purity.

   b. Incubate all original HLCA plates for an additional 22 – 26 h at 37 ± 1° C to obtain 48 h results. After 48-hr incubation, plates with no typical colonies are discarded as negative.

   c. The *L. monocytogenes* screen negative samples are to be further analyzed for other *Listeria spp.*
      i. Streak Neogen® MDS screen negatives to SBA plates.

2) Incubate HLCA purity plates for 22-26 h at 37°C ± 1°C.

3) Incubate SBA for 16-24 h at 35°C ± 2°C.

4) Proceed to the section titled "Confirming *Listeria* and Further Analysis."

b. For suspect *Listeria species* (non-*monocytogenes*) colonies:

i At 24 ± 2 h, suspect colonies for *Listeria spp.* are blue without a white halo.

Figure 11: An HLCA plate featuring suspect colonies for *Listeria spp.* (red arrow) that are blue without a white halo (Photo Credit: Sherre Chambliss and Julie Lynn).

1) If suspect *Listeria spp.* are present on HLCA, streak up to five suspect *Listeria species* (non-*monocytogenes*) colonies to SBA plates. Concurrently, streak the same suspect colonies to fresh HLCA plates for purity.

   a. Incubate all original HLCA plates for an additional 22 – 26 h at 37 ± 1° C to obtain 48 h results. After 48-hr incubation, plates with no typical colonies are discarded as negative.

2) Incubate HLCA purity plates for 22-26 h at 37°C ± 1°C.

3) Incubate SBA for 16-24 h at 35°C ± 2°C.

4) Proceed to the section titled "Confirming *Listeria* and Further Analysis."

Confirming *Listeria* and Further Analysis

1. Examine the SBA plates and HLCA purity plates for typical colonies of *Listeria.*
   a. At 16 - 24 h, examine SBA plates for purity and evidence of contamination.

Figure 12: An SBA plate featuring presumptive colonies *for L. monocytogenes* (Photo Credit: FSIS Midwestern Laboratory).

   b. If using UV positive controls, perform a fluorescence check on the SBA plates for the positive control, presumptive *L. monocytogenes* positive sample and any suspect *Listeria spp.* 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 SBA plates are pure and uncontaminated, perform the proteomic confirmation method.

2. Perform confirmatory tests using a single isolated colony. Commercially available test systems such as Bruker® MALDI Biotyper or validated equivalent systems are to be employed. Refer to the manufacturer's instructions for the use of the instrument, preparation of reagents, and troubleshooting guidance.

   This method allows for the use of each available preparation method (direct, extended direct, and tube extraction) as needed to identify organisms. Refer to the manufacturer's documentation for a full list of organism coverage and thresholds.

3. Confirm the presence of *Listeria monocytogenes* and/or *Listeria spp.*

   a. For Presumptive *Listeria monocytogenes* colonies:
      i A minimum of one colony must be confirmed. If the first selected presumptive SBA colony does not confirm as *L. monocytogenes*, confirmation must be attempted for additional presumptive SBA colonies, if available, until at least three isolates from the test portion have failed confirmation.
         a. Report confirmed samples as **POSITIVE** for *Listeria monocytogenes.*
         b. Any confirmed *Listeria monocytogenes* isolates will be further characterized using Antimicrobial Susceptibility Testing (AST), and Whole Genome Sequencing (WGS).
         c. Report samples that did not confirm as **NEGATIVE**.

d. In the event of inconclusive results for any presumptive *Listeria monocytogenes* sample, submit isolate to Whole Genome Sequencing.

c. **For presumptive *Listeria species* (non-*monocytogenes*) colonies:**

i A minimum of one colony must be confirmed. If the first selected presumptive SBA colony does not confirm as *Listeria spp. (*non-*monocytogenes)*, confirmation must be attempted for additional presumptive SBA colonies, if available, until at least three isolates from the test portion have failed confirmation.

- See Table 5 for a list of available *Listeria* species in the Bruker® kit and report the confirmed *Listeria spp.*
- Bruker® MALDI Biotyper relies on matching the mass spectrum of the sample to its reference library. If there is a *Listeria* species that the Bruker® MALDI Biotyper system isn't validated for, it would likely indicate that the species is "unrecognized" or "not found in the reference library". Report any unconfirmed *Listeria spp.* as **INDETERMINATE**.

Table 5. A list of *Listeria* species that Bruker® MALDI-TOF has been validated to identify.

Listeria aquatica	*Listeria ivanovii*
Listeria booriae	*Listeria monocytogenes*
Listeria cornellensis	*Listeria newyorkensis*
Listeria costaricensis	*Listeria riparia*
Listeria fleischmannii	*Listeria rocourtiae*
Listeria floridensis	*Listeria seeligeri*
Listeria grandensis	*Listeria weihenstephanensis*
Listeria grayi	*Listeria welshimeri*
Listeria innocua	

Key Definition and Confirmation Criteria

Bruker® Matrix-Assisted Laser Desorption Ionization (MALDI) Biotyper (MBT): A method of ionization for mass spectrometry, commonly applied to the analysis and identification of biomolecules.

A genus-level identification requires a minimum score of 1.70, and a species-level identification requires a minimum score of 2.00 when using the Bruker® MALDI Biotyper.

Storage of Cultures

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 or frozen at -20 to -80°C. Fetal calf serum or commercially available cryobead products are appropriate media for frozen storage of *Listeria* spp.

Biosafety Chart

SAFETY INFORMATION AND PRECAUTIONS

1. Required protective equipment: Nitrile or latex gloves, lab coat, safety glasses
2. Hazards

Procedure Step	*Hazard*	*Recommended Safety Procedures*
Preparing samples with Primary Enrichment- Disinfecting intact retail packages with ethanol	Ethanol is acutely toxic by oral, dermal, or inhalation means. Highly flammable.	Wear gloves when using ethanol and avoid breathing the fumes directly. Avoid handling ethanol around heat or open flame.
Preparing samples with Primary Enrichment- Spraying down work area with bleach or equivalent disinfectant	Bleach is corrosive to the skin with prolonged exposure and may cause skin irritation with short-term exposure. Serious eye damage or eye irritation can occur if accidentally sprayed near eyes.	Wear gloves, safety glasses, and a lab coat when using bleach to prevent dermal or eye exposure.
Isolating and Confirming *Listeria monocytogenes*- Using an Ultraviolet (UV) Lamp	Using UV lamps can cause eye irritation and/or damage if eyes are directly exposed. The cornea of the eye can become inflamed or burned. Prolonged UV lamp exposure can also cause skin irritation and/or damage such as burns.	Refrain from staring directly at the UV lamp when in use. Use gloves when fluorescing samples under the UV lamp. Turn off the UV lamp when not in use.
Rapid Screening, Isolation, and Confirmation of *Listeria monocytogenes*- Risk to Pregnant and/or Immunocompromised Individuals	Pregnant women are more susceptible to listeriosis than other healthy adults. Listeriosis may cause miscarriage, premature labor, birth defects, or infant death. Listeriosis can also lead to severe illness or death of the pregnant individual. Listeriosis can cause severe illness or death in immunocompromised individuals.	Follow CDC guidelines for manipulating Biosafety Level 2 (BSL-2) pathogens. Pregnant women and immunocompromised individuals should avoid working with *Listeria monocytogenes* at all. Laboratories should inform pregnant women and immunocompromised individuals of the risks of working with *Listeria monocytogenes*. Reasonable accommodations should be made to ensure the safety of individuals in these health groups.

Appendix A: Alternative Methods

1) **Methodology Variation**
   a) In some rare instances, (hazardous weather or other unanticipated lab closures) it may be necessary to incubate Harlequin® *Listeria* Chromogenic Agar (HLCA) plates for 48 h without recording the 24 h results.

2) **Biochemical Confirmation**

   a) FSIS laboratories can elect to use biochemical confirmation methods (VITEK® 2) for reasons including: Bruker® MALDI Biotyper is unavailable, interruption in reagent supply chain, or results comparison.

   b) To biochemically confirm isolates, inoculate appropriate VITEK® 2 cards (if using VITEK® 2 Compact) or equivalent.

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Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

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This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

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