

FSIS Compliance Guideline:
Controlling *Listeria monocytogenes* in Post-lethality
Exposed Ready-to-Eat Meat and Poultry Products

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Purpose

This compliance guideline provides specific recommendations that official establishments producing post-lethality exposed ready-to-eat (RTE) meat and poultry product may follow to meet the requirements of 9 CFR part 430, the *Listeria* Rule. It also provides information on sanitation, testing for *Listeria monocytogenes* (*Lm*), and prevention of cross contamination of post-lethality exposed, RTE meat and poultry products. This document replaces previous versions of the FSIS *Listeria* Guideline and Q&As.

FSIS has revised this final version of the guideline to address comments on the draft version that was issued in September 2012. It also provides new information on holding products in response to FSIS's policy and procedures on not applying the mark of inspection pending certain test results. FSIS also revised the guidance to make clear that in addition to meeting the requirements of the *Listeria* Rule, establishments must meet the requirements of 9 CFR 416, Sanitation, and 9 CFR 417, HACCP Systems, as well as other applicable regulations.

FSIS also revised the guideline to provide additional information on labeling RTE products and clarifications to labeling guidelines (Attachment 1.1: Resource 1). In addition, FSIS revised the guideline to provide updated information on the impact of multiple processing steps (hurdle effect) on the control of *Listeria* growth, as well as new information regarding controls for reworked product. The updated guideline also provides information on the pros and cons of compositing food contact surface samples at the laboratory. In addition, FSIS updated the guideline to clarify actions establishments should take in response to a *Listeria* spp. positive result in the product when establishments choose to sample products in addition to food contact surfaces (Section 3.6). Updated information is also included on the FSIS RTEPROD sampling program.

This document provides **guidance** to assist establishments in meeting FSIS regulations. Guidance represents **best practice** recommendations by FSIS, based on the best scientific and practical considerations, and does not represent **requirements** that must be met. Establishments may choose to adopt different procedures than those outlined in the guideline, but they would need to support why those procedures are effective in controlling hazards from *Lm* in post-lethality exposed RTE products. By using the recommendations in the guideline, establishments would not need to provide further support for their procedures.

Summary of Changes

Chapter 1: This chapter has been revised to provide clear, easy to follow information regarding the **requirements of the *Listeria* Rule**. Although this information has not changed significantly since the May 2006 version of the Compliance Guideline, FSIS recommends that establishments review this information to ensure that they are in compliance with the regulation. The information may be useful to new establishments that are starting production. In the revised version:

- Step-by-step instructions have been provided, to assist establishments in determining whether their product is covered by the *Listeria* Rule.
- The requirements and recommendations for each control alternative are described.

- In addition, a glossary section has been provided with each chapter to further clarify the meaning of the terms used in the guidance and the *Listeria* Rule.
- Resource 1 ([Attachment 1.2](#)) has been updated to provide information about products that receive a full lethality that are not considered RTE.

Chapter 2: This chapter provides updated **technical information about establishing control alternatives** under the *Listeria* Rule. In the revised version:

- More in-depth information has been provided in [Appendix 2.1](#) regarding validation of post-lethality treatments and antimicrobial agents.
- In addition, sanitation guidelines have been revised to include a description of intensified sanitation conducted in response to positive results.
- The reference section has been updated to provide more information about new technologies to control *Listeria*.
- New information has been provided in [Appendix 2.3](#) on developing establishment employee training programs for implementing the *Listeria* Rule.

Chapter 3: This chapter provides new and updated information on **developing a *Listeria* Control Program** to test for *Lm* or an indicator organism on food contact surfaces (FCS). In the revised version:

- Updated information on routine testing for *Listeria* spp. under the three control alternatives is provided. Although there have been no changes to sampling frequency recommendations for *Listeria* spp., this revised chapter provides further guidance on meeting the recommended sampling frequencies and the number of samples to collect.
- Also, further clarification has been provided regarding FSIS expectations for sample collection and laboratory analysis of the samples.
- Finally, information has been provided on product and non-food contact testing (although not required by the *Listeria* Rule) to provide establishments with more information about the safety of their products and sanitary conditions in their food-processing environments.

Chapter 4: This chapter provides new and updated information on developing **enhanced sampling programs for *Listeria*** in response to positive results from routine sampling. In the revised version:

- A new table is provided ([Table 4.1](#)), clarifying timeframes for follow-up and intensified sampling, as well as hold and test of product.
- Intensified sampling is defined to provide establishments with more information on how to find and address the source of positive results.
- In addition, new information is provided on identifying and addressing *Listeria* trends.

- Findings from Food Safety Assessments (FSA) performed by FSIS in response to *Lm* positives have also been provided to increase awareness of common problems and lessons learned from FSA reviews.

How to Use this Document

The updated information in this revision of the Compliance Guideline should help establishments find specific information on the control of *Lm*, as needed.

- A glossary has been added at the end of each chapter to provide a better understanding of terminology found in the text. Terms in the glossary have been **bolded** the first time they appear in the text.
- Boxes have been provided giving more information about points made in the text.
- Appendices have also been added to the end of each section to provide more detailed information regarding concepts introduced in the text.
- Q&A's have been incorporated into the document to assist establishments in finding specific information.

If the desired information cannot be found within the Compliance Guide, FSIS recommends that users search *Listeria* Q&As in the AskFSIS database or submit questions through [AskFSIS](#). Documenting these questions helps FSIS improve and refine present and future versions of the Compliance Guideline and associated issuances.

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Introduction

Listeria monocytogenes (*Lm*) is a pathogen that can contaminate ready-to-eat (RTE) meat and poultry products and causes the disease listeriosis. Listeriosis is estimated to cause approximately 1,600 foodborne illnesses, 1,500 hospitalizations, and 260 deaths in the U.S. annually (Scallan et al., 2011). In most healthy individuals, **listeriosis** causes flu-like symptoms; however, in highly susceptible populations (e.g., the elderly, pregnant women, and immunocompromised individuals), listeriosis can lead to spontaneous abortion, septicemia, meningitis, and even death. Several outbreaks of listeriosis have been linked to the consumption of ready-to-eat (RTE) meat and poultry products contaminated with *Lm*.

Lm is widely distributed in the environment; it is found in the air, soil, water, dust, and plant material, including silage. As such, *Lm* may enter the environment of processing plants and subsequently contaminate RTE meat or poultry products, as well as other ingredients. *Lm* has ample opportunity to occupy and thrive in various niches in a production facility, such as on floors, in drains, or in standing water. Without proper sanitation and employee hygiene practices, *Lm* can easily cross-contaminate processing equipment, gloves or aprons of employees, and product.

Lm has unique growth characteristics that can make it a formidable pathogen to control in the processing environment. Specifically, *Lm* has the ability to grow in cool damp environments where other pathogens may not and is capable of surviving freezing temperatures. *Listeria* species (*Listeria* spp.) also exhibit heat and salt tolerance. *Lm* is known to form biofilms on food contact surfaces (FCSs) and non-food contact environmental surfaces and, as a result, persists on these surfaces despite aggressive cleaning and sanitizing. Once *Lm* has established a niche, it may persist in the environment for long periods of time until the niche is identified and eliminated.

RTE products are of particular concern for contamination with *Lm* because they may support the growth of the pathogen during refrigerated storage. In addition, since RTE products are often consumed without further cooking, there is a greater possibility of the occurrence of foodborne illness from these products if they become contaminated. Lethality treatments such as cooking meat and poultry products generally eliminate *Lm*; however, RTE products can be re-contaminated by exposure to the environment after the lethality treatment during peeling, slicing, repackaging, and other processing steps. By controlling sanitation in the post-lethality processing environment or implementing interventions in their products, establishments can ensure that their RTE products do not become contaminated with *Lm*.

In 2003, FSIS issued 9 CFR part 430, Control of *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Products (*Listeria* Rule). According to the *Listeria* Rule, RTE products are considered adulterated if they contain *Lm* or come in direct contact with a FCS that is contaminated with *Lm*. FSIS testing has shown that levels of *Lm* in RTE meat and poultry products have decreased as a result of science-based regulations and industry efforts. However, the pathogen continues to contaminate RTE products at low levels, and illnesses from *Lm*-contaminated RTE products continue to occur. Furthermore, contaminated RTE products--both those that support the growth of *Lm* and those that do not--have been shown to cross contaminate other RTE foods at retail, exacerbating the risk of illness. Finally, the infectious dose is thought to be low for highly-susceptible populations. **Therefore FSIS has maintained a “zero tolerance” for the pathogen in RTE products and continues to strengthen programs and recommendations to reduce or eliminate *Lm* from RTE products.**

On December 10, 2012, FSIS issued a *Federal Register* notice (FRN), Not Applying the Mark of Inspection Pending Certain Test Results. The FRN announced that the Agency is changing its procedures and will withhold its determination as to whether meat and poultry products are not adulterated, and thus eligible to enter commerce, until all test results that bear on the determination have been received. The policy and procedures announced in the *Federal Register* became effective February 8, 2013. This guideline provides information for establishments to hold RTE products when FSIS tests product or food contact surfaces for *Lm*.

This guideline provides information that establishments may use to meet the requirements of the *Listeria* Rule. It also provides “safe harbors” that establishments can implement to help ensure that the requirements are met.

Chapter 1

FSIS *Listeria* Guideline: Requirements of the *Listeria* Rule

- 1.1 [Background](#)
 - 1.2 [How Do I Determine whether My Product is Covered by the *Listeria* Rule?](#)
 - 1.3 [The *Listeria* Rule Alternatives](#)
 - [Table 1.1: *Listeria* Control Alternatives](#)
 - 1.4 [Requirements for Establishments Under all Three Alternatives](#)
 - 1.5 [Labeling](#)
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 - 1.2 [Chart of RTE vs. NRTE Products: Resource 1](#)
- Appendices
- 1.1 [Product Types](#)
 - 1.2 [Labeling](#)

This chapter provides information establishments can use to meet the regulatory requirements of 9 CFR part 430 (the *Listeria* Rule).

1.1 Background

After several large outbreaks of [listeriosis](#) starting in the 1980s, FSIS and FDA worked together to implement strategies to decrease foodborne illness from [Listeria monocytogenes \(*Lm*\)](#). In September 2003, FDA and FSIS published the [Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods.](#) This risk assessment indicated that deli meats and hotdogs posed the greatest per serving risk of illness and death from *Lm*. In May 2003, FSIS issued the [“FSIS Risk Assessment for *Listeria monocytogenes* in Deli Meats.”](#) This risk assessment indicated that the **use of a combination of growth inhibitors and post-lethality interventions** to control *Lm* in deli meats exposed to the environment after the lethality treatment **has the greatest impact on lowering the risk of illness or death from *Lm***. The Agency used these risk assessments as resources in developing the regulations to control *Lm* in RTE meat and poultry products.

In 2003, FSIS issued the interim final rule, [Control of *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Products \(the *Listeria* Rule\)](#). The *Listeria* Rule codified the regulations establishments are required to follow to produce safe RTE products. According to the *Listeria* Rule, *Lm* is a hazard that establishments producing [post-lethality exposed](#) RTE products must control. Establishments can control *Lm* in the product through their Hazard Analysis and Critical Control Point (HACCP) plans or prevent *Lm* in the [post-lethality processing environment](#) through a Sanitation Standard Operating Procedure (SOP) or other prerequisite program. According to the *Listeria* Rule, post-lethality exposed RTE products are considered adulterated if they contain *Lm* or come in direct contact with a [food contact surface](#) (FCS) that is contaminated with *Lm*.

The *Listeria* Rule established three [alternative](#) methods establishments can use in controlling *Lm* contamination of post-lethality exposed RTE products.

- Under Alternative 1, an establishment applies a [post-lethality treatment \(PLT\)](#) to reduce or eliminate *Lm* and an [antimicrobial agent or process \(AMAP\)](#) to suppress or limit growth of *Lm* (see [Chapter 2](#) for more information on PLTs and AMAP).
- Under Alternative 2, an establishment applies either a PLT **or** an AMAP.
- Under Alternative 3, the establishment does not apply any PLT, AMAP; instead it relies on its sanitation program to control *Lm*.

These alternatives increase in the stringency of their control from Alternative 3 to Alternative 1 and FSIS samples establishments in Alternative 3 at a higher rate than those in Alternative 1. **The *Listeria* Rule only applies to products that are RTE and exposed to the environment after the lethality step (post-lethality exposed).** The lethality step can be defined as cooking or another process (such as fermentation or drying) that results in a product that is safe for consumption without further preparation.

NOTE: Products that are considered RTE but not post-lethality exposed are not subject to the *Listeria* Rule but are still sampled under the RTEPROD_RAND project code (see [Appendix 3.1](#) for more information on FSIS RTE sampling projects).

1.2 How Do I Determine whether My Product is Covered by the *Listeria* Rule?

Step 1. Determine whether the product is **ready-to-eat (RTE)**.

- A product is considered RTE if there is a standard of identity¹ defining it as fully cooked (e.g., hotdogs or barbecue) or a common or usual name that consumers understand to refer to RTE product (e.g., pâtés), or if it meets the definition in the *Listeria* Rule (9 CFR 430.1).
- HACCP regulations require that the establishment support that its food safety system controls possible hazards in the product, and that the establishment documents this support. For more information, see Section 1.4.
- Examples of RTE products: [deli products](#), [hotdog products](#), whole hams, sausages, meat salads, and other products that have been treated with a lethality step.
- See [Attachment 1.2](#) for further determination if a product is RTE or not ready-to-eat (NRTE).
- **NRTE products are not covered by the *Listeria* Rule.**

Step 2. Determine whether the product is **post-lethality exposed**.

- If the product is RTE, determine whether the product is exposed to the environment after the lethality treatment (e.g., cooking) and before packaging.
- Examples of post-lethality exposure:
 - Product that is exposed to the environment after the lethality step during processing, slicing, freezing, or packaging;
 - Product that is removed from the cooking bag at an official establishment and sliced or cut up and re-packaged; and
 - Product that is acidified/fermented or salt-cured or dried and smoked and then packaged.
- Examples of post-lethality exposed RTE products may include: sliced roast beef, cooked ham for slicing, hotdogs, fermented sausages, cured ham, and jerky.

Step 3. Determine whether the product is covered by the *Listeria* Rule.

- **If product is RTE and post-lethality exposed it is subject to the *Listeria* Rule.**
- **If product is RTE but not post-lethality exposed it is not subject to the *Listeria* Rule.**

Product Considerations
<p>Note: See Appendix 1.1 for more examples.</p> <ul style="list-style-type: none"> • Frozen products may be considered RTE if they do not contain safe handling instructions and they do not need to be cooked for safety (although they may be heated to increase palatability). • Cook-in-bag products that remain in the same bag until the product leaves the establishment are not considered post-lethality exposed. • Hot-filled products at 160°F (or other lethality temperatures), such as fats and lards, are considered RTE but not considered post-lethality exposed. • Soups and other products that are cooked to eliminate pathogens and hot packed in the final packaging are RTE but not post-lethality exposed. • Country cured ham (and other similar products) may be considered either RTE or NRTE, depending on how they are processed and labeled.

¹ Standards of identity for meat and poultry products can be found in 9 CFR Part 319.

1.3 The *Listeria* Rule Alternatives

According to the *Listeria* Rule, *Lm* is a hazard that establishments producing post-lethality exposed RTE products must control through a HACCP plan or prevent through a Sanitation Standard Operating Procedure (SOP) or a prerequisite program (9 CFR 430.4(a). To maintain the sanitary conditions to meet this requirement, establishments must comply with one of three alternatives (9 CFR 430.4(b)).

The *Listeria* alternatives are designed to address post-lethality contamination of *Lm* in RTE products. Each establishment must designate which alternative it intends to implement for a particular product. Each alternative consists of a single control method or combination of control methods which establishments must apply (see [Table 1.1](#)). Establishments may utilize one alternative for all of their products or produce product under multiple alternatives (see the section below on establishments under multiple alternatives). For more information on control measures (e.g., PLT and AMAP), see [Chapter 2](#).

Table 1.1 <i>Listeria</i> Control Alternatives	
Alternative 1 (Alt. 1)	The establishment uses a post-lethality treatment (PLT) to reduce or eliminate <i>Lm</i> in the product and an antimicrobial agent or process (AMAP) to limit or suppress growth of <i>Lm</i> in the product.
Alternative 2, Choice 1 (Alt. 2a)	The establishment uses a PLT to reduce or eliminate <i>Lm</i> in the product.
Alternative 2, Choice 2 (Alt. 2b)	The establishment uses an AMAP to limit or suppress growth of <i>Lm</i> in the product.
Alternative 3 (Alt. 3)	The establishment relies on sanitation alone to control <i>Lm</i> in the processing environment and on the product. There are separate requirements for deli meat and hotdogs under this alternative.

Establishments may also change the production process to meet the requirements for a particular alternative. For example, if an establishment employs only sanitation procedures to control *Lm* (Alt. 3) but later implements an AMAP, it could then meet the requirements for Alt. 2. **Establishments are encouraged to use AMAPs or PLTs, if possible, to reduce the risk of *Lm*.** Further information describing the requirements and recommendations for the three alternatives is provided below.

NOTE: The following sections describe both requirements in the *Listeria* Rule and recommendations to meet these requirements. When the word “**must**” is used, it refers to a requirement. When the word “**should**” is used, it refers to a recommendation.

[Attachment 1.1](#) outlines the 9 CFR 430.4 requirements for Alt. 1, 2, and 3.

Alternative 1 (9 CFR 430.4(b)(1))

Alt. 1 requires the use of a PLT to reduce or eliminate *Lm* **and** an AMAP to suppress or limit the growth of the pathogen.

- The establishment must apply a PLT to control *Lm* in the product and must include the PLT in its HACCP plan.²
- The establishment must validate the effectiveness of the PLT in accordance with 9 CFR 417.4.
- The PLT should demonstrate at least a 1-log decrease before the product is released into commerce.
- The establishment must use an AMAP to control *Lm* in the product and must include the agent or process in the establishment's HACCP plan, Sanitation SOP, or other prerequisite program.
- The establishment must document in its HACCP plan, Sanitation SOP, or other prerequisite program that the AMAP, as used, is effective in suppressing or limiting growth of *Lm*. The AMAP should demonstrate that no more than 2-logs of growth of *Lm* will occur over the shelf life of the product.
- If *Lm* control measures are incorporated into the establishment's Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 9 CFR 416.14. If *Lm* control measures are addressed in a pre-requisite program other than the Sanitation SOP, the establishment must include the program and results of the program in the documentation that the establishment is required to maintain under 9 CFR 417.5.
- Because Alt. 1 includes a combination of controls, the Agency does not require establishments using Alt. 1 to have a testing program for FCS. However, testing is recommended (see [Table 3.1](#)). Testing FCS in Alt. 1 could be minimal and primarily serve as a means to verify that the sanitary conditions in the establishment will not overwhelm the PLT.
- As with all control alternatives, an establishment with products in Alt. 1 must maintain sanitation in the post-lethality processing environment in accordance with 9 CFR 416.

An example of a product that would fall under Alt. 1 would be deli and hotdog products that receive a PLT (such as steam pasteurization after packaging) and has an AMAP (such as the addition of lactates or diacetates in the formulation).

Alternative 2 (9 CFR 430.4(b)(2))

Alt. 2 requires the use of either a PLT (Alt. 2a) or an AMAP that controls the growth of *Lm* over the shelf life of the product (Alt. 2b).

1. Alternative 2, Choice 1 (Alt. 2a)

- The establishment must apply a PLT to control *Lm* in the product and must include the PLT in its HACCP plan.

² According to 9 CFR 417.

- The establishment must validate the effectiveness of the PLT in accordance with 9 CFR 417.14.
- The PLT should demonstrate at least a 1-log decrease before the product is released into commerce.
- As with Alt.1, establishments in Alt. 2a are not required to test FCS; however, FSIS recommends that establishment test the surfaces on a regular basis to demonstrate that its system is in control (for more information on testing for Alt. 2, see [Table 3.1](#)).
- As with all control alternatives, an establishment with products in Alt. 2a must maintain sanitation in the post-lethality processing environment in accordance with 9 CFR 416

An example of a product in Alt. 2a is a hotdog or deli product that is treated with a post-pasteurization treatment after packaging, such as a steam treatment, and DOES NOT contain antimicrobials, such as lactate and diacetate.

2. Alternative 2, Choice 2 (Alt. 2b)

- The establishment must use an AMAP to control growth of *Lm* in the product and must include the agent or process in the establishment's HACCP plan, Sanitation SOP, or other prerequisite program.
- The establishment must document in its HACCP plan, Sanitation SOP, or other prerequisite program that the AMAP, as used, is effective in suppressing or limiting growth of *Lm*. The AMAP should demonstrate no more than 2-logs of growth of *Lm* will occur over the shelf life of the product.
- If *Lm* control measures are incorporated into the establishment's Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 9 CFR 416.4. If *Lm* control measures are addressed in a pre-requisite program other than the Sanitation SOP, the establishment must include the program and results of the program in the documentation that the establishment is required to maintain under 9 CFR 417.5.
- Under Alt. 2b, the establishment must test FCS in the post-lethality environment to ensure that the surfaces are sanitary and free of *Lm* or its indicator organisms (e.g., *Listeria* spp.). It must also indicate testing frequency, identify the size and location of sites to be tested, explain why the testing frequency is sufficient to control *Lm*, and identify conditions for hold and test when an FCS is positive for *Lm* or an indicator organism. Recommended testing frequencies for this alternative are included in [Table 3.1](#).
- As with all alternatives, the establishment must maintain sanitation in the post-lethality environment according to 9 CFR 416.

An example of products in Alt. 2b is deli and hotdog products with antimicrobial agent (AMA) such as lactates and diacetates added to the formulation, but with no PLT. Another example of a product under Alt. 2b would be a frozen RTE product.

Alternative 3: Non-deli or Hotdog Producers (9 CFR 430.4(b)(3)(i))

Under Alt. 3, the establishment does not apply a PLT to reduce or eliminate *Lm* or an AMAP to control the growth of *Lm* in the post-lethality exposed product. Instead, it relies on sanitation alone to control *Lm* in the product.

- The establishment must control *Lm* in its post-lethality processing environment through the use of sanitation control measures, which may be incorporated in the establishment's HACCP plan, Sanitation SOP, or prerequisite program (*Listeria* Control Program).
- If *Lm* control measures are incorporated into the establishment's Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 9 CFR 416.14. If *Lm* control measures are addressed in a pre-requisite program other than the Sanitation SOP, the establishment must include the program and results of the program in the documentation that the establishment is required to maintain under 9 CFR 417.5.
- As with establishments in Alt. 2b, establishments in Alt. 3 must provide for testing FCS in the post-lethality processing area to ensure that surfaces are sanitary and free of *Lm* or its indicator organisms, indicate testing frequency, identify the size and location of sites to be tested, explain why the testing frequency is sufficient to control *Lm*, and identify conditions for hold and test when an FCS is positive for *Lm* or an indicator organism. Recommended testing frequencies are included in [Table 3.1](#).

An example of a product in Alt. 3 is refrigerated chicken nuggets that are not treated with a PLT and are not formulated using AMAs.

NOTE: According to the *Listeria* Rule, products and the processing environment under Alt. 3 are likely to be subject to more frequent verification testing by FSIS than products and the processing environment in Alt. 1 or 2. In fact, Alt. 3 products are sampled at a higher rate in the FSIS risk-based sampling code (RTEPROD_RISK). See Appendix 3.1.

Alternative 3: Deli or Hotdog Producers (9 CFR 430.4(b)(3)(ii))

In addition to meeting the above requirements for Alt. 3 products, there are special requirements for establishments that produce **deli or hotdog** products under Alt. 3.

- Establishments must verify that the corrective actions taken after an initial positive test for *Lm* or its indicator organisms on an FCS in the post-lethality processing treatment are effective. This is achieved by performing follow-up testing for *Lm* or an indicator organism after the FCS positive test that includes a targeted test of the specific site on the FCS that is the most likely source of contamination and additional tests in the surrounding FCS area.
- If follow-up testing yields a second positive result, hold and test products that may be contaminated using a sampling method and frequency that will provide a level of statistical confidence that will ensure that lots are not adulterated.

NOTE: According to the *Listeria* Rule, RTE products are considered adulterated if they are contaminated with *Lm* or pass over a surface that is contaminated with *Lm*. Establishments are required to hold or to maintain control of RTE products that FSIS has tested for *Lm*, and RTE products that have passed over food contact surfaces that FSIS has tested for *Lm*. Establishments may move such products off site provided they maintain control of them (e.g., through company seals).

An establishment in Alt. 3 that produces deli meat or hotdog products will be subject to more frequent FSIS verification testing than one that does not produce such products because deli and hotdog products were ranked as higher risks for *Lm* contamination in the 2003 FDA/FSIS risk assessment.

Examples of deli and hotdog products in Alt. 3 include sliced turkey breast luncheon meat and packaged hotdogs that are not held frozen and not formulated using an AMA.

NOTE: Deli salads and wraps are not considered deli products (according to the *Listeria* Rule) because they are not sliced and are also not typically used in a sandwich.

Establishments under Multiple Alternatives

FSIS recognizes that establishments may produce products under multiple alternatives. These products may be produced under multiple HACCP plans or grouped under a single HACCP plan. Products can be grouped in a single HACCP plan when the hazards, CCPs, and critical limits are essentially the same. Thus, a single HACCP plan could cover hotdogs formulated with and without antimicrobial agents (Alt. 2 and 3), provided that the HACCP plan clearly distinguishes any critical differences. If an establishment produces products using two (or three) alternative Control Programs, FSIS's sampling focus will be on product manufactured under Alternative 3, then 2a and b, then 1.

1.4 Requirements for Establishments Under all Three Alternatives

According to the *Listeria* Rule (9 CFR 430.4(c)), establishments in all three alternatives:

- May use verification testing for *Lm* or an indicator organism (e.g., *Listeria* spp.) to verify the effectiveness of their sanitation procedures in the post-lethality processing environment.
- Sanitation measures for controlling *Lm* and AMAP's or PLT's may be incorporated into the establishment's HACCP plan (required for PLT's) or in its Sanitation SOP or other prerequisite program. When these control procedures are incorporated into the Sanitation SOP or other prerequisite programs, the establishment **must** have documentation that supports the decision in its hazard analysis that *Lm* is not a hazard that is reasonably likely to occur.
- The establishment must maintain sanitation in the post-lethality processing environment accordance with 9 CFR 416.

- If the *Lm* control measures are included in the HACCP plan, the establishment must validate and verify the measures in accordance with 9 CFR 417.4.
- If the *Lm* control measures are included in the Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 9 CFR 416.14.
- If the *Lm* control measures are included in a prerequisite program other than the Sanitation SOP, the establishment must include **the program and the results** produced by the program in the documentation that the establishment is required to maintain under 9 CFR 417.5.
- The establishment must make verification results available upon request to FSIS personnel.

Other Requirements

In addition to meeting the requirements of the *Listeria* Rule, establishments must meet the requirements of 9 CFR 416, Sanitation, and 9 CFR 417, HACCP Systems. Producers of RTE products must support that they are maintaining sanitation in the processing environment according to 9 CFR 416, and preventing or controlling the food safety hazards in their product according to 9 CFR 417.2(a)(1) and documenting the support according to 9 CFR 417.5(a)(1). For RTE products, FSIS recommends that the establishment achieve lethality of pathogens (e.g., *Salmonella*) in the product, and stabilize the product to inhibit the growth of spore-forming bacteria (e.g., *C. botulinum* and *C. perfringens*). The establishment needs to be able to support that its product is RTE at the end of the process. The requirements of the *Listeria* Rule will only be effective as long as sanitation is maintained, and the HACCP plan is effective to control the hazards in the system.

Sanitation problems can lead to *Listeria* harborage and cross contamination of RTE meat and poultry products (see Chapter 4 of this guideline). This cross contamination may overwhelm the effectiveness of the establishment's *Listeria* controls and impact its ability to support its decision that *Listeria* is not reasonably likely to occur in its products. For example, if an establishment has condensation dripping from the ceiling that is not adequately addressed in its sanitation program, harborage of *Lm* could occur in its post-lethality exposed processing environment. This contamination could spread to food contact surfaces and contaminate the product with *Lm*. If the establishment's post lethality treatment is designed to achieve a 1-log reduction of *Lm*, it may be overwhelmed by the additional contamination and no longer be sufficient to ensure the safety of the product. In that case, the establishment may no longer be able to demonstrate that its HACCP system is effective in controlling pathogens.

Likewise, the establishment may find positive results from the food contact surface testing it performs as part of its sanitation SOP to meet the requirements of the *Listeria* Rule. These positive results may indicate that there are sanitation issues in the establishment's environment that need to be addressed to ensure the safety of the product. For example, the establishment has a hole in the wall, allowing insulation to become saturated with water, and a harborage point for *Lm* to form. Through an investigation, the establishment determines that the *Lm* from the hole in the wall could have spread to food contact surfaces, leading to the positive testing result. The establishment takes corrective actions (in accordance with 9 CFR 416.15(a)) by repairing the hole, while at the same time reassessing its Sanitation SOP (in accordance with 9 CFR 416.15(b)) to ensure that cross contamination to food contact surfaces does not occur. In

addition, because the establishment uses its Sanitation SOP to support its decision that *Lm* is not reasonably likely to occur on its product, it reassesses its HACCP plan (in accordance with 9 CFR 417.3(b)(4)) to ensure that its controls for *Lm* are effective in light of this issue.

As these examples show, it is critical that establishments producing RTE products ensure that their systems are working together to control pathogens. A *Listeria* Control Program by itself will not be effective, unless it is used along with the sanitation and HACCP programs to control the hazards from pathogens.

1.5 Labeling

According to the *Listeria* Rule, an establishment that controls *Lm* by using a PLT or an AMAP may declare this fact on the label, provided that the establishment has validated the claim (9 CFR 430.4(e)). The purpose of such claims is to inform consumers about measures taken by the processor to ensure the safety of the product and enable consumers to make informed purchase decisions. Such claims are voluntary and may be of value to consumers, especially those in groups most vulnerable to foodborne illness. Processors need to document their validation of these claims, as described in [Appendix 2.1](#). For further labeling resources, see [Attachment 1.2](#) and [Appendix 1.2](#).

In addition, if an establishment labels the product as RTE (e.g., does not include safe handling instructions, see Attachment 1.1), it is required to process the product to render it RTE, in accordance with 9 CFR 317.2(l) and 381.125(b). To meet these requirements, the establishment's process must be validated to achieve at least a 6.5 log reduction of *Salmonella* for cooked beef, roast beef, and cooked corned beef products (9 CFR 318.17), a 5-log reduction for uncured meat patties (9 CFR 318.23), a 7-log reduction for cooked poultry products (9 CFR 381.150), or other equivalent lethality. FSIS will review the establishment's supporting documentation for its lethality and stabilization processes to verify that the establishment is meeting the requirements.

Alternative means of achieving lethality may also be sufficient, as long as the establishment can support the effectiveness of its process. See the [FSIS *Salmonella* Compliance Guidelines for Small and Very Small Meat and Poultry Establishments that Produce Ready-to-Eat \(RTE\) Products](#) for more information.

1.6 Glossary

Alternative: A method of control for *Lm* adopted by an establishment to meet the requirements of the *Listeria* Rule.

Antimicrobial Agent (AMA): A substance in or added to an RTE product that has the effect of reducing or eliminating a microorganism, including a pathogen such as *Lm*, or that has the effect of suppressing or limiting growth of a pathogen, such as *Lm*, in the product throughout the shelf life of the product. Examples: potassium lactate and sodium diacetate, which limit the growth of *Lm* (9 CFR 430.1).

Antimicrobial Process (AMP): An operation, such as freezing, that is applied to an RTE product that has the effect of suppressing or limiting the growth of a microorganism, such as *Lm*, in the product throughout the shelf life of the product. Other examples are processes that result in a pH or water activity that suppresses or limits microbial growth (9 CFR 430.1).

Cook-in-bag: Product that is cooked in an impermeable package or casing and is not exposed to the environment of the establishment after the lethality treatment.

Deli product: A ready-to-eat meat or poultry product that typically is sliced, either in an official establishment or after distribution from an official establishment, and typically is assembled in a sandwich for consumption (9 CFR 430.1).

Food contact surface (FCS): A surface in the post-lethality processing environment that comes in direct contact with RTE product (9 CFR 430.1).

Hotdog product: A RTE meat or poultry frank, frankfurter, or wiener, such as a product defined in 9 CFR 319.180 and 319.181 (9 CFR 430.1).

***Listeria monocytogenes* (Lm):** A foodborne pathogen that can cause the disease listeriosis in humans.

Listeriosis: A disease caused by *Lm*. In most healthy individuals, listeriosis causes flu like symptoms; however in the elderly, pregnant women and their fetuses, and immunocompromised individuals, listeriosis can lead to spontaneous abortion, septicemia, meningitis, and death.

Post-lethality Exposed Product: Ready-to-eat product that comes into direct contact with an FCS after the lethality treatment (e.g., cooking) in a post-lethality processing environment. Examples of post-lethality exposed products: hotdogs after the casings are removed; cooked roast beef after removing the cooking bag (9 CFR 430.1).

Post-lethality Processing Environment: The area in an establishment into which product is routed after having been subjected to an initial lethality treatment. The product may be exposed in this area as a result of slicing, peeling, re-bagging, cooling semi-permeable encased product with a brine solution, or other procedures (9 CFR 430.1).

Post-lethality Treatment (PLT): A lethality treatment that is applied or is effective after post-lethality exposure. It is applied to the final product or sealed package of product in order to reduce or eliminate the level of pathogens resulting from contamination from post-lethality exposure (9 CFR 430.1).

Ready-to-eat (RTE): A meat or poultry product that is in a form that is edible without additional preparation to achieve food safety and may receive additional preparation for palatability or aesthetic, epicurean, gastronomic, or culinary purposes. RTE product is not required to bear safe-handling instruction (as required for non RTE products by 9 CFR 317.2(1) and 381.125(b)) or other labeling that directs that the product must be cooked or otherwise treated for safety and can include frozen meat or poultry products (9 CFR 430.1).

1.7 References

FDA and FSIS. Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods, September 2003. . Available on the FDA website:
<http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm183966.htm>

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9 CFR part 430 Control of *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Products

Attachment 1.1: Control Requirements for *Listeria monocytogenes*

Requirements	→ Increasing Risk Levels and Frequency of FSIS Verification Testing →				
	ALTERNATIVE 1	ALTERNATIVE 2		ALTERNATIVE 3	
	Post-lethality Treatment AND Antimicrobial Agent or Process	Post-lethality Treatment OR Antimicrobial Agent or Process		Sanitation and Testing Program	
		Choice 1: Post-lethality Treatment	Choice 2: Antimicrobial Agent or Process	Non-deli, Non-hotdog	Deli or hot-dog product
Validate effectiveness of post-lethality treatment (PLT). Must be included as a CCP in the establishment's HACCP Plan and should show at least a 1-log reduction in <i>Lm</i> prior to distribution of the product into commerce	X	X			
Document effectiveness of antimicrobial agent or process: Must be included as part of the establishment's HACCP, Sanitation SOP, or Prerequisite Program and should demonstrate no more than 2-logs growth of <i>Lm</i> over the estimated shelf life.	X		X		
Sanitation Program Requirements			X	X	X
Testing food contact surfaces (FCS) in the post-lethality processing environment for <i>Lm</i> or an indicator organism.			X	X	X
State testing frequency.			X	X	X
Identify size and location of sites to be sampled.			X	X	X
Explain why testing frequency is sufficient to control <i>Lm</i> or an indicator organism.			X	X	X
Identify conditions for Hold-and-Test, when FCS (+) for <i>Lm</i> or an indicator organism.			X	X	X
Additional Sanitation Program Requirements					
Follow-up testing to verify corrective actions are effective after 1 st FCS (+) for <i>Lm</i> or an indicator organism. Includes testing of targeted FCS as most likely source and additional testing of the surrounding area.					X
If follow-up testing yields 2 nd FCS (+), hold products that may be contaminated until problem is corrected as shown by FCS (-) in follow-up testing.					X
Hold and test product lots using a sampling plan that provides statistical confidence that the lots are not contaminated with <i>Lm</i> or an indicator organism. Release, rework, or condemn products based on results. Document results and product disposition.					X
Establishments in all three alternatives must maintain sanitation in accordance with 9 CFR 416.	X	X	X	X	X

Attachment 1.2: Chart of RTE vs. NRTE Products: Resource 1

TYPE	CLASS	HACCP CATEGORY	REQUIRED LABELING	WHAT THE HACCP PLAN MAY ADDRESS
<p>A meat/poultry product (in whole or in part) which has not received an adequate lethality treatment for <i>Salmonella</i> (i.e., raw or partially cooked product). May include cuts of meat and poultry, cured pork products, and NRTE sausage.</p> <p style="text-align: center;"><i>Or</i></p> <p>A meat/poultry product (in whole or in part) which has received an adequate lethality treatment for <i>Salmonella</i>, that is not defined by a standard of identity or a common or usual name that consumers understand to refer to RTE product <u>and</u> does not meet the definition of RTE* in 9 CFR 430.1. May include NRTE ham, casseroles, and other meat or poultry dishes.</p>	<p>Not-ready-to-eat</p>	<ul style="list-style-type: none"> • Raw Product Ground • Raw Product Not Ground • Not Heat Treated Shelf Stable • Heat Treated –shelf stable • Heat Treated but not Fully Cooked Not Shelf Stable • Products with secondary inhibitors Not Shelf Stable 	<p>Product must be labeled with statements such as keep refrigerated, keep frozen, or refrigerate leftovers, if not shelf stable. Use of Safe Handling Instruction (SHI) labeling required.</p>	<ul style="list-style-type: none"> • Use of SHI labeling (Some establishments may have a CCP for SHI labeling application). If it is not obvious that the product is raw and needs to be cooked: • Features on labeling are conspicuous so that intended user is fully aware that product must be cooked for safety. This is best conveyed through the product name (e.g., “Cook and Serve”) but may also be conveyed by the use of an asterisk on the product name that is associated with a statement on the principle display panel or by a burst stating such things as “needs to be fully cooked,” “see cooking instructions,” or “cook before eating.” • Validation that: <ol style="list-style-type: none"> a. Cooking and preparation instructions on the product are sufficient to destroy pathogens. b. Instructions are realistic for the intended consumer.

*A meat or poultry product that is edible without any additional preparation to achieve food safety.

<p>A product containing a meat/poultry component that is RTE in combination with non-meat/poultry components that needs to receive a lethality treatment by the intended user. The final product does not meet the definition of RTE in 9 CFR 430 because it contains raw components. May include meals, dinners, and frozen entrees.</p>	<p>Not-ready-to-eat</p>	<ul style="list-style-type: none"> Heat Treated but not Fully Cooked Not Shelf Stable 	<p>Product must be labeled with statements such as keep refrigerated or frozen. Use of SHI labeling is recommended.</p> <p>NOTE: SHI are not required because the meat or poultry component is RTE. However, FSIS recommends SHI for these products because raw non-meat ingredients are added.</p>	<ul style="list-style-type: none"> Validation that: <ol style="list-style-type: none"> The meat/poultry component received an adequate lethality treatment for pathogens (see Section 1.4). Cooking and preparation instructions on the product are sufficient to destroy pathogens. Instructions are realistic for the intended consumer. Features on labeling are conspicuous so that intended user is fully aware that product must be cooked for safety (e.g., “Cook and Serve”). May also be conveyed by the use of an asterisk on the product name that is associated with a statement on the principle display panel, or by a burst stating such things as “needs to be fully cooked”, “see cooking instructions”, or “cook before eating.” If necessary, hazard analysis should address whether instructions on the label are needed related to cross-contamination (e.g., avoid contact of contents) and prevention of pathogenic growth (e.g., promptly refrigerate leftovers). <p>NOTE: Inspection program personnel are to collect samples as RTE if the establishment does not follow the guidance above.</p>
<p>A meat/poultry product that has received an adequate lethality treatment for <i>Salmonella</i> that may or may not be defined by a standard of identity or a common or usual name that consumers understand to refer to RTE product, and meets the definition of RTE in 9 CFR 430. RTE products that are post-lethality exposed must meet the requirements of 9 CFR part 430. May include hotdogs, deli meat, and RTE sausages.</p>	<p>Ready-to-eat</p>	<ul style="list-style-type: none"> Not Heat Treated Shelf Stable Heat Treated Shelf Stable Fully Cooked Not Shelf Stable Products with secondary inhibitors Not Shelf Stable 	<p>If the product is not shelf stable, labeling such as keep refrigerated or frozen is required. SHI are not required and should not be used because they could be misleading to consumers.</p>	<ul style="list-style-type: none"> Validation that the meat or poultry component received an adequate lethality treatment for pathogens (e.g., a 5-log reduction of <i>Salmonella</i>). The establishment meets the requirements of 9 CFR 430 if the product is post-lethality exposed. Heating (not cooking) instructions may be included. Statements on the principle display panel may indicate that the product is RTE and does not have to be cooked for safety (e.g., “fully cooked,” “heat and serve”).

Appendix 1.1: Product Types

Overview of products covered under *Listeria* Rule

Establishments that produce post-lethality exposed RTE meat and poultry products are covered by the *Listeria* Rule. Accordingly, the establishment should determine the alternative(s) to which it will adhere to in its processes to control *Lm* during the post-lethality exposure.

The following product types, if post-lethality exposed, would fall under the *Listeria* Rule. The classification of deli products and hotdog products, salad/spread/pâté products, cook-in bag products, frozen, and hot-packed products will be described.

I. Deli and Hotdog Products

As defined in 9 CFR 430.1, a deli product is “a ready-to-eat meat or poultry product that typically is sliced, either in an official establishment or after distribution from an official establishment, and typically is assembled in a sandwich for consumption.” RTE hotdog (or hot dog) products are defined in 9 CFR 430.1 as “a ready-to-eat meat or poultry frank, frankfurter, or wiener, such as a product defined in 9 CFR 319.180 and 319.181.” Cooked sausages (e.g., bratwurst), as defined in 9 CFR 319.140, would be considered RTE, but would not be considered to be deli or hotdog products. Post-lethality exposed whole meat and poultry products (e.g., deli loaves) that are destined for slicing at a retail establishment are also considered to be deli products.

NOTE: Deli loaves that are non post-lethality exposed (e.g., cook-in bag) that are sliced at retail are not considered to be deli products because they are not covered by the *Listeria* Rule, see Section III below.

Like all RTE products exposed to the processing environment, deli and hotdog products that are exposed to the post-processing environment are subject to the *Listeria* Rule. If the RTE product is not exposed to the post-processing environment, it is not subject to the Rule. Depending on the method that an establishment chooses to control *Lm* contamination in its processing, deli and hotdog products may be in Alt. 1, 2, or 3.

NOTE: The *Listeria* Rule definition of deli products applies only to deli products produced official establishments. Deli products produced at retail can include deli salads, meats, and other products.

Deli and hotdog products that receive a PLT and AMAP fall under Alt. 1. An example is a hotdog that includes lactates or diacetates in the formulation and is steam pasteurized after repackaging. Deli and hotdog products with antimicrobial agents such as lactates or diacetates added in the formulation, but with no post-process lethality treatment, would fall under Alt. 2b. An example of an Alt. 2a product is a hotdog product that received only a PLT, such as being

Question: A scrapple product receives a full-lethality treatment at the establishment. Is the product required to be RTE?

Answer: No. Unless the product has a standard of identity requiring it to be RTE (9 CFR 319 and 9 CFR 381), it can be considered to be NRTE. NRTE products are required to bear safe handling instructions, and should be labeled with validated cooking instructions. In addition, if the product is NRTE but appears to be RTE, it should be labeled conspicuously so that intended user is fully aware that product must be cooked for safety (see [Attachment 1.2](#)). The establishment’s HACCP plan and intended use statement should also be consistent with a NRTE product (see Appendix 1.2 part II below).

packaged in casings with an antimicrobial agent that reduces the level of *Lm*. If an establishment does not use a PLT or an AMAP in the processing of deli and hotdog products, these products would fall under Alt. 3.

II. Salad/Spread/Pâté Products

Salads/spreads/pâtés are also RTE post-lethality exposed, so they are covered by the *Listeria* Rule. RTE meats that are used in salads receive additional handling after they are removed from their packages and are mixed with other ingredients, thus exposing them to cross-contamination. An establishment producing salads with the meat and poultry components that receive a PLT or antimicrobial agent needs to have supporting documentation showing that the antimicrobial action is sufficient to control *Lm* in all the salad ingredients if it chooses to have its product in Alt. 1 or 2. A salad/spread/pâté product with a final pH below 4.39 in all ingredients of the salad (e.g., due to the salad dressing or other ingredients added) would fall under Alt. 2, if an antimicrobial agent is used. Salads/spreads/pâtés are not considered deli products under the *Listeria* Rule because they are not typically sliced.

III. Cook-in Bag products

A cook-in-bag product such as a cooked ham or poultry roll that leaves the federal establishment intact in its cooking bag is not covered by the *Listeria* Rule because it is not post-lethality exposed. However, once the product is removed from its package, it should be handled using good sanitary controls so that it does not become contaminated with *Lm*.

IV. Frozen Products

Frozen products are covered under the *Listeria* Rule if they are considered RTE and post-lethality exposed. Although freezing controls the growth of *Lm*, the organism can still survive the freezing process. Frozen products generally fall under Alt. 2b (use of an antimicrobial agent or process to control *Lm*). The *Listeria* Rule defines an antimicrobial process as an operation, such as freezing, that is effective throughout the shelf life of the product. Therefore, to meet the definition of an antimicrobial process, and to qualify for Alt. 2b, the product would need to remain frozen throughout its shelf life. If the product is meant to be thawed and held refrigerated either at the establishment or at a retailer, the product would be considered Alt. 3. An example of a frozen product would be RTE sliced chicken strips that are frozen at the establishment and held frozen until prior to consumption. They may be heated by the consumer for palatability prior to eating.

V. Hot-packed Products: Edible Oils and Fats, Lard, and Soups

Edible oils and fats resulting from a rendering process that processes them to a temperature of 180° F and maintains them at 160° F are considered RTE. Rendering is intended to make this meat food product a ready-to-use ingredient in the preparation of other foods, e.g., edible tallow and lard used as shortening. They do not require additional lethality treatment before being consumed. If these products are hot filled at a lethality temperature (e.g., [Appendix A Compliance Guidelines For Meeting Lethality Performance Standards For Certain Meat And Poultry Products](#)) and packaged, they are not considered post-lethality exposed and therefore are not covered by the Rule. However, these products would be considered NRTE and not covered by the *Listeria* Rule if the process calls for partial rendering of the animal fat for tallow or lard and then further processing or finishing rendering in another plant.

Soups and other products that are cooked to eliminate pathogens and hot-packed in the final packaging material are RTE, but are not considered post-lethality exposed. Therefore, the *Listeria* Rule does not apply.

VI. Dehydrated Products

Dehydrated products are considered to be low risk products, because they do not support *Listeria* growth (as long as the water activity is below 0.92). However they may be considered RTE and post-lethality exposed, depending on their intended use. Therefore, they may be sampled under the RTEPROD_RAND and RTEPROD_RISK project codes.

Question: Are lard products covered under the *Listeria* Rule?

Answer: It depends. Lard products that are made from a rendering process are considered to be RTE. If they are hot-filled at a lethality temperature according to Appendix A or other scientific support, they would not be considered post-lethality exposed and would not be subject to the *Listeria* Rule. If they are filled at lower temperatures, then the product is considered post-lethality exposed, and subject to the *Listeria* Rule.

Appendix 1.2: Labeling

I. Post-lethality Treatments (PLT) and Antimicrobial Agents or Processes (AMAP)

According to the *Listeria* Rule, an establishment that controls *Lm* by using a PLT or an AMAP may declare this fact on the label, provided that the establishment has validated the claim (9 CFR 430.4(e)). The purpose of such claims is to inform consumers about measures taken by the processor to ensure the safety of the product and enable consumers to make informed purchase decisions. Such claims are voluntary and may be of value to consumers, especially those in groups most vulnerable to foodborne illness. Processors need to document their validation of these claims as described in [Appendix 2.1](#). An example of a statement that can be made is: “Potassium lactate added to prevent the growth of *Listeria monocytogenes*.” All labeling claims and label changes to add such claims must be submitted for evaluation and approval to the FSIS Labeling and Program Delivery Division.

In addition, antimicrobial agents that are added to RTE products, either to the formulation or to the finished RTE product, and those that are included in the primary packaging material of RTE products must be listed in the ingredients statement of the product. An establishment does not need to submit a label to the Agency for evaluation and approval when it adds an antimicrobial agent (e.g., sodium diacetate) to a product formulation that is approved or listed by FDA and FSIS as safe and suitable, provided that the label can be approved in accordance with the generic labeling regulations in 9 CFR 317.5 and 381.133, (i.e., the product must have a standard of identity in Title 9 of the Code of Federal Regulations (CFR) or the Food Standards and Labeling Policy Book and the labeling must not bear special claims, guarantees, or foreign language). All ingredients including antimicrobial agents require declaration on the label. Establishments may submit for temporary approval to use existing stocks of labels with revised formulations (up to six months) in order to update and produce new labels.

Approval of Labels Bearing Claims

As with all claims on labels, if there is a labeling claim about the use of antimicrobial agents or lethality treatments, the labels must be submitted to the Agency for evaluation and approval before use. Documents for validation of the effectiveness of the PLT or antimicrobial agent must be included with the label application. An establishment cannot put labeling claims of enhanced protection on RTE products that are not post-lethality exposed, such as cook-in-bag that are opened only by the consumer, because these are not covered by the *Listeria* Rule.

Special Considerations for Antimicrobial Agents in Comminuted Beef Products

The standard of identity for ground beef, chopped beef, and their cooked versions does not provide for the addition of ingredients, with the exception of non-fluid condimental seasonings, e.g., salt and pepper. Therefore, these products cannot be formulated with or treated with antimicrobial agents that are classified as having a lasting technical effect, e.g., sodium lactate and sodium diacetate, unless these products are descriptively labeled to reflect the use of the antimicrobial agents. For example, if sodium lactate is added, the product name on the label should be “Ground Beef with Sodium Lactate”.

However, for beef patties, which are standardized products, the regulations permit the addition of ingredients such as antimicrobial agents. Therefore, comminuted beef products formulated with antimicrobial agents and other approved or listed safe and suitable food ingredients can be labeled as “beef patties” and can be generically approved if the labeling does not bear any

special claims, guarantees, or foreign language.

The labeling for other products with standards of identity that permit the addition of antimicrobial agents (e.g., luncheon meats, hotdogs, cooked whole muscle cuts (such as roast beef)) may be approved in accordance with the regulations on generic label approval to reflect the addition of new, approved safe and suitable antimicrobial agents on labeling. The addition applies provided that no special claims, guarantees, or foreign language appear on such labels, per the generic labeling regulations.

II. Differentiating Products as RTE or Not RTE (NRTE)

Some products are expected to be lethality treated and RTE as shipped as part of their common or usual name that consumers understand to refer to RTE product, e.g., pâtés. Other products are defined by a standard of identity as RTE, that is, cooked, e.g., hotdogs. Some products are RTE based on labeling features, including Nutrition Facts, which declare nutrients in a product on a ready-to-serve or ready-to-eat basis. When these factors do not prevail, manufacturers may decide whether to classify products as RTE or NRTE products. However, care should be taken to ensure that is clear whether the product is RTE or NRTE (see [Attachment 1.2](#)).

The following should be taken into account when differentiating RTE from NRTE product:

(1) Decide on the HACCP category that best fits the product based on the processing operations that are involved. The HACCP categories most often used for RTE products include fully cooked—not shelf stable, not heat treated – shelf stable, heat treated – shelf stable, and product with secondary inhibitors – not shelf stable. In the situation where a product has been produced as an RTE product and it is not a product that is defined by a common or usual name that consumers understand to refer to RTE product (e.g., pepperoni) or standard of identity (e.g., hotdog) as a lethality-treated (e.g., cooked/fermented/dried) product, the manufacturer can decide whether the product is RTE or NRTE based on HACCP category. The establishment would need to ensure that documentation exists to support the HACCP category selected by the establishment for the product and that the appropriate category is reflected in the HACCP plan and labeling records. **The establishment's hazard analysis and intended use of the product should also be consistent with a RTE or NRTE product.**

NOTE: It is FSIS's expectation that products in the fully cooked – not shelf stable category will be considered RTE.

(2) Generate data that validate the cooking instructions that appear on the labeling of NRTE products (and include in all the alternative methods of cooking the temperature that the product must reach, i.e., 160°F) to ensure that consumers provide the lethality step. When the product has historically been viewed by the consumers as a RTE product, it is especially important for the establishment to make the distinction between the RTE product and the NRTE product. In addition, the “cooking instructions” should not be the same “heating” instructions that may be used on the labeling for RTE products.

- **Heating** instructions for RTE products should not use the word “cooking,” and should not include end temperatures (e.g. 160 °F), because it could be misleading to consumers.

- **Cooking** instructions for NRTE products should include the internal temperature the product is expected to reach (e.g., 160°F), and the method of cooking (e.g., oven time and temperature) so that the product is safe for consumption by the consumer.

NOTE: Specific cooking instructions are not required for NRTE products, expect the instruction to “cook thoroughly,” included as part of safe handling instructions (SHI). However, FSIS recommends that establishments provide validated cooking instructions on labels of products that are NRTE but appear to be RTE. In addition, if the establishment chooses to label a NRTE product with cooking instructions, it should validate the instructions; otherwise, they may not provide meaningful information to consumers on how to cook the product.

(3) Assess the label to ensure that it adequately reflects the features that are necessary on the principal display panel to convey that the product is a ready-to-cook product, e.g., "cook and serve," "cook and eat," "cook thoroughly," as well as safe handling instructions. **It would not be appropriate to label raw products using terms such as “cooked,” or broiled.** FSIS regulations require SHI if the meat or poultry component is NRTE and the product is not labeled “for further processing.” In comparison, if the meat or poultry component is RTE, but another non-meat or poultry component requires cooking for safety, the display of safe handling instructions is not required, but highly recommended. In addition, the basis for the Nutrition Facts declarations, e.g., serving size, must be on a ready-to-cook basis, not on a ready-to-serve basis (the company has to establish a ready- to-cook basis for serving size if the regulations do not provide one). The reference amount customarily consumed (RACC) for ready-to-cook and ready-to-serve meat and poultry products are found in 9 CFR 317.312 and 381.412, respectively. Nutrition labeling is not changed by this rule, but the serving size will be affected, depending on whether the product is classified as RTE or NRTE.

(4) Consider whether the label for the product can be approved consistent with the regulations on generic label approval (i.e., it is a label for a standardized product that bears no claims, special statements, guarantees, or foreign language). Such labels would not need to be sent to the Agency to be evaluated and approved prior to use.

If a meat or poultry product that is processed to a time/temperature that traditionally is considered to attain a full cook, but the intended use of the product is such that the product is intended to receive a lethality treatment by the consumer, the product does not have to be labeled as RTE unless the product is defined by a standard of identify as an RTE product (e.g., hotdogs, franks, and pork with barbecue sauce). Such product may be identified as an NRTE product, provided that the labeling and validated cooking instructions (SHI) are adequate to discern that the product must be cooked for safety by the purchaser. An example of such product is a cooked, thick-sliced, center-cut ham slice on which the labeling indicates that the product is ready-to-cook and for safety the product must be cooked to attain a minimum temperature. On the other hand, a thin sliced ham product in case-ready packaging may state that the product is RTE without additional cooking and, as such, should not be labeled with cooking instructions. Both products may have been heat treated in the same manner, but the establishment would only have control for *Lm* in the RTE product.

Chapter 2

FSIS *Listeria* Guideline: FSIS Control Measures for *Listeria*

- 2.1 [Post Lethality Treatments \(PLT\)](#)
 - 2.2 [Antimicrobial Agents and Processes \(AMAP\)](#)
 - [Table 2.1: Growth Limits for *Lm*](#)
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This chapter provides technical information about control measures that are used to meet the requirements for the three alternatives and provides examples establishments can use to apply these control measures to their particular product.

2.1 Post-lethality Treatments (PLT)

According to the *Listeria* Rule, [post-lethality treatments \(PLT\)](#) are treatments that are designed to reduce or eliminate levels of *Lm* contamination on RTE products. Establishments may choose to use PLT to meet the requirements of Alt. 1 (use of a PLT and [antimicrobial agent](#) or [antimicrobial process](#) (AMAP) or Alt. 2a (use of a PLT alone). According to the *Listeria* Rule, establishments that use PLTs must include the treatment as a CCP in their HACCP plan and validate the effectiveness of the PLT.

It is FSIS's expectation that PLTs will be designed to achieve at least a 1-log lethality of *Lm* before the product leaves the establishment. The PLT must be validated according to 9 CFR 417.4 and 430.4 as being effective in eliminating or reducing *Lm*. The establishment must also verify the effectiveness of the PLT and other control measures and make these results available upon request to FSIS personnel (9 CFR 430.4(c)(7)). Expected levels of control for PLTs and AMAPs are provided in [Table 2.1](#).

Examples of Post-lethality Treatments (PLT)
<p>PLT for <i>Lm</i> may include:</p> <ul style="list-style-type: none"> • Steam pasteurization, • Hot water pasteurization, • Radiant heating, • High pressure processing (HPP), • Ultraviolet (UV) Treatment,³ • Infrared Treatment, • Drying (Low water activity) (see example 1), and • Other validated processes.

³ Ultraviolet treatment can be used either as a post-lethality treatment or antimicrobial agent or process depending on whether it eliminates, reduces, or suppresses growth of *Lm*.

See the section on validation and verification of PLTs below and [Attachment 2.1](#) for more information.

PLTs could be effective in any post-lethality exposed RTE product, provided a study is performed demonstrating its effectiveness in the product. PLTs can be applied as:

- 1) Pre-packaging treatments, e.g., infrared technology (see Example 2)
- 2) Post-packaging treatments, e.g.,
 - Hot water pasteurization,
 - Steam pasteurization, and
 - High pressure processing (HPP).

Some of the published studies on post-lethality treatments are reviewed in [Attachment 2.1](#). Establishments should refer to the details of these studies if they want to use the intervention methods in their processing operations. The Compliance Guideline will be updated to include studies or other methods as they become available. For more information on using published studies or other methods of validating PLTs, see the validation of PLTs section below and [Appendix 2.1](#).

NOTE: Some AMAs or AMPs may also act as a PLT if they reduce or eliminate the pathogen and control its growth over the shelf life of the product. An example of an AMP that also acts as a PLT is a process such as drying or fermenting, which renders an RTE product shelf stable (see Example 1 below).

Example 1: Drying (low water activity (A_w)) as an AMAP and PLT

Drying is a means to kill *Lm* and help make a product “shelf stable”. Low water activity (A_w) limits the amount of water available to pathogens such as *Lm*, which will not allow them to grow. An A_w less than or equal to 0.92 will not support the growth of *Lm*, and an A_w of 0.85 or less (the A_w for achieving shelf stability) can sometimes even reduce *Lm* numbers. FSIS will consider an A_w of ≤ 0.85 at the time the product is packed to be a post-lethality treatment and an antimicrobial treatment if the establishment provides supporting documentation that *Lm* is reduced by at least 1-log before the product leaves the establishment and that no more than 2-logs growth of *Lm* occurs over the shelf life of the product. See [Table 2.1](#) for growth limits of *Lm*.

Example 2: Pre-packaging Treatment (e. g., infrared technology) as a Post-lethality Treatment

A pre-packaging treatment such as infrared technology can be used as a PLT as long as it is validated to eliminate or reduce the level of *Lm* by at least 1 log. Infrared technologies work by heating water inside microorganisms, causing cell death. However, if there is separation between the treatment and packaging, there is a possibility that the product could be re-contaminated after the infrared treatment. Therefore, sufficient conditions must be met to ensure a hygienic environment after the infrared treatment step to preclude re-contamination, or the post-lethality treatment would not likely be considered effective by FSIS. Some establishments may place the packaging machine right after the radiant heat treatment to

reduce or eliminate product exposure. If the infrared technology or other similar technology (e.g., HPP) is validated to achieve at least a 5-log reduction of *Lm* and other pathogens of concern (e.g., *E. coli* O157:H7 and *Salmonella*), the process would be considered to achieve full lethality and the product would not be considered to be post-lethality exposed.

Sending Product to another Establishment for a PLT

Establishments that produce post-lethality exposed products may send the product to another federally-inspected establishment for PLT. If the product will not be distributed into commerce until after the PLT is applied, it should be labeled “for further processing” or remain under the establishment’s control. The PLT should also be considered as part of the primary establishment’s HACCP program, even if it is applied at a secondary establishment.

Known or suspect *Lm*-positive product may be treated at the establishment or shipped to another establishment for PLT or other reprocessing (see [Section 4.4](#)). If a PLT is used to reprocess *Lm*-positive product, the process should be validated to achieve at least a **5-log reduction of *Lm*** or an indicator organism. If the product is shipped to another establishment for reprocessing, the product should be labeled “for further processing” or remain under establishment control until the PLT is applied to the product.

Validation of PLTs

As previously stated, the PLT must be validated to reduce or eliminate *Lm* from the product (9 CFR 430.4(b)(1)(ii)). The validation should demonstrate at least a 1-log reduction of *Lm* before the product leaves the establishment (unless the PLT is being used to treat contaminated product. See above). Establishments may use published peer-reviewed papers, challenge studies, or in-house studies to validate the effectiveness of PLTs. Published research studies may be used as a reference for validation provided the critical parameters used in the study (e.g., product type or size, the type of equipment, time, temperature, pressure and other variables) match the product or process used by the establishment. In the absence of published peer-reviewed papers, unpublished studies may be used as reference documents, provided there is supporting documentation that the data and analysis of results demonstrate that the specific level of application on specified products or range of products is effective to produce a safe product (e.g., results in at least a 1-log decrease).

FSIS expects the establishment’s HACCP documentation to demonstrate that the post-lethality treatment is adequate to eliminate or reduce *Lm* by at least 1-log. In cases of pre-packaging PLT that is applied to the finished product close to the packaging step (e.g., infrared treatment), the establishment must be able to demonstrate how the level of contamination that may occur between the treatment and the packaging is eliminated. For more information on validation of PLTs and AMAPs, see [Appendix 2.1](#).

2.2 Antimicrobial Agents and Processes (AMAPs)

According to the *Listeria* Rule, AMAPs must suppress or limit the growth of *Lm* throughout the shelf-life of the product. Antimicrobial agents (AMAs) can include lactates and diacetates added in the formulation of the product and growth inhibitors added in the immediate packaging material. AMAPs must be included in the establishment’s HACCP plan, [Sanitation SOP](#), or **prerequisite program** and the establishment must validate that the AMAP is effective as used. **It is FSIS’s expectation that AMAPs are designed to allow no more than 2-logs of growth of *Lm* over the shelf-life of the product.** If the AMAP is included in

the establishment's HACCP plan, the establishment must validate and verify its effectiveness in accordance with 9 CFR 417.4. If the AMAP is included in the establishment's Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 9 CFR 416.14. If the AMAP is included in a prerequisite program other than a Sanitation SOP, the establishment must ensure that the program is effective and does not cause the hazard analysis or the HACCP plan to be inadequate. The establishment must include the program and the results produced by the program in the documentation that it maintains as required in 9 CFR 417.5(a). Expectations for the efficacy of AMAPs are provided in [Table 2.2](#). For further information on validation of AMAPs, see [Appendix 2.1](#).

1. Antimicrobial Agents (AMAs)

AMAs are defined as substances added to RTE products that have the effect of suppressing or limiting growth of *Lm* in the product throughout the shelf life of the product (9 CFR 430.1). AMAs should allow no more than 2-logs of growth over the shelf life of the product. Examples of AMAs include: potassium lactate and sodium diacetate. Growth inhibition achieved by adding antimicrobials to product formulation depends on a variety of factors, such as:

- 1) The level of antimicrobial agent added,
- 2) pH of the product,
- 3) Moisture level of the product,
- 4) Product formulation, and
- 5) Whether the agent was added during formulation or to the finished product.

Some published studies on AMAs are reviewed in [Attachment 2.2](#). **If establishments want to use such studies as part of their validation or support, they would need to identify all of the critical operation parameters in the study and apply them to their process.** See the section below on documenting the effectiveness of AMAPs and [Appendix 2.1](#) for more information.

According to the *Listeria* Rule, the AMAPs must be effective throughout the shelf life of the product (9 CFR 430.1). The shelf life of the product is defined as the amount of time the product can be stored under specified conditions and still remain safe with acceptable quality. Guidance can be found in the following National Advisory Committee for Microbiological Criteria for Foods (NACMCF) report: [Considerations for Establishing Safety-based Consume-by Date Labels for Refrigerated Ready-to-eat Foods](#).

Question: Can modified atmosphere packaging (M.A.P.) be used as an AMP?

Answer: M.A.P. can be used as an AMP if the establishment has documentation that it suppresses growth of *Lm* and other pathogens and their toxins or toxic metabolites throughout the product's refrigerated shelf life

Question: Could curing (156 ppm added nitrite) be considered an AMA?

Answer: Sodium nitrite is primarily used to inhibit *Clostridium botulinum* growth and toxin production in cured meats. Studies have shown an inhibitory effect of nitrite, salt, and vacuum packaging on *Lm* growth in fish. The establishment would have to provide documentation on the inhibitory effect of nitrite on *Lm* in meat and poultry and indicate what other factors, such as salt concentration, are critical for the inhibitory effect.

AMAs can be added to the product during formulation, to the finished product, or to the packaging material. FSIS does not require a specific concentration of inhibitor to qualify as an antimicrobial agent. However, AMAs must be generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) and also must have been found to be safe and suitable by FSIS. Approved antimicrobials for processed meat and poultry products can be found in 9 CFR 424.21 and FSIS Directive 7120.1. The addition of antimicrobials in the formulation must be included in the ingredient statement of the label (see [Section 1.5](#)).

If an AMA is added to the surface of the product, it should be added as close to the final packaging step as possible to ensure the efficacy of the treatment. For example, if an AMA is applied to the surface of the product and the product is sliced, the AMA would no longer be valid as an AMA unless the sliced surface is also treated.

An establishment may also use AMAs that inhibit *Lm* on equipment and FCSs. Using these inhibiting agents on equipment and FCSs can be considered as part of the sanitation program. The use of AMAs on the equipment alone, however, would not qualify the product for Alt. 1 or 2. The establishment would have to add the AMA directly to the product to meet the requirements for either of the alternatives.

Hurdle Concept

Some AMAPs may have increased effectiveness in controlling *Lm* growth when added in combination with other AMAPs. This synergistic effect is commonly referred to as the **hurdle** concept. RTE products with added salt, nitrites, and other additives achieve a water activity, pH, or moisture-protein-ratio that will reduce the level of *Lm* and other pathogens during processing, and continue to inhibit the growth of the pathogens during the refrigerated shelf-life. The added salts and nitrites work together to create hurdles to pathogen growth. These products may not be shelf-stable because they need to be refrigerated during their shelf-life, but because of the combination of water activity and pH attained during the initial lethality treatment, these products may not support the growth of *Lm* during its refrigerated shelf-life.

Example 1: Lactates and Diacetates as AMAs

Lactates and diacetates are antimicrobials that can be added to the formulation of RTE meat and poultry products. These compounds are organic acids that serve to reduce the A_w and pH of the product. FSIS increased the permissible levels of sodium diacetate as a flavor enhancer and as an inhibitor of pathogen growth to 0.25 % (65 FR 3121-3123/2000). The Rule also permits the use of sodium lactate and potassium lactate in fully cooked meat, meat-food products, poultry, and poultry-food products, except for infant foods and formulas, at levels of up to 4.8 % of total product formulation, for the purpose of inhibiting the growth of certain pathogens. These include lactates and diacetates added in the formulation and growth

Question: If an AMA is applied to a product at one establishment, and the product is sent to a second establishment for further processing, can the second establishment claim Alt. 2?

Answer: Yes. The second establishment can claim Alt. 2, as long as it can demonstrate that the processing and sanitary conditions at the second establishment do not impact the effectiveness of the AMA or AMP over the shelf life of the product. To demonstrate its effectiveness, the second establishment would need to obtain documentation from the first establishment regarding levels of the AMA or AMP and demonstrate that the further processing applied to the product does not impact the effectiveness of the AMA or AMP. The second establishment would also need to demonstrate that levels of *Lm* in its post-lethality processing environment would not overwhelm the effectiveness of the AMA or AMP.

inhibitors in the immediate packaging material. Approved antimicrobials for processed meat and poultry products can be found in 9 CFR 424.21 and in FSIS Directive 7120.1. The addition of antimicrobials in the formulation must be included in the ingredient statement of the label. To meet the definition in the *Listeria* Rule, an AMA would need to be effective over the shelf life of the product (therefore, it can not be considered a processing aid), and should allow no more than 2 logs outgrowth of *Lm*.

Example 2: Vinegar as an AMA

Acidulants or added vinegars can be considered as AMAs, as long as the pH of the product is below 4.39, or the establishment can provide other supporting documentation demonstrating the effectiveness of the process. Vinegar serves to control pathogen growth by decreasing the pH of the product. However, *Lm* and other pathogens may still survive in a vinegar-based sauce or other products. Vinegar may also be considered a PLT if at least 1 log of *Listeria* death occurs before the product leaves the establishment, and the establishment can support this reduction. In addition, if the establishment can support that the vinegar results in at least a 5-log reduction of *Lm* after the product is placed in the final package, FSIS will consider the product to be non-post lethality exposed.

2. Antimicrobial Processes (AMP)

AMPs are operations, such as freezing, that are applied to an RTE product that have the effect of suppressing or limiting the growth of a microorganism, such as *Lm*, in the product throughout the shelf life of the product (9 CFR 430.1). Other examples are processes that result in a pH or water activity that suppresses or limits microbial growth.

Examples of Antimicrobial Processes (AMPs) are the following:

- a. Fermentation
- b. Drying
- c. Freezing

FSIS requires establishments to provide adequate supporting documentation as part of any validation when using AMPs to control the growth of *Lm* (see [Appendix 2.1](#) for more information on validation).

[Table 2.1](#) provides growth limits for *Lm*, which can be used to help evaluate the effectiveness of AMPs. If an AMP achieves conditions that would limit the growth of *Lm* based on the table, then the establishment can consider that the process has been validated to control growth of *Lm*.

Table 2.1 Growth Limits for *Lm* (ICMSF, 1996)

	Minimum	Optimum	Maximum
Temperature	-0.4 °C (31.3 °F)	37 °C (98.6°F)	45 °C (113 °F)
pH	4.39	7.0	9.4
Water activity	0.92	---	---

NOTE: Although *Lm* will not grow under the conditions in Table 2.1, it may still survive. In order to meet the conditions for a PLT, establishments would have to provide additional validation demonstrating that *Lm* is reduced or eliminated.

The establishment can place [Table 2.1](#) on file as part of its supporting documentation, demonstrating that the AMP it has selected is sufficient to control growth of *Lm*, and no further scientific support for the process would be needed. However, the establishment should collect in-plant demonstration data in order to meet the second element of validation (see pages 34-35 for a discussion of in-plant demonstration data). In addition, the establishment would also be expected to conduct on-going monitoring and verification activities to demonstrate that it is maintaining the conditions for pH, water activity, or temperature.

Example 1: Fermentation and Drying as an AMP

Fermentation and drying are processes that control the growth of *Lm* and other microorganisms by decreasing the pH and available moisture in the product. These processes are considered AMP if they result in finished product with pH or water activity that suppresses or limits the growth of *Lm*. FSIS will consider starter cultures used in dry or semi-dry fermented sausages to be AMAs if the addition of the starter culture or vinegar results in a finished product with a pH of <4.6, and the establishment documents that this pH level in the specific product suppresses or limits growth of *Lm*. Although Table 2.1 lists a pH of 4.39, the pH of dry and semi dry fermented sausages may be higher (<4.6) and still control *Listeria* growth, because of the hurdle effect of the pH and low water activity (see Section 2.1 for more information about the hurdle effect).

Example 2: Freezing as an AMP

Another antimicrobial process that controls the growth of *Lm* in the post-lethality environment is freezing of RTE products. Freezing prevents the growth of any microorganisms in the product because their cellular activities are arrested, but depending on the method and length of freezing and other factors, some microbial kill can also result. *Lm* is more resistant to freezing than other foodborne pathogens and may survive freezing. Once the product is thawed, cellular activities of microorganisms may resume.

It is important to note that freezing is only effective as an antimicrobial process while the product is frozen. If a product is distributed frozen and then thawed and sold as a refrigerated product, this would not meet the requirement in 9 CFR 430.1 that the antimicrobial treatment is effective throughout the shelf-life of the product. If the product is thawed as part of the preparation process by the consumer, the product will be deemed to have been frozen throughout its shelf-life.

Ensuring the Effectiveness of AMAPs

According to the *Listeria* Rule, establishments must document that the AMAP is effective in suppressing or limit growth of *Lm* over the shelf life of the product (9 CFR 430.4(b)(1)(ii)). The documentation should demonstrate that no more than 2-logs of growth occurs over the expected shelf-life of the product. The documentation for the effectiveness of the AMAP can be included in the establishment's HACCP plan, Sanitation SOP, or prerequisite program. Establishments may use published peer-reviewed papers, challenge studies, or in-house studies to support the effectiveness of AMAP. For more information on scientific supporting documentation, see [Appendix 2.1](#).

2.3 Sanitation

All RTE establishments are required to maintain sanitation in their environment, according to 9 CFR 416. Sanitation is the foundation for an effective *Listeria* Control Program. Establishments in Alt. 3 rely on sanitation alone to control *Lm* in their post-processing environment; therefore, it is critically important that they maintain sanitary controls. They are also required to verify sanitation by testing food-contact surfaces for *Lm* or an indicator organism (see [Chapter 3](#)). Maintaining effective sanitation is also important for Alt. 1 and 2 establishments because PLTs and AMAPs are validated to provide certain levels of reduction or control growth of *Lm*. **If levels of *Lm* are not controlled by proper sanitation, they could overwhelm the effectiveness of PLTs and AMAPs.** Therefore, it is important that all establishments producing post-lethality exposed product maintain sanitation in their environments and verify its effectiveness.

According to the *Listeria* Rule, sanitation measures for controlling *Lm* or an indicator organism may be incorporated into the establishment's HACCP plan, Sanitation SOP, or other prerequisite program. If *Lm* control measures are included in the Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 9 CFR 416.14. If sanitation measures are incorporated into a prerequisite program other than the Sanitation SOP, the establishment must ensure that the program is effective and does not cause the hazard analysis or the HACCP plan to be inadequate. The establishment must include the program and the results produced by the program in the documentation that the establishment maintains, as required in 9 CFR 417.5. It is expected that establishments will develop procedures for both routine and intensified sanitation in the event that *Lm* or an indicator organism is found on a FCS or in the product. **Sanitation actions should be escalated if repeated positives are found, indicating *Listeria* trends.** See [Chapter 4](#) and [Appendix 2.2](#) for more information on *Listeria* trends and sanitation.

Question: How do I maintain sanitation if my establishment produces raw and RTE product in the same room?

Answer: In some instances, small and very small establishments may not have the physical space to have separate RTE and raw processing areas. There are numerous sanitation considerations for separating processes by time or space, such as:

- Thoroughly cleaning and sanitizing between raw and RTE processing;
- Scheduling RTE processing on alternate days or scheduling RTE processing before raw processing;
- Using separate equipment for RTE and raw processing or scheduling equipment for RTE processing first, then for raw processing;
- Assigning different personnel for RTE and raw processing or having personnel clean hands thoroughly and use new coats, gloves, and hairnets and sanitized boots for RTE processing;
- Restricting movement of personnel during RTE processing;
- Using color-coded coats and locating coat racks for coats used in RTE area in designated space;
- Maintaining procedures for movement of personnel and equipment to prevent *Listeria* contamination; and
- Not allowing RTE product to come in contact with surfaces or raw products in coolers.

Reworked Product

In some cases, establishments may [rework](#) product from previous shifts. Although this is an acceptable practice, reworked products may be more likely to be contaminated than other products because of the increased handling of the products. The establishment may also rework products that are returned from another location (e.g., an off-site warehouse). The establishment should have documented procedures as part of its hazard analysis to evaluate the returned product on receipt, to ensure it has not been temperature abused or otherwise contaminated before being returned to the establishment. In addition, the establishment should have sanitary controls in place as part of its Sanitation SOP to ensure that reworked product is not contaminated during reprocessing or repackaging of the product. Establishments would also need to take into account their production of returned and reworked product when developing their *Listeria* Control Programs (see Chapter 3) and determining what products to hold when FSIS samples products or food contact surfaces for pathogens.

2.4 Expected Levels of Control

1. Antimicrobial Agents and Post-lethality Treatments

[Table 2.2](#) shows the expected level of control ([log reduction](#)) for establishments using PLTs and AMAPs in Alt. 1 and 2. Establishment validation studies or supporting documentation should demonstrate that these levels of control are achieved, at a minimum, in order for the PLT, AMAP to be considered effective (see [Appendix 2.1](#) for more information on designing validation studies). As indicated in the table, establishments that achieve higher levels of control will be sampled relatively less by FSIS than establishments that achieve a lower level of control.

Table 2.2 Expected Control Levels for Post-lethality Treatments and Antimicrobial Agents or Processes under Alternatives 1 & 2.			
[Levels of reduction or inhibition achieved to control <i>Lm</i>]			
Level of Control/ Treatment	Increased	Minimum	Not Accepted
Post-lethality Treatment (reduction should be achieved prior to distribution of the product into commerce)	2-logs or greater reduction	At least 1-log reduction	Less than 1-log reduction (At this level of reduction, the PLT is not eligible unless there is supporting documentation)
Antimicrobial Agent or Processes (growth must be limited over the shelf-life of the product)	Allows no more than 1-log growth	Allows no more than 2-logs growth	Allows greater than 2-logs growth (At this level of growth, the AMAP is not eligible unless there is supporting documentation)

How to use Table 2.2

For PLTs, the expectation is that establishments will achieve a minimum of at least a 1-log reduction in *Lm* prior to distribution of the product into commerce. If the establishment achieves an increased level of control (a 2-log or greater reduction), they will be sampled less frequently by FSIS. If they do not achieve at least a 1-log decrease, the PLT would not be eligible as a PLT under the *Listeria* Rule unless there is supporting documentation. In addition, an establishment using a PLT achieving less than 1-log reduction would not be eligible to apply for the labeling claim regarding enhanced protection from *Lm* (see [Section 1.5](#)).

For AMAPs, the expectation is that establishments will demonstrate a minimum of no more than 2-logs of growth over the estimated shelf-life. If the establishment demonstrates an increased level of control (1-log or less of growth over the shelf-life), then FSIS will sample the product less frequently. If the establishment demonstrates more than 2-logs of growth over the shelf-life, then the AMAP would not be considered eligible as an AMAP for purposes of the *Listeria* Rule, unless there is further supporting documentation.

NOTE: Establishments producing products that allow greater than 1-log growth of the pathogen during its shelf life will not be eligible to apply for the labeling claim regarding enhanced protection from *Lm*.

2. Sanitation Controls

Regardless of which alternative an establishment chooses, per 9 CFR 430.4(c), establishments are responsible for maintaining their sanitation programs and may use microbial testing for *Lm* or an indicator organism to verify the effectiveness of their sanitation program by testing food-contact surfaces (FCSs). Establishments in Alt. 2b and 3 are required to test their FCSs to verify sanitation in the environment, and FSIS recommends that establishments in Alt. 1 and 2a test their FCSs, as well. As stated previously, establishments are expected to implement intensified sanitation, and escalate their sanitation actions in response to positive results. Information on intensified sanitation can be found in [Appendix 2.2](#), and recommended testing frequencies to verify sanitation are discussed in [Chapter 3](#).

2.5 Training

A clearly written, fully-implemented training program is critical to the success of any food safety program designed to control *Listeria*. A *Listeria* Control Program, including implementation of HACCP and Sanitation SOP, will only be effective if employees understand the program, their role, and are able to perform the duties required of them in the program. This applies to new and existing employees involved in all stages of production, from sanitation to food handling to record keeping. Individuals that develop or reassess or modify HACCP plans must be trained in accordance with 9 CFR 417.7(b); however it is important that all employees be trained in basic sanitation.

An establishment's *Listeria* training program should include a broad, basic training program for all employees regardless of their job duties, as well as more specialized training programs for employees that handle product and staff involved in cleaning and sanitation. In some cases, employees that may be involved in more than one of these activities should be trained appropriately. The training should be tailored to meet specific needs of the establishment.

NOTE: A clearly written, fully-implemented training project is critical to the success of any *Listeria* Control Program. A *Listeria* Control Program will only be effective if employees understand the project, understand their roles, and are able to perform the duties required of them in the project.

For more information on developing training programs, see [Appendix 2.3](#).

2.6 New Technology and New Ingredient Review

FSIS believes that the facilitation of the use of new technology and new ingredients represents an important means of improving the safety of meat, poultry, and egg products. The Agency defines “new technology” and “new ingredients” as new ingredients or technologies or new applications of equipment, substances, methods, processes, or procedures affecting the slaughter of livestock and poultry, and processing of meat, poultry, and egg products. FSIS evaluates whether new technology and new ingredients affect product safety, inspection procedures, inspection program personnel safety, or if they would require the waiver of a regulation.

Substances used as new technology or new ingredients must also meet the requirements for safety and suitability under the Agency’s food ingredient approval process. While the Food and Drug Administration (FDA) has the responsibility for determining the safety of food ingredients and additives, as well as prescribing safe use, FSIS has the authority to determine that new ingredients and new uses of ingredients are suitable for use in meat, poultry, and egg products.

FDA and FSIS have a Memorandum of Understanding (MOU) regarding the review, approval, and listing of food ingredients and sources of radiation used in the production of meat, poultry, and egg products. This agreement establishes the working relationship to be followed by FSIS and FDA in responding to requests for the sanctioning of the use of food ingredients and sources of radiation subject to regulation by FDA and intended for use in the production of meat, poultry, and egg products. This review is normally done simultaneously by both agencies. The MOU information can be found at: [Memorandum of Understanding between FDA and FSIS](#)

The FSIS Innovations (New Technology) Staff within the Risk, Innovations, and Management Division (RIMD) in the Office of Policy and Program Development (OPPD) reviews new technology and new ingredients that can be applied in meat, poultry, and egg processing to facilitate the introduction of the new technology in establishment or plant operations. New technology and new ingredients for use on post-lethality RTE meat, poultry, and egg products to control the growth of *Lm* should be sent to the New Technology Staff for review. FSIS issued the document [“Guidance Procedures for Notification and Protocol Submission of New Technology”](#) to aid in the submission of applications for review of new technology and new technologies by FSIS. Those to which FSIS has “no objection” to their use in FSIS establishments are posted on the FSIS website at: [New Technology Tables](#).

A listing of ingredients that have been reviewed and approved by FDA and FSIS are available in [9 CFR Part 424, Subpart C, 424.21 “Use of food ingredients and sources of radiation.”](#) This regulatory listing of approved ingredients is now updated quarterly through revisions of [FSIS Directive 7120.1 “Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products”](#) to expedite the posting of new approved substances.

The above technology and ingredient reference resources should be used when considering the use of a technology or ingredient.

2.7 Glossary

Antimicrobial Agent (AMA): A substance in or added to an RTE product that has the effect of reducing or eliminating a microorganism, including a pathogen such as *Lm*, or that has the effect of suppressing or limiting growth of a pathogen, such as *Lm*, in the product throughout the shelf life of the product. Examples include potassium lactate and sodium diacetate, both of which limit the growth of *Lm* (9 CFR430.1).

Antimicrobial Process (AMP): An operation, such as freezing, applied to an RTE product that has the effect of suppressing or limiting the growth of a microorganism, such as *Lm*, in the product throughout the shelf life of the product (9CFR 430.1). Antimicrobial agents and processes are referred to together as **(AMAP)**.

Log Reduction: A 90% reduction of a pathogen. For example, a 2-log₁₀ reduction is a 99% reduction of a pathogen.

Post-lethality Treatment (PLT): A lethality treatment that is applied or is effective after post-lethality exposure. It is applied to the final product or sealed package of product in order to reduce or eliminate the level of pathogens resulting from contamination from post-lethality exposure (9 CFR 430.1).

Prerequisite Program: A procedure or set of procedures that is designed to provide basic environmental or operating conditions necessary for the production of safe, wholesome food. It is called “prerequisite” because it is considered by scientific experts to be prerequisite to a HACCP plan (9 CFR 430.1).

Rework: Rework is the process of recooking, reprocessing, or repackaging the product. This could also include temporary packaging of the product. FSIS considers any process that removes the product from the package and exposes it to the environment as rework.

Sanitation Standard Operating Procedure (Sanitation SOP): Written procedures for sanitation that describe all of the procedures the establishment will perform daily, before, and during operations, sufficient to prevent direct contamination or adulteration of products, according to 9 CFR 416.12(a).

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Attachment 2.1: Post-Lethality Treatments

NOTE: Mention of trademarks or commercial names does not constitute endorsement by USDA.

I. Steam Pasteurization and Hot Water Pasteurization

Post processing contamination of RTE meat and poultry is mostly confined to the surface. Pasteurization by steam and hot water acts on the surface microbial contaminants by the action of heat. Studies on surface pasteurization using steam or hot water were shown to be effective in reducing this contamination.

Studies by Murphy et al., (2003a) showed that post-cook hot water pasteurization and steam pasteurization resulted in a 7 log₁₀ reduction of *Lm* in inoculated vacuum packaged fully cooked sliced chicken. The reduction was effective when single-packaged breast fillets, 227 gm-packaged strips, and 454 gm-packaged strips were heat treated at 90° C in a continuous steam cooker or hot water cooker for 5, 25, and 35 minutes respectively. These investigators developed a model called ThermoPro that could predict the thermal lethality of pathogens in fully cooked meat and poultry products during post-cook in-package pasteurization (Murphy et al., 2001, 2003b, 2003c). The model was developed using *L. innocua* and verified for *Lm*.

Information gathered from the summary or abstract:

Post-lethality treatment: hot water pasteurization or steam pasteurization

Products: fully cooked chicken breast fillets and strips

Procedure: fully cooked products were surface inoculated with *Lm*, vacuum packaged and pasteurized

Equipment used for the pasteurization treatment:

Steam pasteurization: pilot-scale steam cooker

Hot water pasteurization: pilot-scale hot water cooker

Temperature of pasteurization: 90°C

Reduction of *Lm*: 7-log reduction

Products and time of pasteurization that resulted in 7-log reduction

Product	Time of pasteurization (min)
Single-packaged breast fillets	5
227g-package strips	25
454 g-package strips	35

II. Pre-Package Pasteurization and Post-Package Surface Pasteurization

Pre-package surface pasteurization treatment of fully cooked meat removed from its packaging wrap and inoculated with *Lm* resulted in a 1.25 to 3.5-log reduction with a treatment time of 60-120 sec at 475 to 750° F air temperature (Gande and Muriana, 2003). Surface pasteurization was applied on cooked whole and split roast beef, whole corned beef, and whole and formed ham using a radiant oven. Pre-package pasteurization (60 sec) combined with post-package submerged water pasteurization using formed ham (60 or 90 sec), turkey bologna (45 or 60 sec), and roast beef (60 or 90 sec), resulted in a 3.2 to 3.9-log reduction for ham, 2.7 to 4.3-log reduction for bologna, or a 2.0 to 3.75-log reduction for roast beef. The level of reduction varied depending on the method of inoculation, type of product used, treatment temperature, and residence time.

Muriana et al., (2002) used a stainless steel water bath to submerge cooked RTE deli-style whole or formed turkey, ham and roast beef, removed from their package, inoculated with *Lm* and vacuum packaged. Results show a 2 to 4-log decrease in the levels of *Lm* in inoculated products post-cooked at 195-205° F for 2-10 min.

Treatment of processed foods with acidified sodium chloride (ASC) is another example of pre-packaging treatment. ASC is an antimicrobial agent that is approved for use on processed meat food products (unless precluded by standards of identity in 9 CFR 319), prior to packaging of the food for commercial purposes (21 CFR 173.325(f)). It is applied as a dip or spray at levels that result in a sodium chlorite concentration of 500 to 1,200 ppm in combination with any GRAS acid at levels sufficient to achieve a pH of 2.5 to 2.9. It is approved as a secondary direct food additive and considered as a processing aid, with very temporary or short term technical effect (bactericidal antimicrobial activity), after which it rapidly degrades to leave no long term residues or actives remaining (Kemp, Alcide Corp., personal communication, 2003). Because of this, it does not have to be included in the ingredient listing of the label. Marsden et al. (2000, unpublished), evaluated sodium chlorite (1,200 ppm) with 0.9% citric acid for its effectiveness in reducing *Lm* on retail sausages. Results show that a water wash gave a 1.2-log reduction of *Lm*. An ASC dip for 15 sec provided a 1.0-log reduction better compared to water wash. ASC exposure time of 30 sec gave 1.1 and 1.6-log reductions over the water wash control, for spraying and dipping, respectively. Spray wash or dipping was found to be comparable in antibacterial effectiveness against *Lm*.

II. High-Pressure Processing

High-pressure processing (HPP) is a technology that subjects food to elevated pressures, with or without the addition of heat, to inactivate microorganisms and extend microbiological shelf life. This technology provides a means of ensuring food safety for those products that are difficult to heat treat due to organoleptic effects. HPP was shown to inactivate pathogens without any thermal or chemical effects and, at the same time, preserve the quality of the product. Raghubeer and Ting (2003) evaluated the efficacy of HPP in inactivating *Lm* in retail-packaged samples of sliced ham, turkey, and roast beef obtained from a manufacturer, and repackaged in 25-g portions. Results show that an inoculum of about 10^4 *Lm* cocktail in these 3 products and HPP treatment at 87,000 psi for 3 minutes showed no recovery of *Lm* after 61 days of storage at 34° F. No pressure-injured cells were detected. No adverse organoleptic effects were detected on the 3 HPP treated products during the 61-day shelf life study. No signs of spoilage were seen on all 3 products after 61 days of storage, and for 100 days for ham and turkey. According to the investigators, the normal shelf life of these products is 30 days, so the HPP treatment extended the shelf life of the products.

Attachment 2.2: Antimicrobial Agents or Processes

I. Use of Antimicrobial Ingredients including Bacteriophages, Lactates, Acetates, Diacetates, and Ozone

Bacteriophages are viruses that infect bacteria, and cause cell death. Bacteriophage preparations may be sprayed on RTE products to reduce or eliminate *Lm*. These preparations (a mixture of equal proportions of six different individually purified lytic-type bacteriophages specific against *Lm*) are applied as a spray at a level not to exceed 1 ml of the additive per 500 cm² product surface area.

Guenther et al., (2009) showed that *Lm* pathogen-specific bacteriophages could reduce bacterial counts by up to 5 logs when applied to the surface of hot dogs (sausages) and sliced turkey breast (cold cuts).

Ozone is an antimicrobial gas usually applied in an aqueous solution to products, food contact surfaces as a continuous spray (e.g., belts, moving tables), and non food contact environmental surfaces. Currently, the use of ozone is permitted by FDA and FSIS (21 CFR 173.368, FSIS Directive 7120.1) for use with all meat and poultry products, including RTE meat and poultry products.

Buege et al., (2004) showed 1.0 to 2.4 log reductions (average 1.5) of *Lm* when 0.6 ppm ozone for 30 seconds was applied to ham, salami, meatloaf, natural casing wieners, and skinless wieners.

Studies have shown that lactic acid and acetic acid have significant antimicrobial activity in broth and food systems. Sodium and potassium salts of these acids, when added to processed-meat formulations, are also known to potentially inhibit pathogenic bacteria, especially *Lm*. These antimicrobials inhibit growth of pathogens by inhibiting their metabolic activities.

Seman et al., (2002) developed a mathematical model capable of predicting the growth or stasis of *Lm* in commercial cured meat products using a response surface method. The model can be used by manufacturers in the determination of the appropriate amounts of potassium lactate and sodium diacetate to be added to cured meat products that are organoleptically sensible and will not support the growth of *Lm*.

Thirty products were formulated by using a variety of raw material sources such as pork trimmings, trimmed turkey breast halves, and four-muscle ham. Varying amounts of potassium lactate and sodium diacetate were added to the meat formulation and the meats were processed into different products. After chilling, the products were stripped of their casings, sliced into 25-g slices, placed into pouches, and inoculated with *Lm* by applying it to the surface of 100g of cured meat (four slices).

Sodium chloride content was found to have a negative correlation to growth rate. The investigators provided a final regression equation predicting the growth of *Lm* in cured RTE meat products stored at 4° C. The investigators used predictive model performance factors and a simple linear regression analysis to evaluate the model generated in this study. They verified the accuracy of the model by comparing it with actual *Lm* growth data from an independent challenge study conducted with four different commercial RTE meat products using similar storage conditions. Performance factors calculated and evaluated for control products (those

not containing potassium lactate and sodium diacetate) indicated that on the average, the predicted growth of *Lm* exceeded those of the observed values by about 24%.

The study also emphasized the importance of **moisture content** in the application of lactates and diacetates as antimicrobial agents. The article reports that **“The results show that increasing amounts of potassium lactate syrup and sodium diacetate decreased the growth rate of *Lm*, while increasing finished product moisture increased the growth rate. Sodium chloride content was not significant but was found to have a negative correlation to growth rate.** This study provided a useful model in determining the target amounts of potassium lactate and sodium acetate for cured meat product formulations to inhibit the growth of *Lm*. The calculations would also require knowledge of the finished product sodium chloride and moisture contents.”

Recall Alert

An investigation of a 2007 recall of RTE cooked chicken products contaminated with *Lm* showed that the establishment failed to maintain sanitation, and antimicrobial agent failed to suppress *Lm*. The moisture levels were higher in the product than in the establishment's supporting documentation, which could have allowed *Lm* growth.

Table 2 from the study shows that different finished product moisture levels, amount of sodium chloride, and lactate and diacetate result in different levels of *Lm* growth rate.

% Salt	% Sodium Diacetate	% Potassium Lactate Syrup	% Product Moisture	<i>Lm</i> Growth Rate (wk ⁻¹)
1.50	0.15	7.0	74.0	0.0
1.50	0.05	2.5	74.0	0.0991
2.20	0.20	4.75	64.5	0.0
2.20	0.10	0.25	64.5	0.1338

The investigators advised that this validated model is specific to the products designed for the study and the *Lm* strains used. Testing of this model in other environments and with other *Listeria* spp., and to formulations that are outside the model's limits may result in different maximum growth rates.

This study (Seman et. al., 2002) provided a useful model in determining the target amounts of potassium lactate and sodium acetate for cured meat product formulations to inhibit the growth of *Lm*. The calculations would also require knowledge of the finished product sodium chloride and moisture contents. The investigators advised that this validated model is specific to the products designed for the study and the *Lm* strains used. Testing of this model in other environments and with other *Listeria* spp., and to formulations that are outside the model's limits may result in different maximum growth rates. This study was used as the basis for the Opti.Form *Listeria* Control Model.

The Opti.Form *Listeria* Control Model is a unique tool used to calculate the levels of lactate and diacetate required to retard the growth of *Lm* in cured meat and poultry products. The model is based on the study detailed in the paper by Seman et al., 2002, above. The model includes:

- Instructions on how to use the model,
- Explanation on the development of the model,
- Information on the anti-microbial effects of lactate and diacetate,

- Lactates and diacetates and use of these products,
- Regulations and labeling, and
- Literature references.

The model can be accessed by visiting the Purac website at:

<http://www.purac.com/EN/Food/Calculators/Listeria-Control-Model.aspx>

Bedie et al., (2001) evaluated the use of antimicrobials, including in frankfurter formulations, on *Lm* populations during refrigerated storage. Fully cooked and cooled frankfurters were inoculated with 10^3 to 10^4 CFU /cm² of *Lm* after peeling and before vacuum packaging. Samples were stored at 4° C for up to 120 days and sampled for testing on assigned days. Results were as follows:

Antimicrobial	Level (%)	<i>Lm</i> Growth Inhibition
Sodium lactate	3	70 days no pathogen growth
Sodium diacetate	0.25	50 days no pathogen growth
Sodium acetate	0.25, 0.50	20 days no pathogen growth
Sodium lactate	6	120 days no growth and reduced pathogen growth
Sodium diacetate	0.5	120 days no growth and reduced pathogen growth
Inoc. Control	0.0	Increased to 6 logs in 20 days

Note: Sodium acetate is approved as a flavor enhancer, not as an antimicrobial agent.

No pathogen growth refers to zero increase in the number of inoculated *Lm* cells (bacteriostatic), while reduced pathogen growth refers to a decrease in the number of inoculated *Lm* cells (bactericidal) in the product. In this study, tables showed that the reduction varied with storage days, but was up to 1.0 log on some days. Antimicrobials were found to have no effect on pH, except for sodium diacetate, at 0.5%, which reduced the initial pH. Using the formulations and conditions in the study, establishments can add 3% sodium lactate in the frankfurter formulation and obtain no growth of *Lm* up to 70 days at refrigerated storage of 4° C. If the lethality treatment is adequate to eliminate *Lm*, then the only probable source of *Lm* would be from exposure of the product during peeling and repackaging. However, the establishment's sanitation program may keep the numbers to a very low level, and 3% sodium lactate included in the formulation would inhibit the growth of *Lm* during the product's refrigerated shelf life. Levels of sodium lactate at 6.0% and sodium diacetate at 0.5% showed a reduction of the pathogens; however, these levels are above the permitted levels.

A study by Samelis et al., (2002) used similar treatments, processing, and inoculation procedures and frankfurter formulations as the previous study described above. However, in this study, combinations of antimicrobials were used, and in combination with hot-water treatment. Hot-water treatment involved immersion of frankfurters, with two product links in a package to 75 or 80° C for 60 sec. Storage at 4° C yielded the following results:

Treatment	Levels (%)	<i>Lm</i> Growth Inhibition
Sodium lactate	1.8	35-50 days no growth
Sodium lactate + sodium acetate	1.8 0.25	120 days no growth; 35-50 days growth reduction
Sodium lactate +	1.8	120 days no growth; 35-50 days growth

Sodium diacetate	0.25	reduction
Sodium lactate + Glucuno-delta- lactone	1.8 0.25	120 days no growth, 35-50 days growth reduction
Hot water treatment (80° C, 60 s) + Sodium lactate	1.8	Inoc. population reduced by 0.4-0.9 log CFU/cm ² , and 50-70 days growth reduction by 1.1-1.4 CFU/ cm ²
Hot water treatment (80° C, 60 s)		Increase in growth to about 6-8 logs in 50 days
Inoculated Control, no treatment		Increase in growth to about 6 logs in 20 days and 8 logs thereafter up to 120 days

Note: Sodium lactate was used as a 3% of a 60% (wt/wt) commercial solution. Glucuno-delta lactone is approved as an acidifier and a curing accelerator, but not as antimicrobial. Sodium acetate is approved as a flavor enhancer, not as an antimicrobial agent.

Glass et al., (2002) evaluated sodium lactate and sodium diacetate on wieners and cooked bratwurst containing both beef and pork supplied by a commercial manufacturer. Antimicrobial solutions used were sodium lactate and sodium diacetate singly or in combination at varying concentration. Wieners were repackaged in gas-impermeable pouches, then surface-inoculated with *Lm* mixture on multiple areas of the surface of each link. Packages were vacuum-sealed and stored at 4.5° C for up to 60 days.

Two types of cooked bratwurst from a commercial manufacturer were evaluated: bratwurst that was cured and naturally smoked and bratwurst that was uncured and unsmoked. Bratwurst was stored at 3 or 7° C for up to 84 days. The surface treatment, consisting of dipping wieners into solutions containing up to 6% lactate and up to 3% diacetate for 5 seconds, did not delay pathogen growth, indicating that dipping wieners in the lactate/diacetate solutions is not an efficient way to apply the antimicrobials. However, the inclusion of lactates and diacetates in the formulation was found effective in inhibiting growth of *Lm*. Results were as follows:

Product	Sodium Lactate (%)	Sodium Diacetate (%)	<i>Lm</i> Levels (CFU/pkg)
Bratwurst uncured, unsmoked	3.4	0.1	Growth delayed for 4-12 weeks at 7 and 3° C storage, respectively.
	2.0	0.0	Growth delayed for 1-2 weeks at 7 and 3° C
Bratwurst cured, smoked	3.4	0.1	Growth inhibited for 12 weeks at 7 and 3° C
	0.0	0.0	Growth up to 1 log after 4 weeks at 7 and 3° C
Wieners	3.0	0.0	Growth inhibited for 60 days at 4.5° C
	1.0	0.1	Growth inhibited for 60 days at 4.5° C

A study by Porto et al., (2002) used freshly processed peeled frankfurters in vacuum sealed packages obtained from a commercial manufacturer. Two formulations of links were used in the study: one with added 2 or 3% potassium lactate and the other without added potassium lactate. Frankfurters were aseptically removed from their original package, repackaged, and inoculated

with a mixture of *Lm*. The packages were vacuum-sealed to 95 kPa and incubated at 4 and 10° C.

Results show that the addition of 2% or 3% potassium lactate in frankfurters can appreciably enhance safety by inhibiting or delaying the growth of *Lm* during storage at refrigeration or abused temperatures. The viability of the pathogen was influenced by pH and the levels of lactate added, but not by the presence of indigenous lactic acid bacteria.

Potassium Lactate (%)	Inoculum CFU/pkg	Storage Temp. (°C)	Days Storage	<i>Lm</i> Levels (CFU/package)
2.0	20	4	90	Remained at about 1.6 log
3.0	20	4	90	Remained at about 1.4 log
3.0	500	4	90	Remained at about 2.4 log
0.0	20	4	90	Increased to about 4.6 log
0.0	500	4	90	Increased to about 5.0 log
2.0	20	10	60	Remained at about 1.4 log
3.0	20	10	60	Remained at about 1.1 log
0.0	20	10	60	Increased to about 6.5 after 28 days, declined to about 5.0 after 60 days
3.0	500	10	60	Remained at about 2.4
0.0	500	20	60	Increased to about 6.6 log after 40 days and declined to about 5.5 log after 60 days

II. Growth Inhibitor Packaging

Growth-inhibitor packaging is an intervention which delivers an active antibacterial agent to the surface of an encased sausage product. By incorporating this special coating onto the internal surface of cellulose casings, the antilisterial treatment is transferred to the surface of the processed meat/sausage during thermal processing. Upon removal of the casing, the treatment remains active on the meat surface, providing effective protection against inadvertent *Listeria* contamination during subsequent peeling and packaging processes. Growth-inhibitor packaging, used in conjunction with functional HACCP and Good Manufacturing Practices, provides the industry with one more tool to control the risk of *Lm* contamination of RTE meat and poultry products.

Studies on meat formulations for hotdogs using NOJAX® AL™ (Viskase Corporation, 2003) showed that the use of the casings provide a lethality hurdle to the growth of *Lm*, not just an inhibitory effect. The lethality impact is delivered within the first hours/days of the sausage/hotdog package life. This impact is dependent on many variables, but is generally in the range of 1 – 2 log decrease of *Lm* at high levels of inoculation. This performance has been observed in challenge studies conducted on hotdogs drawn from commercial full-scale trials at a number of commercial processing plants. In high-inoculation trials, NOJAX AL has been combined with conventional growth inhibiting additives, and the lethality impact is obtained and then maintained throughout the product life cycle. In these same trials, without growth inhibiting additives, this casing produces lethality but in several weeks the remaining *Lm* begin to grow. NOJAX AL is available in the U.S., and has been approved by both FDA and USDA for its key component, nisin. This GRAS component must be included in the ingredient statement via a label change request to the FSIS Labeling and Program Delivery Division. Because this is a

naturally derived polypeptide, there are storage and use-by criteria that will have to be adhered to by the user for maximum benefit. Casing shelf-life is about 60-90 days, with a not to exceed temperature of 85° F.

This technology can be applied to most hotdogs and sausages that are encased in cellulose casing. This casing intervention can be used in any instance where casing is used as a mold for processed meat and poultry during thermal processing. This would include cellulose, plastic, and, possibly, natural casing. As part of a manufacturer's decision to use this technology, benefits are: 1) no capital costs or new equipment; 2) no change in processing steps or plant reconfigurations; 3) no impact on flavor, texture, or package appearance, and 4) minor labeling change to ingredient statement.

Since this is a surface treatment, cost will be proportional to the surface to volume ratio of the product: the larger the sausage diameter, the lower the cost per pound. In general, economic analyses put the cost of this lethality intervention at about 2-3 cents per pound of finished product, with a mid-range target price of 2.5 cents per pound for a traditional 10-to-the-pound retail pack of hotdogs.

Janes et al., (2002) investigated the effect of nisin added to zein film coatings (Z) coated onto cooked RTE chicken against *Lm*. Cooked chicken samples inoculated with *Lm* were dipped into Z dissolved in propylene glycol or ethanol, with or without added nisin (1,000 IU/g) and/or 1% calcium propionate and stored at 4°C or 8°C for 24 days. After 16 days at 4°C, *Lm* was suppressed by 4.5 to 5 log CFU/g with zein film coatings with nisin. The most effective treatment in the study for controlling *Lm* on the surface of RTE chicken was found when using edible zein film coatings containing nisin at a storage temperature of 4°C.

A processing plant would use film coatings by fully processing the meat products, then coating them with the films. Coating can be done by spraying or dipping the processed meat products and then allowing them to dry. Zein coatings on the meat products can be dried by circulating air around the meat product using a fan. Finally, the dried coated meat products can be packaged with the usual plastic film material and refrigerated. The study by Janes et. al. has not been tested in commercial poultry processing conditions.

Some general observations from the published studies on antimicrobials:

- Lactates, acetates, and diacetates were found more effective in inhibiting growth of *Lm* when used in combination than when used singly.
- These antimicrobials (described in the guideline) were found more effective when used to the maximum allowable concentration. However, higher concentrations of antimicrobials used in the formulation may affect the sensory qualities of the product, such as flavor and texture, which would necessitate sensory evaluation of treated products.
- When used in combination, the amount needed to inhibit growth may be reduced.
- These antimicrobials were found to have listeristatic activity more than listericidal activity, i.e., they prevent growth of the pathogen more than reduce the number of cells of the pathogen, and therefore may not be effective against gross contamination of a product. The establishment's sanitation program should control gross contamination of

the processing environment and equipment. Addition of antimicrobials would be effective only as part of the overall HACCP strategy.

- Including these antimicrobials in the formulation was found to be more effective in inhibiting listerial growth than dipping products in solutions of antimicrobials.
- The antimicrobial activity of lactates and diacetates when used singly or in combination is affected by the level of contamination of the meat product surface and processing factors such as pH, moisture, water activity, fat, nitrite, salt content, time and temperature of storage, and packaging atmosphere.
- Application of the treatments used in these studies is limited to the formulations, products, and treatments used in the studies. Applying these studies to other products and formulations may result in different rates of growth inhibition. Therefore, the establishment should verify the effectiveness of the antimicrobials used in these studies for other processed meat products and other storage temperatures.
- Antimicrobials used in the formulation should have an effective antilisterial activity throughout the commercial shelf life of the product. Currently, the targeted commercial shelf life of refrigerated cooked meat products in the U.S. is 75 to 90 days.
- Using post-packaging thermal treatments in addition to antimicrobials was found to increase the total antilisterial effects of the antimicrobials.
- These antimicrobials were found to be more effective in smoked products formulated with sodium nitrite or in products stored at strict refrigeration temperatures.
- These antimicrobials may be a cost-effective antilisterial method that very small establishments can use.

Appendix 2.1 Validation

- I. Validation
- II. Scientific Support
 1. Published Processing Guidelines
 2. Scientific Articles from a Peer-Reviewed Journal
 3. Challenge or Inoculated-Pack Studies
 4. Validated Predictive Microbial-Modeling Programs
 5. Establishing the Shelf-life of the Product
- III. In-plant Demonstration
- IV. Validation Examples

I. Validation

Validation is the process of demonstrating that the HACCP system as designed can adequately control identified hazards to produce a safe, unadulterated product. **There are two distinct elements to validation:**

- 1) **The scientific or technical support for the HACCP system (design).** This consists of having scientific and technical documentation that demonstrates that the designed process can control the identified hazard. In other words, will the HACCP **work in theory**?
- 2) **The initial practical in-plant demonstration proving the HACCP system can perform as expected (execution).** This consists of having records that demonstrate that the HACCP plan achieves what it is expected to achieve. In other words, **does the plan work in practice**?

Validation encompasses activities that make up the entire HACCP system. Validation is an important component to the development of a HACCP system but has particular importance for products produced under the *Listeria* Rule. Validation, as it relates to the requirements in the *Listeria* Rule, will be covered in this Appendix. In particular, considerations for scientific support and in-plant data for AMAPs, and PLTs will be covered. Further recommendations can be found in the [FSIS Compliance Guideline: HACCP Systems Validation, May 2013](#).

II. Scientific Support

The **first element of validation is scientific support** (design). There are several types of scientific support that would be considered acceptable for validating an AMAP, PLT, or other treatment. These include:

- Published processing guidelines
- Regulatory performance standards

Question: Can establishments use the studies cited in the Compliance Guidelines for validation as they use the Compliance Guidelines in Appendices A and B in the Final Rule for certain meat and poultry products to validate cooking and cooling (stabilization) processes?

Answer: Yes, provided the product, processing procedures, and ingredients are equivalent to those in the studies. For example, if the pH and concentration of antimicrobial in the study were both considered critical, then the product must have that pH and contain the antimicrobial in the concentration used in the study.

- A scientific article from a peer-reviewed journal,
- A challenge or inoculated-pack study,
- Unpublished data gathered in-house, and
- Validated predictive microbial-modeling program.

The scientific documentation should identify:

- The purpose,
- The experimental procedure (including microbial testing methodology),
- The hazard studied,
- The product type, size, formulation, and composition (i.e., water activity, pH, fat, moisture level, salt level, and if applicable, antimicrobial level),
- The processing steps that will achieve the specified reduction or prevention of growth of the pathogen, and
- The critical operational parameters (i.e., the factors affecting microbial reduction in the processor's HACCP system), including:
 - The model and type of equipment,
 - Concentration,
 - Time,
 - Temperature, and
 - Pressure.
- How the critical operational parameters can be monitored, and
- The level of reduction or prevention achieved by the post-lethality treatment or antimicrobial agent applied.

Question: What records would the Agency require for products with formulations that are inherently antilisterial, but that may not be formulated specifically for that purpose (e.g., BBQ and pickled meats, precooked bacon, beef snack sticks)? Would the establishment be required to make changes to the HACCP plan, Sanitation SOP, or pre-requisite project to account for the antilisterial benefit of the formulation/process?

Answer: FSIS would expect the establishment to have scientific support (e.g., citations to published data) that the product characteristics (e.g. moisture level, pH, or salt levels) result in at least a 1-log decrease of *Listeria*. Inclusion of the process in the HACCP plan would only be required for a PLT. If the process controls *Listeria* growth, it could be included in the Sanitation SOP or prerequisite project.

Question: Does an establishment need to provide additional validation information beyond what is in the Compliance Guidelines with regard to freezing, pH and water activity to satisfy the first part of validation, scientific support?

Answer: No. The establishment needs to validate the process in relation to *Lm*, except when these values are below the limit of *Lm* growth: pH below 4.39, water activity below 0.92, and temperature below -0.4°C, as stated in the Compliance Guidelines. However, the establishment must have the supporting documentation on-file and must conduct monitoring and verification activities.

Care should be taken to ensure that the scientific support documents are sufficiently related to the process, product, and hazard identified in the hazard analysis. The supporting documentation should be complete and available for review. Failure to take these steps would raise questions about whether the HACCP system has been adequately designed and validated.

To be effective, the process procedures should relate and adhere to the critical operational parameters in the supporting documentation. Critical operational parameters are those parameters of an intervention that must be met in order for the intervention to operate effectively and as intended. Critical operational parameters include product type or size, the type of equipment, time, temperature, pressure, and other variables used in the study needed to result in equivalent levels of reduction of *Lm*.

It is important that the critical operational parameters in the establishment's actual process match those in the scientific support because such characteristics affect the PLT efficacy; for example: pH, water activity, and the presence of preservatives may all affect the PLT efficacy. If one or more of the parameters are not addressed in the process or if one or more parameters differ from those used in the scientific support, then the establishment should document a justification for the differences.

1. Published Processing Guidelines

This guideline (the FSIS *Listeria* Guideline) is an example of a published processing guideline that can provide adequate supporting documentation for an establishment's control processes for *Lm*. For example, [Table 2.1](#) contains growth limits for *Lm*, which can be used by establishments to help support the effectiveness of AMPs. If an AMP achieves conditions that would limit the growth of *Lm* based on the table, and the establishment meets the other criteria in the guidelines that would limit pathogen growth (e.g., maintaining sanitation), then the establishment can consider that the process has been validated to control growth of *Lm*. The establishment can place [Table 2.1](#) on file and no further scientific support for the process would be needed. However, the establishment should collect in-plant demonstration data in order to meet the second element of validation (see pages 34-35 for a discussion of in-plant demonstration data). In addition, [Attachment 2.1](#) and [Attachment 2.2](#) contain summaries of journal articles that may be used to support the efficacy of PLTs or AMAPs, respectively. These attachments are not considered adequate support on their own, however, because they do not provide the details of each study that an establishment needs to determine if the study is representative of the actual process. For this reason, if an establishment chooses to use one of the articles provided in Attachment 2.1 or Attachment 2.2., FSIS expects that the establishment will have a full copy of the original article on file. Establishments may also keep [Table 3.1](#) on file to support that they are meeting the requirements of the *Listeria* Rule related to Alternative 2, Choice 2 (2b) and Alternative 3 processes. Establishments can keep this table on file as part of the supporting documentation needed to explain why the testing frequency they have selected is sufficient to control *Lm* or an indicator organism according to 9 CFR 430.4(b)(2)(iii) (E) and (3)(i)(E).

In addition, both [Appendix A](#) and [Appendix B](#) of the final rule, "[Performance Standards for the Production of Certain Meat and Poultry Products](#)", [FSIS Guidance on Safe Cooking of Non-Intact Meat Chops, Roasts, and Steaks, April 2009](#) and the [Time-Temperature Tables for Cooking Ready-to-Eat Poultry Products](#) may be used to support the reprocessing of contaminated products, as described in [Section 4.4](#). Although Appendix A, the FSIS Guidance on Safe Cooking of Non-intact Meat Chops, Roasts, and Steaks, and the Time-Temperature

Tables for Cooking Ready-to-Eat Poultry Products are designed to achieve reductions in *Salmonella*, establishments are not expected to validate that these processes also achieve reductions in *Lm* because *Salmonella* is considered an indicator of lethality for *Lm*.

2. Scientific Data/Information

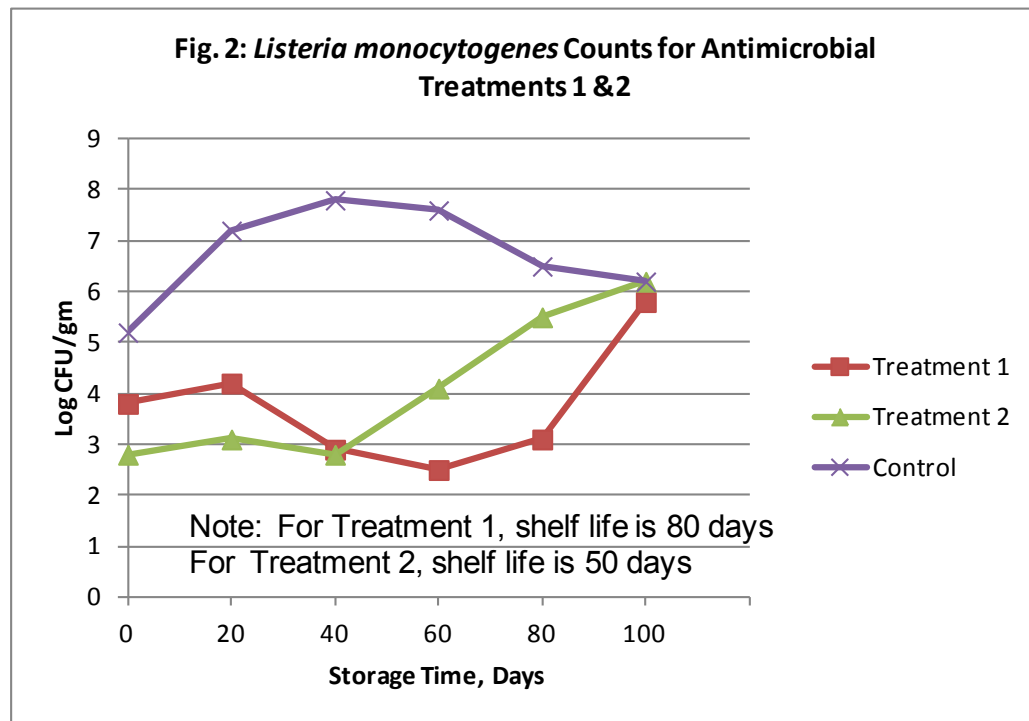
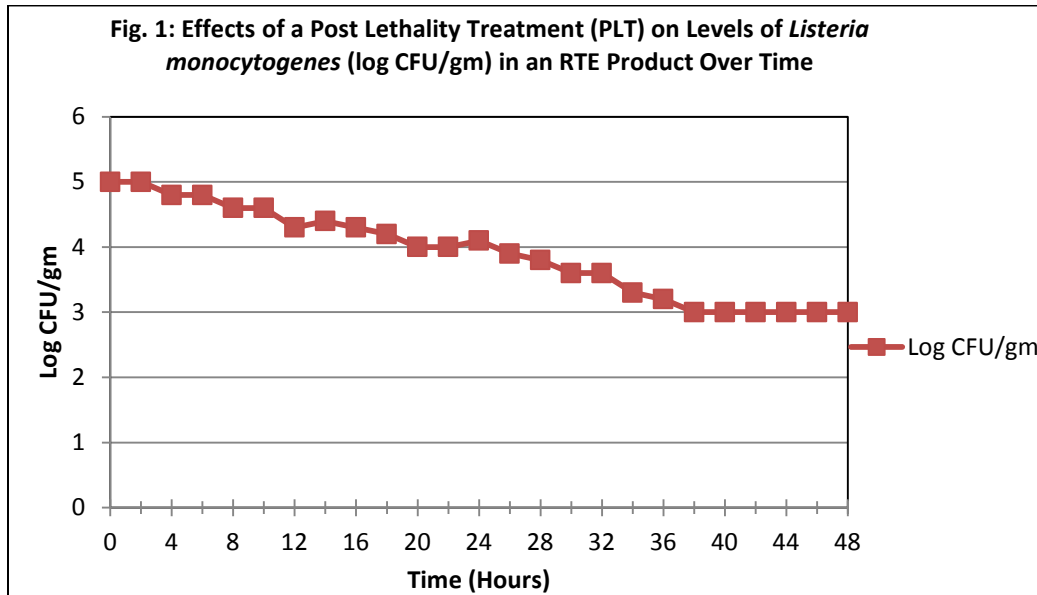
Peer-reviewed scientific data that describes a process and the results of the process can provide adequate supporting documentation for the establishment's process. This type of support could include journal articles, graduate student theses, or information found in a textbook. All of these types of scientific data go through a process of evaluation involving qualified individuals within the relevant field. In addition to describing the microbiological results of the process, the data may describe the role intrinsic and extrinsic product factors play on the growth of microorganisms. For example, a textbook may contain data on the growth limits of certain pathogens based on a food product's water activity and pH. For journal articles, the study should relate closely to the establishment's process with regards to species, product characteristics, and equipment. The establishment should use the **critical operational parameters** (see Section II above), cited in the journal article, that achieve the required or expected lethality or stabilization. If the establishment uses parameters that differ from those cited in the journal article, it should provide additional support for those parameters. For biological hazards, the scientific article should contain microbiological data specifying the level of pathogen reduction achieved by the intervention strategy for the target pathogen identified in the hazard analysis. A lack of microbial data in the scientific support could raise questions concerning whether the process design has been adequately validated.

There are a number of published journal articles, theses, or textbooks available that can be accessed on-line or through a library system. Again, the establishment should ensure that the study closely relates to the establishment's process. An establishment that uses products, treatments or variables other than those used in the referenced studies should perform its own studies (or use another method of scientific support) to ensure effective reduction of *Lm*. For example, if a published study uses a ham product, and the establishment produces a turkey product with a different formulation, the establishment should not use the study alone as its scientific support. In order to support the safety of its process, it would need to use a different study, perform its own study, or use another form of scientific support. Likewise, if an establishment uses a process such as drying for 10 days, and the study shows that drying for 20 days is effective, it would not be appropriate for the establishment to use the study, alone, as scientific support. The establishment would need to provide other support demonstrating that 10 days would be effective in controlling *Lm* and other pathogens in their particular product type.

3. Challenge or Inoculated-Pack Studies

In the absence of a published processing guideline, published peer-reviewed paper, or predictive microbial-modeling program that would contain information needed for validation, unpublished studies may be used. In order for an unpublished paper to provide sufficient support, the study would need to be well designed, and the results would need to demonstrate that the specific level of application on specified products or range of products is effective to produce a safe product. For more information on design of challenge studies see the article "[Parameters for Determining Inoculated Pack/Challenge Study Protocols](#)" published by the National Advisory Committee on Microbiological Criteria for Foods in the Journal of Food Protection in 2010.

Examples of the effects of a post-lethality treatment and an antimicrobial process or treatment over time are shown in Figures 1 and 2, respectively:



A challenge study is a study that documents the adequacy of control measures in a process. This involves inoculating the target organism (e.g., *Lm* or an appropriate surrogate organism) into a product to determine the effect of control measures such as post-lethality treatment or antimicrobial agent or process on the reduction or growth of the organism. Challenge studies should be conducted by a microbiologist trained in performing challenge studies, in a laboratory to avoid the possible spread of contamination in an establishment. The number of organisms

before and after the application of the control measure is counted to determine the effect of the control measure. The study determines the effect using different processing variables such as time, temperature, pressure, concentration, acidity, pH and others. Challenge studies are performed under laboratory conditions, which means that the scale of the study is adjusted, based on the capacity of the laboratory (i.e., fewer products may be tested, and a water bath may be used rather than a hot-water pasteurizer).

The challenge study is often the most definitive means of scientific support. The study should be done on the same product or very similarly formulated product, closely replicating conditions in the real production environment.

- For an antimicrobial agent or treatment, the challenge study should be designed to demonstrate that *Listeria* growth does not occur over the product shelf life. (see establishing a Product's Shelf-life below).
- For a PLT, the challenge study should demonstrate a specific log reduction of *Listeria* effective from day 0 to the point before the product leaves the establishment.

If challenge studies are used as supporting documentation by the establishment, it is important that they use product that has similar physical characteristics to that being produced by the establishment (i.e., pH, Aw, etc.) and processing (and intervention) steps that are similar to those utilized by the establishment.

For example:

- If a challenge study examines the effect of steam pasteurization or hot-water pasteurization, the time and temperature of treatment may be critical components of the study. In order for the study to be used as supporting documentation, the establishment would need to apply the same or similar time and temperature treatment.
- For high pressure pasteurization, pressure is a critical variable. The establishment would need to apply the same pressure as specified in the study.
- For the use of chemical additives as antimicrobial agents, pH, acidity, and concentration may be additional critical variables. The establishment would need to demonstrate that they are applying the same levels as specified in the study.

All challenge studies should be based on a sound statistical design and should also employ positive and negative controls. *Listeria innocua* strains are usually employed as a nonpathogenic surrogate for *Lm*. The inoculum level should be at least two logs greater (if possible) than the log reduction to be demonstrated. The inoculum should be composed of a cocktail of 3-5 *Listeria* strains, including some strains known to be relatively resistant to the

Question: Many dried meat products do not support the growth of *Lm*, and *Lm* present on the product will die. If challenge studies are conducted to demonstrate the death of some identified amount of *Lm*, will FSIS consider the products to fall under Alt. 1?

Answer: When challenge or inoculation studies incorporated into the establishment's HACCP plan demonstrate both elimination of *Lm* before product leaves the establishment and that *Lm* growth is not supported during the shelf life, those products likely will fall under Alt. 1.

treatment. The levels of *Listeria* should be measured at day 0 (initial level) and remaining levels measured daily or at regular intervals (Day 1, 2, 3) to the end of the shelf life (or until the point when product would leave the establishment).

Listeria isolates used in challenge studies should relate to the type of meat or poultry product. They could be from foodborne illness outbreaks or from meat or poultry processing environments. If possible, one of the strains should be from a product as similar as possible to the product to be challenged, e.g., a strain isolated from a specific luncheon meat should be included in challenge studies for luncheon meats. A single strain of *L. innocua* may be used if the strain is known to be particularly resistant to the treatment (~2 fold more resistant) being tested (e.g., *L. innocua* M1 for studies evaluating heat treatments).

One way of obtaining isolates is to purchase strains from culture repositories. These include the [American Type Culture Collection](#) (ATCC), the [National Collection of Type Cultures](#) (NCTC). Cornell University hosts the ILSI *Lm* strains collection, which provides researchers with a standard set of *Lm* isolates, thus allowing for comparison of data on *Listeria* physiological and genetic characteristics generated in different laboratories. These isolates are grouped into two separate sets, including one diversity subset (25 isolates) and one matched human and food isolate subset (17 isolates, 2 of which are also included in the diversity subset) representing isolates from human listeriosis outbreaks and cases. More information on the ILSI *Listeria* strain collection, including a list of all isolates in the collection, source information, year of isolation, serotype, and ribotype information is available on Dr. Wiedmann's website at: <http://foodscience.cornell.edu/cals/foodsci/research/labs/wiedmann/ils-na-strain.cfm>.

4. Validated Predictive Microbial-Modeling Programs

Establishments may use the results of modeling programs to satisfy the first part of validation, scientific support. If the establishment:

- Inputs accurate values into the modeling program, and
- The modeling program has been validated for the type of product in question, and
- The results of the modeling program show adequate control of *Lm*,

then the establishment does not need additional scientific support such as a challenge study. If the pathogen modeling program was developed from the manufacturer of an antimicrobial agent, the establishment can contact the manufacturer to determine whether the model has been validated for their particular product and process.

The following are some key points regarding the use of microbial pathogen modeling programs:

- Modeling programs can be obtained from published studies or from the manufacturer of an antimicrobial agent. Information and guidance on the application of the antimicrobial agent may be obtained from the manufacturer.
- Establishments can also seek guidance from University Extension Service specialists or authors of the modeling programs on how to use a modeling program.
- If using a modeling program to determine the amount of antimicrobial agent to use, follow the directions with regards to salt content, moisture level of the finished products, and other information needed. For example, a modeling program may ask to confirm that the product is a cured product because the model is only valid for cured products. It will

ask for the following: Shelf life of product in days, product specification, salt content (%) and finished product moisture content (%). The program will calculate the amount of lactate/diacetate to be used and the log suppression of *Lm* based on the information provided.

- Growth models on the use of antimicrobial agents are available mostly for cured products. For uncured products where there are no growth models, validation studies need to be conducted per product.
- Verify the effectiveness of the antimicrobial agent/process used by testing for *Lm* growth during the shelf life of the product, at a certain frequency.
- Maintain and monitor records of validation, verification, and corrective actions for deviations from the effective application of antimicrobial agents/processes.

5. Establishing the Shelf-life of the Product

As stated in [Section 2.2](#), the AMAP must be effective throughout the shelf life of the product (9 CFR 430.1). The shelf life of the product is defined as the amount of time the product can be stored under specified conditions and still remain safe with acceptable quality. In order to demonstrate effectiveness of control measures over the shelf life of the product, the establishment would need to establish their expected shelf life through a challenge study, shelf-life study, or other supporting documentation such as predictive microbial modeling. This study or other supporting documentation should demonstrate that the AMAP is effective in controlling growth over the product's shelf life. **Although establishments are not required to label their product with a "use-by" date, or other information indicating the shelf life of the product, a prudent establishment would use this labeling to help ensure that the product is not consumed after the shelf life is complete.**

An establishment may perform the shelf-life study or provide other supporting documentation establishing the shelf life of the product. A microbial shelf-life study is one that measures the increase or decrease in the number of the target organism or pathogen during storage. For an AMAP, a shelf-life study is important to perform as part of the challenge study, because it determines the time (in days) the growth of *Lm* is controlled. Both refrigeration temperatures (e.g., 40°F) and a slightly abusive temperature (e.g., 45°F) should be used in the shelf-life study in order to ensure that if *Lm* is present and viable, growth will occur and can be measured throughout shelf life. This slightly abusive temperature also represents the worse-case conditions that could occur during cold-chain storage and handling.

Some of the factors that should be considered in the shelf life study of a product with an added AMAP to determine that the agent or process is effective in limiting or suppressing growth of *Lm* are:

1. Suppression of *Lm* growth in product during shelf life – growth should be lower in the product with added antimicrobial than growth in the untreated control. Although the Compliance Guidelines set a maximum of less than 2 log growth of *Lm* during the shelf life of product with added antimicrobials for the purposes of the challenge study, it is best to target a lower amount of growth than this.
2. The rate of growth of *Lm* in product-- the *Lm* growth-rate in product with added antimicrobial should be slower than the growth rate in product without added antimicrobial.

3. Temperature for holding product during the shelf life study – Most studies use the temperature that the product is normally held during storage as the temperature during shelf life studies e.g., refrigerated temperature of 38-40 ° F. Shelf life studies can also use or include a temperature of 45 ° F to hold product since this reflects consumer handling.

A resource article for conducting challenge studies for validation of antimicrobial agents is the Considerations for [Considerations for Establishing Safety-based Consume-by Date Labels for Refrigerated Ready-to-eat Foods](#)(NACMCF, 2004). This article gives guidance on how to determine the shelf-life of a RTE product containing an added antimicrobial agent that is supposed to suppress *Lm* growth during the refrigerated shelf-life. Most studies use the temperature which the product is normally held during storage as the temperature during shelf life studies, e.g., refrigerated temperature of 38-40° F. As described above, shelf-life studies also should use or include a temperature of 45° F which reflects consumer handling. The NACMCF document recommended to using a higher temperature for shelf-life studies because foods can encounter a range of temperatures below and above 45° F, with higher temperatures more likely in grocery store cases and during consumer handling. Therefore these temperatures more accurately reflect reality. In addition, establishments should extend the time period of their study (e.g., 2.5 times the shelf-life) to determine the safety if consumers hold the product longer.

NOTE: A product with an added antimicrobial agent demonstrating *Lm* growth of <2 log at a storage temperature of 38-40° F and at 45° F or above would be viewed by FSIS as more protective of public health than another product showing the same growth only when stored at 38-40° F.

III. In-Plant Demonstration Data

The **second element of HACCP systems validation is initial in-plant validation** which may include in-plant observations, measurements, microbiological test results, or other information demonstrating that the *Lm* control measures, as written into a HACCP system, can be executed within a particular establishment to achieve the process's intended result.

As of the date of this guideline, FSIS realizes that some establishments may not have kept their initial in-plant demonstration documents from when HACCP was originally implemented. Those establishments that have not will be allowed the time to assemble their in-plant demonstration documents. The Agency will describe and explain these documents in a future Federal Register Notice that it intends to issue when it finalizes the Compliance Guideline on HACCP systems validation. Until the Federal Register Notice issues and further instructions are given to FSIS personnel, FSIS will not cite the lack of in-plant validation data as the only reason for the documentation of noncompliance.

In cases where the process specifications described in the supporting documentation are implemented in the same or similar enough way (see box below) in the establishment's process, and when the scientific supporting documentation used contains microbiological data specifying the level of pathogen reduction achieved by the intervention strategy for the target pathogen identified in the hazard analysis, the establishment should:

- Identify the critical operating parameters in the scientific support, AND
- Translate them in the HACCP system, AND

- Demonstrate that the critical operating parameters are being met by gathering 90 days of execution data.

Generally, establishments should use the same critical operational parameters as those in the support documents. In some circumstances, establishments may be able to support using critical operational parameters that are different from those in the support documents (e.g., higher concentrations of antimicrobials or higher thermal processing temperatures). In these cases, establishments should provide justification supporting that the levels chosen are at least as effective as those in the support documents. In addition to ensuring that the levels chosen are at least equally as effective, establishments should also ensure the levels are also safe and suitable per FSIS [Directive 7120.1](#).

By demonstrating that the critical operating parameters are being met through the collection of execution data, the establishment will have addressed the second element of validation – in-plant demonstration data without the need for further microbiological data. In cases where the process specifications described in the supporting documentation **are not implemented** in the same or similar enough way in the establishment's process, or when the scientific supporting documentation used **does not contain** microbiological data specifying the level of pathogen reduction achieved by the intervention strategy for the target pathogen identified in the hazard analysis, the establishment should:

- Validate that the intervention as modified actually achieves the effect documented in the scientific supporting documentation (Element 1), AND
- Validate that the modified critical operating parameters are being met, AND
- Validate the intervention's effectiveness under actual in-plant conditions.

NOTE: Microbiological data (e.g., challenge studies or in-plant data) is encouraged but not required to comply with the minimum initial validation requirements provided the establishment has adequate scientific supporting documentation (e.g., journal articles) to meet the first element of validation. In addition the establishment would need to follow the parameters in the scientific support, and demonstrate that it can meet the critical parameters during operation (the second element of validation). In order to meet the second element of validation (in-plant demonstration data) the establishment would need to gather data (such as monitoring records of water temperature for a hot water pasteurization process or of water activity resulting from a drying process) over the initial 90 days demonstrating the critical operational parameters are being achieved.

The establishment should develop the appropriate execution data during the initial 90 days of implementing a new HACCP system, or whenever a new or modified food safety hazard control is introduced into an existing HACCP system as identified during a reassessment. During these 90 calendar days, an establishment gathers the necessary execution data to demonstrate critical operating parameters are being achieved. In essence, the establishment would repeatedly test the adequacy of the process steps in the HACCP system to establish that the HACCP system meets the designed parameters and achieves the intended result as described in the HACCP Final Rule. These execution data become part of the validation supporting documentation along with the scientific support used to design the HACCP system.

For examples of the type of scientific support and in-plant demonstration data that would be expected for different types of *Lm* controls, please see the validation examples taken from the [FSIS Compliance Guideline on HACCP Systems Validation](#) on the following pages.

IV. Validation Examples

Product	Hazard	Process	Critical Operational Parameters	Validation	
				Scientific Supporting Documentation	Initial In-plant documentation
Post-lethality exposed ready-to-eat meats	<i>Listeria monocytogenes</i>	Prerequisite program – Sanitation SOPs	<p><i>Listeria</i> Control Program for food contact surfaces.</p> <p>Sanitary design of equipment and sanitary zone concept.</p> <p>Frequency for collecting samples and number of samples that should be collected per line.</p>	<p>Joint Industry Task Force on Control of Microbial Pathogens in Ready-to-Eat Meat and Poultry Products. 1999. Interim Guidelines: Microbial Control During Production of Ready-to-Eat Meat and Poultry Products, Controlling the Incident of Microbial Pathogens.</p> <p>Sanitary Design Assessment Fact Sheet</p> <p>Tompkin, R.B. 2004. Environmental Sampling – A tool to verify the effectiveness of preventive hygiene measures. <i>Mitt Lebens Hyg.</i> 95:45-51.</p> <p>Tompkin, R.B. 2002. Control of <i>Listeria monocytogenes</i> in the food processing environment. <i>J Food Prot.</i> 65: 709-725.</p> <p>FSIS. 2012. Compliance Guidelines to Control <i>Listeria monocytogenes</i> in Post-lethality Exposed Ready-to-eat Meat and Poultry Products.</p>	<p>In plant monitoring records for 90 day period mapping food contact surface swab results for <i>Listeria spp.</i> collected on different processing dates and at different times and locations a 90-day period to potentially find hard-to-control areas in the plant and to support ongoing verification testing frequency after the initial validation period*.</p> <p>Assessment of sanitary design of equipment in the post-lethality environment using the AMI Sanitary Equipment Design worksheet and changes to <i>Listeria</i> Control Program based on assessment.</p> <p>Identification of all possible food contact surfaces.</p>

NOTE: Establishments may also collect environmental swab samples on different processing dates and at different times during the 90-day initial validation period to potentially find hard-to-control areas and niches within the establishment.

Product	Hazard	Process	Critical Operational Parameters	Validation	
				Scientific Supporting Documentation	Initial In-plant documentation
Post-lethality exposed ready-to-eat smoked turkey deli meat with skin on*	<i>Listeria monocytogenes</i>	Hot water Pasteurization	Hot water temperature at 195°F; product submersed for at least 6 minutes.	Muriana, P.M., Quimby, W., Davidson, C.A., Grooms, J. 2002. Post package pasteurization of ready-to-eat deli meats by submersion heating for reduction of <i>Listeria monocytogenes</i> . <i>J. Food Prot.</i> 65(6): 963-969.	In plant monitoring records for 90 day period demonstrating time and temperature can be consistently achieved. In plant monitoring records for 90 day period in which temperature of water is mapped and measured at increased frequencies to support monitoring procedures and frequencies.

***NOTE:** Reduction of *Lm* was found to be less for smoked turkey deli meat with skin-on using these time/temperature parameters than smoked turkey deli meat without skin, although the log reduction was > 1 log. For products subject to 9 CFR 430, it is FSIS expectation the post-lethality treatment will be designed to achieve at least a 1-log lethality of *Lm* before the product leaves the establishment.

Appendix 2.2: Sanitation

- I. Introduction
- II. Pre Operational Sanitation Procedures
- III. Operational Sanitation Procedures
 1. Controlling Temperature and Air Handling Units
 2. Equipment Design
 3. Traffic Control
 4. Employee Hygiene
 5. Controlling Cross Contamination
- IV. Sanitation During Construction
- V. Intensified Sanitation in Response to Positives
- VI. Determining the Effectiveness of the Sanitation Program

I. Introduction

The cornerstone of the *Listeria* Rule is sanitation within the post-lethality environment. All other layers of antimicrobial interventions (antimicrobial agents, post-lethality treatments, antimicrobial processes) are built upon the effective design of the establishment's sanitation program to control *Lm* and **will not** be effective if the sanitation program is poorly designed.

Understanding the growth/survival characteristics is critical to the success of controlling the pathogen. *Lm* is more heat-resistant than most foodborne pathogens. It can survive freezing and drying. *Lm* resists high salt levels, nitrite, and acid and can grow in vacuum packaged products. Most importantly, the pathogen can grow in a damp, cool environment. Once the bacteria attaches to a surface it can form a biofilm and establish a niche, or harborage site, which can become more resistant to superficial cleaning regimens. Bacteria can then spread from the niches to food-contact surfaces and product.

The critical components of an effective sanitation program to control *Lm* can be divided into the following major categories. These include:

- Pre-operational cleaning and sanitizing procedures that are effective in preventing *Lm* from forming niches or harborage sites in the processing environment.
- Operational sanitation procedures to prevent cross-contamination in the RTE processing environment.
- Intensified cleaning and sanitizing procedures in response to positive sampling results.
- Documentation and verification of cleaning and sanitizing procedures.

Establishments are required to develop and implement the Sanitation SOP regulatory requirements, 9 CFR 416.12 through 416.16. Proper and effective sanitation involves both cleaning and sanitizing, and verifying that the cleaning and sanitizing were effective. This involves developing and implementing written sanitation standard operating procedures (Sanitation SOPs). Sanitation SOPs could be viewed as the first step to designing a total system, including the HACCP plan that will prevent, eliminate, or reduce the likelihood of pathogenic bacteria from entering and harboring in the plant environment.

Sources, Harborage, and Control of *Lm* Contamination

An effective sanitation program should prevent contamination of food contact surfaces and prevent the formation and growth of *Lm* in a niche, especially in areas where the product is post-lethality exposed. A **niche is an area where *Listeria* has grown to high numbers, such as a harborage site** within the plant. Harborage sites provide an ideal place for *Lm* to establish and multiply. Factors that may affect the formation of niches include:

- equipment design,
- construction activities,
- operational conditions that move product debris into difficult to clean locations,
- mid-shift cleanup,
- high pressure during cleaning, and
- product characteristics that require excessive rinsing.

Certain strains can become established in a processing environment for months or years. *Lm* can be spread from these sites and re-contaminate food or food contact surfaces between the lethality step and packaging.

Therefore, the sanitation procedures should target the known reservoirs and harborage sites within the RTE processing environment.

Examples of reservoirs and harborage sites of *Lm* in RTE processing environment

- Drains, hollow rollers on conveyors, on-off valves and switches, worn or cracked rubber seals around doors, vacuum/air pressure pumps, lines, cracked tubular rods on equipment, air filters, condensate from refrigeration units, floors, standing water, open or gully drains, ceilings and over head pipes, overhead rails and trolleys, chiller and passageway walls and doors, chiller shelving, roller guards, door handles, boots, ice makers, saturated insulation (wet or moldy), trolley and forklifts, compressed air, in-line air filters, trash cans, cracked hoses, wet, rusting or hollow framework, walls that are cracked, pitted, or covered with inadequately sealed surface panels, maintenance and cleaning tools, space between close fitting metal-to-plastic parts, and space between close fitting metal-to-metal parts.
- Filling or packaging equipment, packaging film or wrappers, solutions (e.g., brine) used in chilling food,
- Peelers, slicers, shredders, blenders, brine chillers, casing removal system, scales, or other equipment used after heating and before packaging, Spiral or blast freezers, conveyors,
- Bins, tubs, wagons, totes, or other containers used to hold exposed product.

II. Pre-operational Cleaning and Sanitation Procedures

Typically, effective sanitation can be distilled down to the nine following steps. This is an example outline. Cleaning should be intensified during periods of construction and if repetitive positives are found.

- 1) Perform **dry cleaning of the equipment**, floors, conveyor belts, and tables to remove meat particles and other solid debris. Some equipment, such as slicers and dicers, will require disassembly so that parts can be cleaned thoroughly.
- 2) **Wash and rinse floor.**
- 3) **Pre-rinse equipment** (rinse in same direction as product flow). Pre-rinse with warm or cold water – less than 140°F (hot water may coagulate proteins or “set soils”).
- 4) **Clean, foam, and scrub equipment.** Always use at least the minimum contact time for the detergent/foam. Guidance should be provided concerning the location of possible niches and written instructions provided concerning the cleaning method.
NOTE: Live steam for cleaning is not acceptable at this step since it may bake organic matter on the equipment.
- 5) **Rinse equipment** (rinse in same direction as product flow).
- 6) **Visually inspect equipment** to identify minute pieces of meat and biological residues.
- 7) **Sanitize floor and then equipment** to avoid contaminating equipment with aerosols from floor cleaning. Care should be taken in using high pressure hoses in cleaning the floor so that water won't splash on the already cleaned equipment. Use hot water, at least 180°F, for about 10 seconds to sanitize equipment. Sanitizers (e.g., acidic quaternary ammonia) may be more effective than steam for *Lm* control.
- 8) **Rotate** sanitizers periodically. Alternating between alkaline-based and acid-based detergents helps to avoid “soapstone” and biofilms. This also helps change the pH to prevent adaptation of bacteria to a particular environment. Portable high-pressure, low volume cleaning equipment (131°F (55°C) with 20-85 kg/cm² pressure and 6- 16 liters/minute) can also be used.
- 9) **Dry.** Removing excess moisture can be done most safely and efficiently by air drying. Reduced relative humidity can speed the process. Avoid any possible cross-contamination from aerosol or splash if a method other than air drying (e.g., using a squeegee or towel) is used.

Recommended Frequencies for Cleaning and Sanitizing Procedures	
Area	Recommended Cleaning Frequency
All processing equipment, floors and drains, waste containers, totes, wagons, RTE storage areas	Daily
Walls, condensation drips pans, RTE coolers	Weekly
Freezers	Semi-annually

Sanitizers

Cleaning and sanitizing are vital to any effective sanitation program. Thorough cleaning should be followed by sanitizing. Generally, the cleaning step is to remove all waste materials and soils, and the sanitizing step is to destroy all microorganisms. Careful consideration should be given to selecting both cleaning and sanitizing solutions. It is important to use solutions that are compatible with the equipment materials, such as stainless steel or heavy plastics, and solutions that are effective in destroying the type of bacteria commonly associated with the type of products produced in the establishment.

Rather than relying on a single sanitizer, **rotating** sanitizers will help prevent the development of microorganisms resistant to a particular sanitizer. The concentration and application processes for all sanitizers approved for use in meat and poultry establishments are referenced in Title 21 Code of Federal Regulations (21 CFR), Part 178, section 178.1010. All cleaners and sanitizers commercially available should have, at the minimum, the following information either on the label or available on a specification sheet that must accompany the product:

Product Description

- **** Instructions on how to use the product (concentration, method of application, contact time, temperature)**
- Properties
- Safety Information

Additional information that is sometimes available includes:

- Benefits
- Quality Assurance Statements

****Effectiveness against *Listeria*.**

Some manufacturers provide labeling in both English and Spanish, which makes the products more user friendly in various environments. At least one manufacturer also has commercially available color coded products that are easy to associate with a particular cleaning or sanitizing task.

Recommendations for sanitizers inactivating *Lm* in biofilms on stainless and plastic conveyor belts:

- Chlorine and iodophors are not effective inactivating *Lm* in biofilms on stainless steel.
- The most effective sanitizers are acidic (not neutral) quaternary ammonium compounds, peracetic acid, and chlorine dioxide.
- The less effective are the mixed halogens and acid anionics sanitizers, which were less effective than the sanitizers listed in the 2nd bullet above.
- And the least effective sanitizers were chlorine, iodophors, and neutral quaternary ammonium compounds.

III. Operational Sanitation Procedures to Prevent Cross Contamination Between Raw and RTE Post-Lethality Environment

1. Controlling Temperature and air handling units

- Maintain temperature in processing areas and packaging rooms as stated in the HACCP plan, Sanitation SOPs, or Prerequisite Programs.
 - Maintain cold temperature (<50° F) in packaging room for products that are to be refrigerated or frozen, as stated in the HACCP plan, Sanitation SOPs, or Prerequisite Programs to prevent *Lm* growth in the RTE processing environment.
 - Monitor temperatures as stated in the HACCP plan, Sanitation SOPs, or Prerequisite Programs.
- Establish positive air pressure movement out of the RTE room into the raw processing areas.
- Clean cooling units and air handling units at some specific frequency.
- Immediately address and correct problems of dripping condensation and standing water. Production of RTE products should be stopped during repairs and corrective actions for these problems. The equipment and processing area should be cleaned and sanitized after all the repairs and corrective actions are finished.

2. Equipment Design

- Evaluate the equipment to ensure that it can be easily dismantled for cleaning and is durable.
- Investigate for potential *Lm* harborage sites, such as hollow rollers.
- If new equipment is purchased, select equipment designed to enhance cleaning
 - All areas and parts should be accessible for manual cleaning and inspection or be readily disassembled.
 - Closed conveyor designs are more difficult to clean. Equipment on the processing line should be as easy to clean as possible.
 - Avoid hollow conveyor rollers and hollow framing. If hollow material is used, have a continuous weld seal instead of caulk.
 - Select food contact surfaces that are inert, smooth, and non-porous.
 - Equipment should be self-draining or self-emptying.
- Maintain equipment and machinery by adopting a regular preventive maintenance schedule (QA should verify performance)

- Damaged, pitted, corroded, and cracked equipment should be repaired or replaced.
- Repair parts or machinery in a manner that prevents food deposits that are not easily removed with normal cleaning.
- Use separate tools for RTE equipment only. Sanitize them before and after each use.
- If compressed air is used, maintain and replace in-line filters regularly.
- Use lubricants that contain listericidal additives, such as sodium benzoate. *Lm* can grow in lubricants that are contaminated with food particles.
- Clean maintenance tools (including wrenches, screws, and tool boxes) on a regular basis. Consider designating certain tools for raw and RTE areas.

3. Traffic Control

One critical component of an effective sanitation program is control of the movement of personnel and raw product to prevent cross-contamination of RTE finished product and FCSs within the post-lethality environment. Establishments should examine product routes from heat treatment or other antimicrobial control steps to eliminate *Lm*, to final packaging. The following are steps that can be used to develop control procedures.

Establish traffic patterns to eliminate movement of personnel, meat containers, meat, ingredients, pallets, and refuse containers between raw and finished product areas. If possible, employees should not work in both raw and RTE areas. If they must work in both areas, they must change outer and other soiled clothing, wash and sanitize hands, and clean and sanitize footwear.

- If possible, use air locks or vestibules between raw and RTE areas.
- Use foam sanitizing spray systems on either side of the RTE room door on a timed system or triggered by entry/exit.
- Clean, dry floors are preferable to foot baths at the point of entry because effective concentrations of disinfectant are difficult to maintain and may become a source of contamination.
 - If foot baths are absolutely necessary:
 - Wear rubber or other non-porous boots.
 - Maintain them properly, so that they are clean and maintain effective levels of sanitizer.
 - Solutions should contain stronger concentrations of sanitizer than normally used on equipment (e.g., 200 ppm iodophor, 400-800 ppm quaternary ammonia compound).
 - Use a minimum depth of 2 inches.

NOTE: Chlorine is NOT recommended for foot baths because of rapid inactivation, especially if cleated boots are used. The accumulation of biological material adhering to the cleats inactivates (or reduces) the bioavailability of chlorine, making it less effective. Monitor and maintain the strength of the chlorine solution, if used.

4. Employee Hygiene

Development of employee hygiene procedures to prevent the contamination of FCSs should be the responsibility of management. The employee should be responsible for preventing contamination of food products and the management should be responsible for ensuring that the employee is properly trained and maintains good practices.

- Employee responsibilities and actions should include:
 - Using a 20 second hand wash, allowing the soap suds to be in contact with the hands for this period of time, after using restroom facilities.
 - Washing hands before entering the work area, when leaving work area, and before handling product.
 - If gloves are worn:
 - Gloves that handle RTE product should be disposable.
 - Dispose immediately and replace if anything other than product and FCS is touched.
 - Dispose of gloves when leaving the processing line.
 - Remove coats, gloves, sleeves and other outer clothing when leaving RTE areas.
 - Do not wear coats, gloves, sleeves or other outer clothing inside restrooms or cafeterias.
 - Do not store soiled garments in lockers.
 - Do not eat in the locker room or store food in lockers because food may attract insects and vermin.
 - Do not store operator hand tools in personal lockers. This equipment must remain in the RTE area at all times.
 - Do not allow employees who clean utensils and equipment for raw materials to clean RTE utensils and equipment, if possible.
 - The tools to clean utensils and equipment for raw materials must be different than those used to clean RTE utensils and equipment. In either case, the intent is to prevent cross contamination of finished product.
 - Management responsibilities should include:

- Providing hand washing facilities at proper locations.
 - Ensuring that the employee receives proper hygiene instruction before starting – use of hand soaps and sanitizers, no-touch dispensing systems, and boot and doorway sanitizing systems.
 - Developing a system for monitoring employee hygiene practices.
 - Developing a system for tracking the training, testing, and certification.
 - Retraining employees before placing them back into production if they are absent from the job or have failed to follow acceptable hygiene practices. This will help ensure that the employees are following current, acceptable hygiene habits.
- Do not permit maintenance employees in RTE areas during operations if possible, primarily because they may cause direct product contamination or adulteration if they touch or lay their “dirty” equipment hands onto food contact surfaces. If this is not possible:
 - Consider the need to cease operations until a full cleaning and sanitizing is done, or,
 - Require maintenance personnel to change outer clothing and any other soiled clothing, use separate tools for raw and RTE areas (or wash and sanitize tools and hands prior to entering RTE areas) and wear only freshly cleaned/sanitized footwear in such areas.
 - Use separate equipment, maintenance tools and utensils for the RTE and raw areas. If not possible, there should be a time separation between raw processing/handling and RTE processing in order prevent cross contamination of finished product.

5. Controlling Cross Contamination

- For establishments processing RTE products, establish procedures to ensure that other non-meat or non-poultry RTE ingredients do not cause cross-contamination with *Listeria*.
- Maintain an effective rodent and insect infestation preventive and Control Program. Rats, mice, and insects are sources of *Listeria* and other microbial contamination.
- Eliminate standing water which can facilitate the spread of *Lm* into other areas of the plant. Sanitizer boluses can be used to sanitize standing water on a continuing basis.
- Discard products that touch environmental surfaces, such as products falling on the floor, if the product cannot be properly re-conditioned (e.g., by washing).
- Pallets can serve as a source of cross-contamination – pallets for raw materials should not be used in RTE areas or used for finished product.
- Do not allow condensation to build up or drip over exposed RTE product.

- Do not spray high pressure hoses near exposed product. Aerosols could develop that could contaminate the product.
- Do not allow employees to store knives, gloves, or equipment in their lockers. Provide designated storage areas for these items.
- Employees should not wear gloves, coats, or aprons in the restroom or break areas.
- Drains from the “dirty” or “raw” side should not be connected to those on the “clean” or “cooked” side.

Dual Jurisdiction Establishments

Because FSIS-regulated products are susceptible to *Lm* outgrowth:

It is advisable, due to the food safety nature of FSIS-regulated product, to separate processing areas for FSIS-regulated products and FDA-regulated products by time or space, such as scheduling processing on different days. If that is not possible, schedule FSIS product processing first, then FDA product processing. If FDA product is produced first, a complete clean-up and sanitizing before starting FSIS product processing is recommended.

Because of the risk for cross contamination, consider assigning different personnel for FSIS and FDA products and processing areas, if possible, especially if both are conducted on the same day. If not possible, have personnel clean hands thoroughly, and use unused, clean coats, new gloves and hairnets, and sanitized boots for FSIS and FDA processing.

IV. Sanitation During Construction

Dust generated by construction activities can move throughout the plant on air currents or be transferred by people or equipment traveling through the construction area into other areas of the establishment. A study by De Roin et al., (2003) showed that *Lm* in dust can survive and grow, once in contact with meat surfaces. Construction or maintenance activities that can result in *Lm* contamination of RTE product of FCS include removal of drains, removal of floor coatings, removal of a wall or ceiling that has absorbed moisture, movement of potentially contaminated materials through RTE areas or areas that directly connect with RTE processing areas, and exposure of areas typically not accessible for cleaning. Tompkin (2002) considers the potential of introduction of *Lm* into the RTE processing environment from an outside source or through disturbance of a harborage site (e.g., the process of replacing floor drains, walls, or cooling units) as a great concern.

Control of the Environment during Construction

If possible, suspend operations during construction. Otherwise:

- Dust from construction can be difficult to detect and control. Therefore, increased monitoring of product, food-contact surfaces, and the environment is recommended during and after these disruptive events.
- Establish negative air pressure in the construction area in order to ensure that air does not flow from the construction area into the plant.

- Temporary partitions can be established to protect the undisturbed areas of the plant from construction dust and debris.
- Cover any construction debris when moving out of the construction area.
- Do not move debris through RTE processing areas or areas that directly connect to RTE processing areas, if possible.
- Schedule construction during non-processing hours.
- Conduct intensified cleaning and monitoring of food contact and environmental surfaces after construction is complete.

Control of the Environment after Construction

- Schedule removal of all construction equipment, barriers, and final debris after production hours.
- Perform a thorough clean-up and increased sanitation sampling at pre-operational inspection. Continue intensified cleaning and monitoring of food contact and environmental surfaces until food contact surfaces test negative for 3 consecutive days.

V. Intensified Cleaning and Sanitation Following a Positive *Listeria* Sample

The following are actions that can be taken during intensified cleaning. Not all steps may be necessary to address contamination. Actions should be escalated to address consecutive positives.

If positives occur, consider:

- Thoroughly cleaning and scrubbing sites where positives were found.
- Identifying all possible harborage sites and cross contamination pathways. Clean and sanitize harborage points and address cross contamination.
- Removing equipment parts and soaking overnight.
- Increasing the frequency of all less than daily sanitation procedures (e.g., walls and ceilings).
- Scrubbing surfaces where product residue accumulates. Pay special attention to gaps, cracks, rough welds, and crevices in equipment.

If positives continue to occur, consider:

- Disassembling equipment and soaking of parts in quaternary ammonia overnight.
- After cleaning and sanitizing of larger pieces of equipment, applying steam heat via an oven at 160°F and holding for 20-30 minutes.
- Fogging the room with a sanitizer solution.
- Replacing rusty, pitted, peeling tools or parts of equipment with new, smooth-surfaced ones. These rusty, pitted tools and equipment parts serve as ideal harborage places for *Lm* to grow and multiply.

If positives still continue to occur, consider:

- Identifying harborage points in equipment, such as spiral freezers and slicers, and repairing or replacing.
- Thoroughly cleaning all areas of the establishment, including raw and non post-lethality exposed areas, to address possible harborage sites leading to contamination of RTE areas.
- Repairing or replacing leaky roofs, broken and cracked equipment, floors, overhead pipes, and cooling units, fans, doors, and windows. Suspend operations during repairs or replacement. FSIS recommends testing the environment for *Listeria spp.* after repairs are finished.
- Constructing new walls to separate raw and RTE areas. If drains or air handling units lead to raw areas or outside, consider rerouting.

VI. Determining the Effectiveness of the Sanitation Program

Establishments can verify the effectiveness of their sanitation program through monitoring the implementation of their pre-operational and operational procedures in their Sanitation SOP. The most basic level of daily verification occurs within the post-lethality environment by monitoring the effective implementation of cleaning/sanitizing of FCSs and observing whether operational sanitation procedures are implemented to prevent cross-contamination (9 CFR 416.13(c)). Maintaining daily records to document the implementation and monitoring of the Sanitation SOP procedures targeted to the RTE environment is also a regulatory requirement to track the effectiveness of the sanitation program (9 CFR 416.16(a)). In addition, observation of employee hygiene practices within the RTE area is required to verify compliance with the Sanitation Performance Standard and prevent cross-contamination (9 CFR 416.5(c)). There are also requirements in the *Listeria* Rule for sampling for *Lm* or an indicator organism to verify sanitation. These are discussed in the main body of the *Listeria* Guideline.

It is also important that establishments take steps to prevent future contamination events. This can include reassessing and modifying the Sanitation SOP for specific pieces of equipment or areas of the establishment, increasing cleaning and sanitation frequency, and repairing or replacing equipment or areas of the establishment that may represent harborage sites for *Lm*.

Non-regulatory methods to verify the effectiveness of the Sanitation SOP include the use of total plate counts and ATP bioluminescence, as well as organoleptic inspection. **It is important to note that these methods can not be used to replace testing performed for *Lm* or an indicator organism to meet the requirements of the *Listeria* Rule.**

Total Plate Counts (TPC)

Visual verification combined with Total Plate Counts (TPCs) can determine both observable contamination and the level of bacterial contamination. Since TPC results are available in about 24 hours, and cannot be obtained at the time of inspection, their value lies in the measurement of the level of contamination. The level of contamination on cleaned and sanitized equipment should be very low (e.g., less than 100 CFU/in²). The level of contamination may assist the establishment in determining the source of *Listeria* contamination and the effectiveness of the Sanitation SOP. Establishments may be able to use the results from TPC monitoring to indicate areas where *Listeria* spp. testing should be performed.

ATP Bioluminescence Testing “Lightning”

The use of adenosine triphosphate (ATP) bioluminescence swab testing on FCSs can also be a measurement tool to verify sanitary conditions. Most food residue and all microbes are rich in ATP and detecting microorganisms through ATP bioluminescence analysis is one method to test for sanitation effectiveness. The more ATP present, the greater the amount of bioluminescent light emitted. A microprocessor transforms the data into a digital readout for the luminometer’s display and quantifies the light output into a 2 digit zone. The product manufacturer specifies the “acceptable” and “unacceptable” zone. The ATP test can detect contamination that is not observable, is a rapid test, and results are available immediately prior to the start of operations.

It is important for the establishment to verify that the cleaning and sanitizing procedures are effective. In addition, the recordkeeping should be used for data analysis and the establishment should evaluate the monitoring records for trends. 9 CFR 416.14 requires that each official

establishment routinely evaluate the effectiveness of the Sanitation SOP and the procedures therein. Therefore, trend analysis, evaluation, and appropriate revision of the Sanitation SOP, should be conducted, as necessary, to remain effective and current with respect to changes in facilities, operations, equipment, utensils, personnel, and equipment within the post-lethality environment.

Records of Sanitation Procedures

The following sanitation records are required by 9 CFR 416.16:

- Keep records of the implementation of Sanitation SOPs.
- Maintain monitoring records of Sanitation SOPs.
- Maintain records of corrective actions taken if adulterated product or a direct FCS noncompliance occurs. Ensure appropriate disposition of products, restore sanitary conditions to prevent recurrence, and record the date of the noncompliance and the initials of the plant employee conducting the corrective action.
- Records must be maintained for 6 months, and may be stored electronically.

References

De Roin, Mark, S.C. C. Foong, P. M. Dixon, J. S. Dickson. 2003. Survival and recovery of *Listeria monocytogenes* on ready-to-eat meats inoculated with a desiccated and nutritionally depleted dustlike vector. *J. Food Protection*. 66: (6): 962-969.

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Appendix 2.3: Training

- I. Introduction
- II. Suggested Training Programs
 - a. Hand washing
 - b. Cross contamination
 - c. Cleaning and sanitizing
 - d. Equipment maintenance
 - e. Sampling
 - f. Facilities
- III. General Guidance on Training Programs
- IV. Reference Materials

I. Introduction

Basic training for all staff should include an overview that defines *Lm*, the differences between *Listeria* spp. and *Lm*, and an explanation of why *Lm* is a public health concern in post-lethality exposed ready-to-eat products. Training should also include a discussion about locations where *Listeria* can be found in a processing facility, with an emphasis on common harborage sites. Employees should understand why they should be concerned about *Listeria*, considering the perspective of both the health of the consumer and the interests of the company. Providing employees with a broad knowledge base regarding *Listeria* will be beneficial to any *Listeria* Control Program. For example, the very simple but relevant principle that employees can unknowingly bring *Listeria* into a ready-to-eat processing facility on their shoes may not be clear to all employees if training does not address that *Listeria* is ubiquitous in the environment.

II. Suggested Training Programs

Specific company-wide policies affecting *Listeria* control should be discussed in a basic training course, such as rules requiring protective smocks of a certain color to be worn in certain areas of the establishment or rules about traffic patterns in the plant. Tailoring your training program to your establishment, your products, and your needs is crucial.

a. Hand Washing

All personnel should be instructed in proper hand washing techniques. Adopt a descriptive hand washing policy and display clear instructions in all restrooms and at all sinks. Instructions may be for a 20-second hand wash, for example, or to wash hands as long as it takes to sing "Happy Birthday." A thorough hand washing policy should also include instructions as to when employees should wash their hands, such as after breaks, or before gloving.

b. Cross Contamination

Although a basic *Listeria* overview training course for all employees may address cross contamination principles, a more focused cross contamination training course should be directed at employees handling product. Encouraging all employees to be aware and identifying potential harborage sites can limit lost product and reduce risk. Areas for discussion within this course should include the importance of keeping ready-to-eat and raw products separated, from receiving to storage, including food preparation, packaging, and display. General hygiene practices should be discussed, including specific requirements for outer garments, gloves, and

shoes. Training should also include common practices that can result in cross contamination, such as an employee sneezing into his or her hand and not washing his or her hands immediately afterwards. The take home message for cross contamination training is that employees must always be aware of how their actions may impact food safety.

c. Cleaning and Sanitizing

Just as the importance of cleaning and sanitizing cannot be overemphasized, so too is the case for an employee training program that addresses proper cleaning and sanitizing. Employees must not only be shown how to do their job, but they should understand why they are cleaning and sanitizing equipment and utensils and non-food contact surfaces, as well as understand the public health implications of improper cleaning and sanitizing. In addition to the principles of cleaning and sanitizing, the importance of following instructions as to the proper concentration and temperature when preparing chemicals, and the importance of cleaning before sanitizing should also be discussed. Employees need to know specifically what equipment and utensils to sanitize, with special emphasis placed on known harborage sites. The cleaning and sanitation training program should also include a discussion of the importance of disassembling equipment completely when cleaning, as well as instructions as to how often to clean.

d. Equipment Maintenance

Personnel using equipment and utensils, cleaning and sanitizing equipment and utensils, or involved in the maintenance of equipment and utensils should all be made aware of the importance of a thorough examination for cracks, rust, or pitting which result in non-smooth surfaces. While management may be aware of the importance of looking, for example, for cracks in knives or imperfections in gaskets, the employees that actually handle that equipment may not be aware of these potential *Listeria* harborage sites. Maintenance personnel should also have training that discusses common improper practices, such as the use of duct tape for equipment repair, which can be a source of contamination and a harborage site for *Listeria*.

e. Sampling

Every *Listeria* control training program should include training targeting personnel involved in the establishment's sampling program. Employees should be thoroughly trained in the "when", "where" and "how" to sample, as well as the "why." For example, the employee should understand that the environmental swabs he or she takes may lead to the identification and elimination of harborage sites. It is also critical that any employee taking samples should be trained in proper aseptic technique procedures.

f. Facilities

Facilities maintenance personnel should be informed that *Listeria* thrives in moisture and that it is important that they vigilantly look for leaking roofs, drips, standing water, and condensation. Personnel should be instructed in the procedures to follow if they observe facilities issues that can result in the presence of excessive moisture or water, such as who to notify and what action to take.

III. General Guidance on Training Programs

Training may be delivered in a variety of formats, including handouts, demonstrations, PowerPoint presentations, and on-the-job training, and should be "hands-on" whenever

possible. It should be delivered in the most appropriate language or languages to meet the needs of its employees so that all employees can fully understand it. For example, training in company sanitation procedures should include a description and demonstration of the procedure to be performed, monitoring procedures, and how to respond to problems.

The frequency of training is also very important: all new employees should be trained upon hiring as part of the establishment's new employee orientation prior to starting work. A refresher training course for current employees should be conducted at least once a year to ensure that each employee is properly trained for the job position held. Additional training may be necessary for employees whose duties change. Adequate time for training should be allocated, rather than attempting to fit in training during down time. It is important that all employees clearly understand their roles in the production of safe products upon completion of the training.

All aspects of training should be documented, including course contents, who received the training, and when training was given. Even after training is completed, the establishment still maintains the responsibility for ensuring that the training has been implemented correctly. Establishments should verify that employees are implementing the training, as instructed, on the job. This can be accomplished by performing periodic in-house audits where employees are observed to see if they are implementing what they have been trained to do. A review of in-plant records to verify that, for example, equipment has been cleaned at the proper frequency, or that sanitizers have been mixed according to directions, will also indicate if training was effective. The establishment should also have a process in place to address employee training deficiencies, such as retraining.

A final suggestion on implementing a successful *Listeria* training program is to identify a way to get employees involved and vested in the importance of *Listeria* control and the protection of public health. One way to do this is to have a rewards program where employee incentives, such as a "Food Safety Employee of the Month," are established to recognize outstanding effort in promoting the establishment's overall mission of producing a safe, wholesome product. Opening up *Listeria* training or the Control Program to employee suggestions may yield some very interesting and useable findings. Employees can be very insightful sources of information for improvements to your *Listeria* Control Program since they are often able to observe situations that managers do not.

IV. Reference Materials

These resources can be ordered from using the [Food Safety Resources for Small and Very Small Plant Outreach: Order Form](#).

FSIS Resources:

1. FSIS Compliance Guideline: Controlling *Listeria monocytogenes* in Post lethality Exposed Ready-to-Eat (RTE) Meat and Poultry Products (Document)
2. [HACCP-10: Generic HACCP Model for Heat-Treated, Shelf-Stable Meat and Poultry Products](#)
3. [HACCP-12: Generic HACCP Model for Fully Cooked, Not Shelf-Stable Meat and Poultry Products](#)

4. [HACCP-15: Generic HACCP Model for Not Heat-Treated, Shelf-Stable Meat and Poultry Products.](#)

Pennsylvania State University Resources:

1. [Control of *Listeria monocytogenes* in Small Meat and Poultry Establishments. DVD and booklet](#)
2. [Control of *Listeria monocytogenes* in Retail Establishments. DVD and booklet.](#)
3. Implementation of a Post-Packaging Heat Treatment to Reduce *Listeria monocytogenes* on Ready-to-Eat Meat Products for Very Small and Small Establishments. DVD and booklet.

Chapter 3

FSIS *Listeria* Guideline: *Listeria* Control Program: Testing for *Lm* or an Indicator Organism

- 3.1 [Sampling for *Lm* or an Indicator Organism](#)
- 3.2 [Design of the *Listeria* Control Program](#)
- 3.3 [Routine Sampling Program](#)
- 3.4 [Frequency of Sampling and Explanation of this Frequency](#)
- 3.5 [Sample Collection and Laboratory Testing Methods](#)
- 3.6 [Other Routine Sampling](#)
- 3.7 [Glossary](#)
- 3.8 [References](#)

Attachments

- 3.1 [Possible Food Contact and Non-Food Contact Sites](#)

Appendices

- 3.1 [FSIS RTE Sampling Program](#)
- 3.2 [FSIS Sampling Procedure](#)
- 3.3 [Sample Collection and Laboratory Testing Methods](#)

This chapter provides information on sampling and testing for *Lm* or an indicator organism and design of the *Listeria* Control Program. It also provides information on sampling frequency and other routine sampling.

3.1 Sampling for *Lm* or an Indicator Organism

According to the *Listeria* Rule, establishments in all three alternatives may use verification testing for *Lm* or an indicator organism (e.g., *Listeria spp.*) to verify sanitation in their post-lethality processing environment (9 CFR 430.4(c)(1)). Establishments in Alt. 2b and 3 are required to test their **food contact surfaces (FCS)** in order to verify sanitation in the environment (9 CFR 430.4(b)(2)(iii)(A) and (3)(i)(A)). Testing FCSs is encouraged for establishments in Alt. 1 and Alt. 2a. **If a product or FCS tests positive for *Lm*, then the product will be considered adulterated and the product must be reworked or destroyed** Establishments are required to hold or maintain control of RTE products that FSIS has tested for *Lm* or RTE products that have passed over food contact surfaces that FSIS has tested for *Lm*. Establishments may move such products off-site provided they maintain control of them (e.g., through company seals).

NOTE: A finding of *Listeria spp.* on a FCS indicates conditions where *Lm* may be present, but the product is not considered adulterated. However, establishments are expected to take corrective action, according to their control alternative, to address *Listeria spp.* positives so that product does not become adulterated.

3.2 Design of the *Listeria* Control Program

Establishments may control *Lm* through their HACCP plan, Sanitation SOP, or prerequisite program. Establishments that choose to control *Lm* through their Sanitation SOP or prerequisite program may do so through the use of a *Listeria* Control Program. The *Listeria* Control Program can be incorporated as part of the Sanitation SOP or designed to work with the Sanitation SOP

and HACCP plan as a prerequisite program. It is expected that the *Listeria* Control Program will be designed based on the relative risk of the product, depending on the alternative. It is also recommended that establishments take corrective and preventive actions and perform enhanced sampling in response to positives (see [Chapter 4](#)).

NOTE: If the establishment does decide to use its *Listeria* Control Program as a basis for decisions in the hazard analysis, the establishment should follow the project. If the establishment deviates from the project then FSIS may find that the establishment can no longer support its decision that *Lm* is not reasonably likely to occur in the product. The establishment would need to provide further justification as to why the product is unlikely to be contaminated with *Lm*.

If the establishment chooses to use a prerequisite program for controlling *Lm* in the environment, it must be included as part of the documentation the establishment maintains under 9 CFR 417.5 (see 9 CFR 430.4(c)(6)). Establishments may use the results from their *Listeria* Control Program or other prerequisite program as support for the decision in their hazard analysis that *Lm* is not a hazard reasonably likely to occur in their product.

The *Listeria* Control Program should be designed to meet the requirements of the *Listeria* Rule. For establishments in Alt. 2b and 3, the *Listeria* Rule (9 CFR 430.4(b)(2)(iii) and (3)(i)) requires that establishments:

- Provide for testing of FCS sites,
- Identify conditions under which the establishment will hold and test product,
- State the frequency that testing will be done,
- Identify the size and location of the sites that will be sampled, and
- Provide an explanation of why the testing frequency is sufficient to control *Lm*.

In addition, Alt. 3 deli and hotdog processors are required to perform follow-up sampling and hold and test product after a second positive (9 CFR 430.4(b)(3)(ii)(B)). The *Listeria* Control Program should also include information about the sampling and testing methods that are used to analyze the samples, and actions taken in response to positive test results, including disposition of contaminated product. Also, although not required, if [non food-contact surfaces \(NFCS\)](#) and product samples are collected as part of the establishment's routine sampling program, they should be described in the *Listeria* Control Program (Sections [3.3-3.6](#) and [4.1-4.3](#)).

***Listeria* Control Program Considerations**

- *Listeria monocytogenes* (*Lm*) is the foodborne pathogenic species of the bacterial genus *Listeria*. Most establishments choose to test for *Listeria* spp. (i.e., Genus *Listeria*) because they are indicators for *Lm*.
- Establishments are expected to have **Routine** and **Enhanced Sampling Programs**.
- Step-by-step **sample collection and laboratory methods** should be included.
- The establishment should list all of the **food contact surface (FCS)** samples they will collect as part of their *Listeria* Control Program.
- The establishment's **Hold and Test** program should be included as part of the *Listeria* Control Program.

Question: My establishment tests FCS for *Listeria* spp. and found a positive result. Are we required to further analyze the sample to determine if it's positive for *Lm*?

Answer: No. There is no requirement that establishments further analyze *Listeria* spp. positives on FCS to determine if they are positive for *Lm*. However, the establishment is required to take corrective actions, depending on their control alternative (see [Chapter 4](#) for more information).

Parts of the *Listeria* Control Program

The following outline provides considerations that should be taken into account by establishments when designing a *Listeria* Control Program. Establishments are encouraged to include any additional considerations in designing a *Listeria* Control Program that are unique to its specific process.

- ✓ **Types of products produced** (HACCP programs considered under the *Listeria* Control Program).
- ✓ ***Listeria* Control Alternative(s)** used for each product.
- ✓ **Organism to be sampled** (*Lm*, *Listeria* spp., or *Listeria*-like organisms).
- ✓ **Routine Sampling Program** ([Section 3.3](#)).
 - List of sites that will be sampled (all possible food contact sites should be identified for Alt.2b and 3 establishments, see page 3-5).
 - Number and frequency of samples collected and explanation for this frequency ([Section 3.4](#)).
 - Size of each site that will be sampled.
 - Sampling and testing method ([Section 3.5](#)).
 - Step by step collection method.
 - Type of analysis performed (detailed laboratory analysis methods should be maintained by the lab).
 - Sampling for non FCS and product (if performed). See [Section 3.6](#).
 - Number and frequency of samples collected.
 - Response to positive results.
- ✓ **Enhanced Sampling Program** ([Chapter 4](#))
 - Follow-up testing ([Section 4.1](#))
 - Timeframe for follow-up sampling (e.g., after the 1st FCS positive).
 - Number of samples collected.
 - Response to positive results (corrective and preventive actions (details should be included in the establishment's Sanitation SOP)).
 - Intensified testing ([Section 4.2](#))
 - Timeframe for intensified testing (e.g., after the 2nd FCS positive).
 - Number of samples collected.
 - Response to positive results.
 - Intensified sanitation (details should be included in the establishment's Sanitation SOP).
 - Number of consecutive negatives to demonstrate that the process is back in control or that sanitary conditions have been restored.
 - Conditions for re assessment of the establishment's HACCP plan in response to positives.
- ✓ **Hold and Test Program for product** ([Section 4.3](#))
 - Conditions for hold and test.
 - Organism to be sampled.
 - Type of analysis performed.
 - Number and type of products to be sampled (statistically based program required for Alt. 3 deli and hotdog producers).
 - Product disposition in case of a positive result.

<p>NOTE: Recommended timeframes for performing follow-up sampling and intensified sampling are included in Table 4.1.</p>
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3.3 Routine Sampling Program

As part of their *Listeria* Control Program, establishments are expected to have both routine and enhanced sampling programs. The routine sampling program should include all of the procedures the establishment will follow when collecting routine samples. As part of the routine sampling program, the establishment should identify the sites they will sample, the frequency of sampling, the number of samples they will collect, the size of the sampling sites, the sampling method, and procedures for sampling NFCS and product (if performed). Establishments should collect samples on first and second shift if RTE post-lethality exposed product is produced on both shifts.

NOTE: The *Listeria* Rule requires establishments in Alt. 2b or 3 to test their FCS for *Lm* or an indicator organism. Testing product alone would not be sufficient to meet the requirements of the *Listeria* Rule.

In the routine sampling program, establishments can test for *Lm* or an indicator organism (e.g., *Listeria* spp.). For more information on testing methods, see [Section 3.5](#). As previously stated, if a product or FCS tests positive for *Lm*, then the product will be considered adulterated and the product must be reworked or destroyed. A finding of *Listeria* spp. on a FCS indicates conditions where *Lm* may be present and grow, but the product is not considered adulterated. There is no requirement that establishments perform a [confirmation test](#) on samples that test positive for *Listeria* spp. to determine if the sample is positive for *Lm*. **However, because many tests for *Listeria* spp. are screening tests for *Lm*, a positive result could mean that *Lm* is present, just not confirmed by the test.** Therefore, establishments are expected to take corrective actions and to follow up on *Listeria* spp. positives according to their control alternative, so that product does not become adulterated.

Food Contact Surface (FCS) Sampling

As stated previously, according to the *Listeria* Rule, establishments in Alt. 2b and 3 are required to provide for testing of FCSs in the post-lethality processing environment to ensure that the surfaces are sanitary and free of *Lm* or an indicator organism (9 CFR 430.4(b)(2)(iii)(A) and (3)(i)(A)).

Establishments are also required to identify the **size and location** of the sampling sites (9 CFR 430.4(b)(2)(iii)(D) and (3)(i)(D)). FSIS recommends that establishments in Alt. 1 and 2a also test their food contact surfaces. The sites that the establishment will test can be included in the *Listeria* Control Program.

The expectation for establishments in Alt. 2b and 3 is that all possible FCSs in the post-lethality processing area will be identified.

This includes surfaces which may come into contact with food on a regular basis as well as those that may come into contact on an intermittent basis. FSIS recommends that the establishment list all possible FCS in their *Listeria* Control Program. This means that the establishment should identify items that the product touches (e.g.,

Question: Would product racks, sticks, and screens that RTE products are cooked on need to be included as product contact surfaces for *Listeria* sampling?

Answer: Yes, the racks, sticks, and screens that are used for RTE product would be considered food contact surfaces, after the product has been cooked. Even though the racks, sticks, and screens are subjected to high temperatures along with the product, they may be handled when being removed from the oven and may be placed in a cooler as the product is cooled, so it is possible they could become contaminated after cooking.

equipment, utensils, and gloves). For example, it is not necessary to list each individual glove, but “gloves” should be listed as a sampling site. This will assist the establishment in identifying all areas that could harbor bacterial pathogens such as *Lm*. By including all possible FCS, the establishment could decrease the likelihood that FSIS would find the food safety system inadequate.

NOTE: It would not be sufficient for establishments to collect product samples in lieu of food contact surface samples to meet the requirements for Alternative 2b or 3. The *Listeria* Rule requires establishments to collect food contact surface samples to meet the requirements for those alternatives (9 CFR 430.4(b)(2)(iii)(A) and (b)(3)(i)(A)).

Sample Collection Considerations

Establishments should design their sampling programs so that they collect a combination of random and discretionary samples. Initially, **samples should be collected at random**, to ensure that all FCS have an equal probability of being sampled. Random sampling should be used after an establishment has started production or begins processing a new product to verify that their system is effective. The establishment should have plans in place so that representative samples of all FCS will be sampled over a specified period of time.

Once the establishment has generated data demonstrating that their control system is effective, the establishment should adopt a more **risk-based sampling** program. The risk-based sampling should include **discretionary samples** that are collected along with the random samples. These samples can be collected at the discretion of the sample collector based on positive results or other conditions as observed at the establishment. For example, if the establishment is collecting 3-5 samples per line as part of the routine sampling program, 1-2 of the samples should be discretionary while the others should be collected randomly. Discretionary samples should be collected if the sample collector observes conditions that could lead to harborage or cross contamination in the post-processing environment (e.g., backed-up drains, sanitation issues, and condensation dripping over equipment). Establishments should also sample more frequently in areas where sanitation issues have been identified, and use the results of their sanitation monitoring testing (e.g., APC or bioluminescence) to identify sampling sites. Discretionary samples can also be collected to demonstrate the effectiveness of the establishment’s corrective actions. The results from the discretionary samples can be linked to the sample collector’s

Question: Each piece of equipment may have multiple sampling sites. Does the establishment need to identify every site it will sample on the equipment, or just identify the piece of equipment as a sampling site?

Answer: The establishment just needs to identify the piece of equipment. However, the establishment should recognize that the equipment may have both food contact and non food contact sites, and should sample these according to their *Listeria* Control Program.

Question: An establishment produces both FDA-regulated and USDA-regulated product using the same food contact surfaces on different days. Can the establishment collect food contact surface samples as required by the *Listeria* Rule when FDA product is being produced?

Answer: No. Collecting the samples when FDA product is being produced would not meet the requirements of the *Listeria* Rule, because the surfaces do not come into direct contact with FSIS product that day. However, collecting food contact surface samples when FDA product is being produced would provide the establishment with useful information regarding its overall sanitary practices.

observations, providing more information about sources of harborage or cross contamination in the establishment.

If positive samples are found, the establishment should take corrective actions and collect follow-up samples according to their alternative. In addition, the establishment should **target** the sites during future routine discretionary sampling, to ensure that the contamination has been addressed. For more information on follow-up sampling see [Chapter 4](#).

Examples of FCSs may include:

- Conveyor belts,
- Slicers,
- Utensils,
- Tubs,
- Trays, and
- Racks.

A table of other possible FCSs and NFCs is provided in [Attachment 3.1](#). As indicated in the table, depending on the establishment's process some surfaces that would normally be NFCs may be considered FCSs if they come into direct contact with the product. For example, employees' gloves should be identified as FCSs if employees directly handle the product with their gloves. Also, some NFCs are adjacent to products (e.g., equipment sides) and are more likely to contaminate product (see [Section 3.6](#) for more information on sampling NFCs).

Size of the Sampling Sites

FSIS recommends that establishments sample a **12"x12" area**, when possible. If the sampling site (e.g., tool or control button) is smaller than 12"x12" then a smaller size can be sampled. This sampling size is recommended to provide a representative sample of the equipment and is the same as the sample size FSIS uses when collecting samples (see [Appendix 3.2](#)). Therefore, it should help provide similar opportunity for detecting contamination as the FSIS sampling method, when used in conjunction with sampling and analysis methods meeting FSIS expectations (see [Section 3.5](#)).

3.4 Frequency of Sampling and Explanation of this Frequency

According to the *Listeria* Rule, establishments in Alt. 2b and 3 are required to state the frequency of testing and include an explanation of why the testing frequency is sufficient to maintain control of *Lm* or an indicator organism (9 CFR 430.4(b)(2)(iii)(C) and (E) and (3)(i)(C) and (E)). Specifying the sampling frequency is also recommended for establishments in Alt. 1 and 2a. The sampling frequency should be based on the following criteria:

- a) Alternative,
- b) Establishment size or volume (large, small, very small)⁴,
- c) Whether or not the establishment produces deli meats and hotdogs, and
- d) Past history and observed patterns of contamination.

⁴ Large establishment are those with 500 or more employees, small establishment are those with 10 or more employees, but fewer than 500 employees, and very small establishments are those with fewer than 10 employees or annual sales of less than \$2.5 million.

Other factors to consider are type of product, how often product is produced, production volume, product flow, traffic patterns, age of the processing facility, and whether raw product is produced in the same room as RTE products (or produced using the same equipment). Establishments can use the minimum sampling frequencies in Table 3.1 below to meet the requirements of the *Listeria* Rule. Establishments may prefer to increase their testing frequency in response to positives or *Listeria* trends (see [Section 4.5](#)).

Table 3.1 Minimum Routine Sampling Frequencies for Testing of Food Contact Surfaces (FCS) for Alternatives 1, 2, and 3.

Alternative	Daily Production Volume Ranges (lbs)**	Food Contact Surface (FCS) Testing
		Minimum Frequency*
Alternative 1		2 times/year/line (every 6 months)
Alternative 2a and 2b		4 times/year/line (quarterly)
Alternative 3 Non-deli, non-hotdogs		1 time/month/line (monthly)
Alternative 3 Deli, hotdogs HACCP Size:		
Very small	1-6,000	1 times/month/line (monthly)
Small	6,001 – 50,000	2 times/month/line (every 2 weeks)
Large	50,001->600,000	4 times/month/line (weekly)

*At least **3-5 samples** per production line should be sampled each time (every 6 months, quarterly, monthly, biweekly or weekly).

**Establishments producing deli or hotdogs under Alt. 3 may decide to collect samples based on HACCP size or production volume.

Frequency Determinations: How to use Table 3.1

The table lists FSIS expectations for minimal sampling frequencies to meet the *Listeria* Rule. Establishments should consider these frequencies when determining their sampling frequency for their routine sampling program. Establishments can keep this table on file as part of the supporting documentation needed to explain why the testing frequency they have selected is sufficient to control *Lm* or an indicator organism according to 9 CFR 430.4(b)(2)(iii) (E) and (3)(i)(E). The table has been updated to provide Alt. 3 deli and hot dog

Question: Can the food contact surface samples be composited (combined) at the laboratory, so that one result is obtained from all 5 samples?

Answer: Yes. Establishments may request that laboratories composite food contact surface samples in order to decrease costs. However, establishments should collect the samples individually to avoid cross contamination and take corrective actions for all sites that are part of a composited sample if a positive result occurs, as described on page 95

producers with the option of using daily production volume ranges or establishment HACCP size to determine sampling frequencies. Basing the sampling frequency on the production volume provides more risk-based sampling frequencies and is similar to FSIS sampling programs. If the establishment chooses to follow the testing frequency based on daily production volume, it is important that it **modifies the documentation** associated with its sampling programs. It would not be sufficient for the establishment to make modifications to its testing frequency without changing its programs and supporting documentation.

When the establishment is using the sampling frequencies specified in the table, at least **3-5 FCS samples per production line** should be sampled each time (every 6 months, quarterly, monthly, biweekly, or weekly). The samples should be taken at different days throughout the year, quarter, month, or week, and on different shifts (e.g., 1st and 2nd shift) to ensure that the samples are truly representative of processing conditions. The frequencies listed in the table are based on a typical processing schedule (5 days a week). Establishments that produce intermittently may be able to support sampling less frequently depending on the production schedule. Establishments operating under multiple alternatives that use the same FCS during a production day (clean-up to clean-up) should use the testing frequency for the highest risk product. For example, if an establishment produces hotdog products under Alt. 1 and deli products under Alt. 3 using the same equipment on the same processing day, they should sample at the frequency listed for Alt. 3.

Sample Frequency Considerations

- **Intermittent Production:**
Establishments that produce RTE product intermittently may be able to justify sampling at a lower frequency, based on the number of days they produce.

For example, assuming that if there are 20 production days in a typical production month (excluding weekends), and an establishment produces RTE product 1-2 days a week, then it may be able to justify sampling quarterly rather than monthly.
- **Representative:** Samples should be representative of conditions at the establishment and collected over different shifts and seasons.
- **Sampling Frequency:** Establishments are expected to increase their sampling frequency in the event of a positive or other event (e.g., construction) in the establishment.

NOTE: Once an establishment has identified a sampling frequency, it should follow the frequency it has selected. If sampling is not performed at the stated frequency, the establishment would need to provide support that their surfaces are sanitary and free of *Lm*.

As stated previously, the sampling frequencies for FCS testing suggested in the Table 3.1 are recommended minimum frequencies. **These sampling frequencies should be increased, or additional intensified samples should be added, based on a change in risk including the following:**

- a) **Construction activities,**
- b) Change in the HACCP plan or addition of a new HACCP plan,
- c) Addition of a new product,
- d) Roof leaks, condensation, equipment breakdowns, or other events that could change or increase the probability of product contamination,
- e) Increased positives from routine sampling, or

- f) Increased aerobic plate count (APC) or bioluminescence counts indicating sanitation issues.

NOTE: Establishments operating under multiple alternatives that use the same FCS during a production day (clean-up to clean-up) should use the testing frequency for the highest risk product. For example, if an establishment produces hotdog products under Alt. 1 and deli products under Alt. 3 using the same equipment on the same processing day, they should sample at the frequency listed for Alt. 3.

Question: Our establishment produces a tamale product (meat and cheese filling wrapped in a corn husk). Would this product be considered post-lethality exposed?

Answer: Yes. The corn husk is not considered a sealed package. Therefore the tamale would be considered a post-lethality exposed product and FCSs that come in direct contact with the product should be sampled.

Question: Our establishment produces a product that is cooked in a casing that is clipped on the ends. The product is not removed from the casing until it reaches the consumer. Would this product be considered cook-in bag, and therefore not post-lethality exposed?

Answer: It depends. If the establishment can provide documentation from the manufacturer of the casing material demonstrating that it is not permeable to microorganisms and microorganisms can't penetrate the packaging at the clip, then the product would be considered non post-lethality exposed (or cook-in bag). If the casing is considered semi-permeable (permeable to microorganisms) then the product would be considered post-lethality exposed.

Question: Our establishment produces pickled pig's feet. Would this product be considered post-lethality exposed?

Answer: No, pickled pig's feet are typically not considered post-lethality exposed because the product is packaged in a jar with a brine and pickle solution that causes reduction of *Lm* and does not allow growth. As long as the establishment can provide supporting documentation that at least a 5-log decrease of *Lm* is achieved by the pickle solution, the product would not be considered post-lethality exposed.

Question: Our establishment uses brine to cool post-lethality exposed RTE product. Should we sample the brine?

Answer: Yes. If the brine comes in direct contact with RTE product, it should be sampled as a FCS. If the product is packaged in an impermeable membrane, the brine should be sampled as a NFCS.

3.5 Sample Collection and Laboratory Testing Methods

Sampling using proper collection technique is important to ensure that low levels of *Lm* or *Listeria* spp. are detected in the post-lethality processing environment. **It is also important that results are accurate and reliable, so they can be used to support the decision made in the hazard analysis that *Lm* is not reasonably likely to occur in the product.**

The establishment should provide written instructions for collecting food contact, environmental surface or product samples, and the samples should be collected using [aseptic techniques](#) (see box below). The instructions can be included as part of the establishment's *Listeria* Control Program. In [Appendix 3.2](#), the sampling procedures used by FSIS during IVT and RLM sampling to sample FCSs, NFCs, and brine used to chill RTE product are provided. Establishments may use these methods, or adjust the methods based on the needs of the establishment. FSIS expectations for sampling and testing methods are provided below. Further sampling and testing considerations are included in [Appendix 3.3](#). See the box on the next page for FSIS expectations for sampling methods.

Sampling Methods

Aseptic Technique: Sampling should be performed by a person trained in aseptic technique and samples should be collected using sterile sponges or other sampling devices.

Sample size: A 12"x12" area should be sampled, when possible, for FCS and NFCS surfaces. If the surface area is smaller than 12"x12", then the entire surface should be sampled.

NOTE: Cotton-tip swabs and other smaller sampling devices are not recommended for sampling large areas (12"x12") because they may become easily saturated with microorganisms. If these devices are used, FSIS recommends collecting a smaller sampling size according to the manufacturer's instructions to equal a 12"x12" area.

Sample collection: The sponge or sampling device should be hydrated with sterile neutralizing buffer, Dey Engley (DE) broth, or another sterile broth that contains components that can neutralize the effects of sanitizers that may be present in the sample.

When to collect samples: Some samples can be collected at pre op, but most samples should be collected at least 3 hours into operations, if possible, to allow *Lm* to work its way out of the equipment. If the establishment typically produces RTE product for less than 3 hours then the samples can be collected less than 3 hours into operations.

Sample integrity: Samples should be stored under refrigeration before analysis. Samples should be properly labeled to avoid confusion regarding testing results.

Brine sampling: Some establishments use brine to cool or inject into RTE product. Depending on whether the finished product surface is directly exposed to brine after the lethality step, the brine solutions could be considered either as food contact or environmental samples.

Sample compositing: FCS samples may be composited (combined) in order to conserve establishment's resources. If compositing is performed, FSIS recommends that no more than 5 samples be composited, and **separate** sponges (or other sampling device) be used to collect each sample, to avoid possible cross contamination. One laboratory test can then be performed on the 5 separate samples, decreasing the cost to the establishment.

In addition, individual locations for the composite sample should be noted to assist in determining the site of contamination to facilitate follow-up testing. **If a composited sample tests positive, the establishment should consider all the sites represented by the sample as positive and take corrective actions accordingly.** During follow-up sampling of FCSs, the sites should be **re-sampled individually**, along with additional swabs in the area. For more information on compositing, see Appendix 3.3.

Handling and shipping of samples: If the samples will be analyzed by an in-house lab, testing should be initiated immediately after collection. If not tested by an in-house lab, the testing should be initiated within 2-3 days of collection. If this is not possible, the establishment should provide evidence that another strategy does not compromise the sensitivity of the method. The samples should be stored under refrigerated conditions (33 – 45 °F), and in no case be allowed to freeze, which could kill organisms captured on the sampling device. Samples should be placed into insulated shipping containers and sent refrigerated to the laboratory. Lastly, the identity of the sample should be maintained during testing to ensure that sites are correctly identified.

Testing Methods

Establishments may test for *Lm*, *Listeria* spp., or LLO. Testing can be performed either in-house or at a third-party laboratory (see [Appendix 3.3](#)). **However, if the testing is performed at a third-party laboratory, the establishment should be familiar with the method used by the lab, have the method on file at the establishment, and know whether it meets FSIS expectations for testing methods.**

If an establishment uses the testing results to support the decision made in its hazard analysis that *Lm* is not reasonably likely to occur in its product, then it is important that the results are reliable and accurate. Further information on testing methods can be found in [Appendix 3.3](#).

The following are FSIS's expectations for testing methods:

1) **An enrichment step is used** to allow for recovery of injured organisms and growth of *Listeria* to levels that can be detected by most testing methods. Many commonly used testing methods are unable to detect levels below 100 cells/sample. Therefore, it is important that the enrichment step be designed to allow low levels of cells that may be present in the sample to grow to detectable levels. It is also important to allow injured cells time to recover so that they can be detected by the testing method. In most cases, at least an 8-hour enrichment is needed to achieve adequate levels of *Lm* growth for detection. A one-hour resuscitation step is not an enrichment step, and would likely not be sufficient to detect low levels of *Listeria* spp. or *Lm*.

NOTE: Direct plating methods (e.g., media that is added directly to an agar plate or dehydrated media) that do not include an 8-hour enrichment step would be unlikely to detect low levels of *Listeria* spp. or *Lm*.

2) **The entire sponge or sampling device is analyzed.** Some methods involve testing just a small part of the broth or other diluent used to hydrate the sponge or sampling device. Studies have shown that bacteria are likely to be trapped on or in the interior of the sponge or other sampling device. Therefore, FSIS suggests that the whole sponge or sampling device be included in the enrichment step. Analyzing the entire sampling device will help ensure that cells that are present will be detected.

3) **The method has been validated.** All screening methods should either be used by a regulatory body (e.g., FDA Bacterial Analytical Manual (BAM)), or validated by a recognized independent body (e.g., AOAC, AFNOR, ISO, NordVal, Microval). A validated method from a scientifically robust study using the FSIS *Lm* qualitative method as a reference method, or other validated cultural methods is also acceptable, but would be subject to FSIS review.⁵ In addition to guidance provided by the recognized independent bodies mentioned above, FSIS has provided guidance on the design of validation studies for pathogen testing methods in the [FSIS Guidance for Test Kit Manufacturers, Laboratories: Evaluating the Performance of Pathogen Test Kit Methods](#).

NOTE: It is not sufficient for methods to be AOAC or ISO validated alone. To meet FSIS expectations for testing methods, the method should also include an enrichment step and analyze the entire sponge or sampling device.

⁵ Submit request for review of methods to [AskFSIS](#).

FSIS Review of Sampling and Testing Methods

As part of FSIS Food Safety Assessments (FSA), Enforcement, Investigations, and Analysis Officers (EIAOs) will review the **sampling** and **testing** methods used by the establishment to determine if they meet FSIS expectations. If an establishment chooses not to use a validated methodology for food-contact and other environmental-surface testing, or if the quality of the testing results provided by the laboratory is in question, the establishment may be assuming a greater risk of allowing adulterated product into commerce. Should FSIS question the sampling or testing methodology, it may choose to review the establishment's scientific basis for using these methods. In such a circumstance, the establishment could be subject to focused verification checks, including a review of recordkeeping, observation of production, and the collection of product and environmental sampling by FSIS.

3.6 Other Routine Sampling

Although **not** required by the *Listeria* Rule, establishments may choose to include sampling for indirect and NFCS and product as part of their *Listeria* Control Program. Sampling indirect and NFCS and product can give the establishment more information about possible harborage and cross contamination pathways in their environment. For more information on harborage and cross contamination, see [Chapter 4](#).

Testing Indirect and Non Food Contact Surfaces (NFCS)

As previously stated, establishments may choose to test indirect and NFCS samples as part of their *Listeria* Control Program, although they are **not** required by the *Listeria* Rule. FSIS samples indirect and NFCSs during RLM and IVT sampling, so by sampling these areas, the establishment can find harborage points before they are found by FSIS. Some examples of indirect and NFCS sites are included below. Other examples are included in [Appendix 3.1](#).

NOTE: If an NFCS tests positive for *Lm*, the product is **not considered adulterated**, however a positive finding could indicate insanitary conditions in the environment. Likewise, if a NFCS tests positive for *Listeria* spp., the product is not considered adulterated, however the establishment should address positive results to ensure that harborage and cross contamination to FCSs and product does not occur.

Indirect FCS sites include the following:

- The sides of conveyor belts,
- Equipment frame-work, and
- Table legs or other areas that are near or adjacent to food processing sites.

NFCS sites include the following:

- Drains,
- Floors,
- Walls, and
- Ceilings.

NOTE: NFCS samples may be collected anywhere in the establishment where RTE products are stored or held (e.g., coolers, freezers, loading docks, and trucks). NFCSs may also be collected in areas associated with post-lethality processing, such as equipment storage and wash rooms, spice rooms, and ingredient rooms.

Establishments can set their own frequency for NFCS sampling (e.g., weekly or monthly) based on their processing schedule or past history of positives. While there is **no requirement** that establishments perform follow-up testing in response to indirect or NFCS samples, it is important that establishments address the source of positives (e.g., by cleaning and sanitation) to ensure that harborage and cross contamination of product does not occur.

Product Testing

Although product testing is **not** required by the *Listeria* Rule (except under hold and test conditions for Alt. 2b or 3), establishments may decide to test product as part of their *Listeria* Control Program. Product testing can be used as a verification of the effectiveness of establishments' PLTs, AMAPs, and sanitation control measures. Also, as most of FSIS testing is of product (RTEPROD_RAND and RTEPROD_RISK project codes), testing by the establishment can help to detect product contamination before it is found through FSIS testing.

Many establishments choose to test product quarterly as part of their *Listeria* Control Programs. However, product testing is not a substitution for the food contact surface testing required by the *Listeria* Rule. **Establishments that test products still need to test food contact surfaces to meet the requirements for Alternatives 2b and 3 (9 CFR 430.4(b)(2)(iii)(A) and (b)(3)(i)(A)).** Product testing protocols are typically designed and validated for a 25-gram analytical portion (i.e., the portion of the collected sample that is actually tested). Before testing larger analytical portions from single or multiple composited samples, ensure that the testing method has been validated for use with the larger portion. FSIS has begun compositing 5x25 gram samples collected during RLM sampling into a 125 gram portion. The FSIS [Microbiology Laboratory Guidebook](#) (MLG) Chapter 8 methods have been updated to include validated methods for the larger sample size and may be used by establishments to analyze a 125 gram test portion. However, establishments may continue to analyze a 25 gram sample, as 25 grams is the size of the sample FSIS analyzes for the RTEPROD_RAND and RTEPROD_RISK project codes.

Establishments can set their own frequency for product testing (e.g., quarterly or twice yearly), based on the establishment's processing schedule or past history of positive results (except in hold and test conditions).

NOTE: The establishment should hold all product lots until its test results are received. This will prevent exposure of the consumer to a potential food hazard. Retaining the product being tested also will eliminate the cost of a recall to the establishment.

Product that tests positive for *Lm* would be considered adulterated and the establishment would be expected to destroy or rework the product with a process that is destructive of *Lm*.

If a product tests positive for *Listeria* spp., the establishment should provide the following documentation to demonstrate that the product is not positive for *Lm*:

- Testing data demonstrating that the original isolate is not positive for *Lm*,
- A sampling plan that provides a level of statistical confidence that each product is not contaminated with *Lm* (e.g., testing for *Lm* using a sampling plan recommended by the ICMSF, see Section 4.3), or
- Documentation showing that the product has been reprocessed using a process validated to achieve at least a 5-log reduction of *Lm*.

A finding of *Listeria* spp. in the product can indicate insanitary conditions where the product could become contaminated with *Lm*. Therefore, the establishment should review its Sanitation SOP and HACCP plan to ensure that *Lm* is controlled in its environment, and that cross contamination of the product is unlikely to occur. FSIS will review the establishment's sanitation records, observations of sanitation, and sanitation NRs, and if it finds that the establishment's Sanitation SOP is inadequate or its corrective actions are ineffective, an NR will be issued (according to 9 CFR 416.12 or 416.15), or an IVT may be scheduled at the establishment.

If the establishment does not provide compelling documentation that the product is not contaminated with *Lm* (as described above), FSIS may not be able to determine that the product is not adulterated, and it may take a regulatory control action, under 9 CFR 500.2(a)(3). If the agency determines that product is adulterated by being produced under insanitary conditions, and the product is in commerce, the agency may request a recall.

Production lot

A production lot is the amount of product that may be impacted by a product or FCS positive test result. As previously stated, establishments are required to hold or maintain control of RTE products that FSIS has tested for *Lm* or RTE products that have passed over food contact surfaces that FSIS has tested for *Lm*. Establishments may move such products offsite provided that they maintain control of them (e.g., through company seals). A production lot is typically defined as all product produced from clean-up to clean-up unless the establishment can support a smaller lot size. If the establishment performs a complete cleaning and sanitizing (following the procedures in its Sanitation SOP) between lots, the lot size could be reduced. Factors that should be taken into account when determining lot size include RTE source materials used, frequency of cleaning and sanitizing, and processing steps.

NOTE: An establishment may reduce its lot size on a day when FSIS collects a sample, in order to facilitate holding the product, as long as the change does not interfere with FSIS's ability to collect a representative sample.

Products produced in the same room could be considered part of the same or different processing lots, depending on how the lots are separated. If the processing lines can be considered microbiologically and physically independent of one another (i.e. equipment, personnel, utensils, and RTE source materials are not shared among the lines), then they can be considered different lots. An example of a common source material could be chicken in a chicken salad that is taken from the same package over multiple lots. If a FCS tests positive on one line, and the establishment has supporting documentation that there is not cross contamination among the lines, then lots produced on the other lines may not be implicated.

Likewise, products produced on the same line could be considered different processing lots, if they are separated by a complete cleaning and sanitization, as well as the other factors described above.

NOTE: Products stored in a common cooler would not necessarily be considered part of the same lot. However, the establishments Sanitation SOP should address possible cross contamination, especially if RTE and raw products are held in the same cooler.

3.7 Glossary

Aseptic Technique: A sample-collection procedure performed under sterile conditions. The samples are collected using sterile sampling swabs, buffer, gloves, and other sampling supplies. Aseptic technique should be used to avoid cross contaminating samples, and keep contamination from spreading between sampling sites during sampling.

Confirmation Test: A series of tests, often following a positive screening test, used to definitively identify the target organism.

Food Contact Surface (FCS): An area in the post-lethality processing environment that comes in direct contact with post-lethality exposed RTE product.

Indirect Food Contact Surface: An area in the post-lethality processing environment that is adjacent to a FCS, but does not come in direct contact with the product.

***Listeria monocytogenes* (Lm):** A foodborne bacterial pathogen that can cause the disease listeriosis in humans.

***Listeria* spp.:** Members of the genus *Listeria*, which includes both pathogenic (*Lm*) and non pathogenic strains. The presence of *Listeria* spp. indicates conditions where *Lm* could be present or grow. Further confirmation tests would be needed to determine if *Listeria* spp. positive tests are also positive for *Lm*.

***Listeria*-like organism (LLO):** An indicator for *Lm*. LLO tests usually employ traditional *Listeria* culture enrichment and isolation media to screen for bacteria that have biochemical characteristics typical for but not necessarily exclusive to *Listeria* spp. Many LLO methods are based on the ability of *Listeria* species to hydrolyze esculin or other compounds, resulting in a color change to the broth or solid media (usually to dark brown or black). LLO could include *Enterococcus* spp. and *Lactobacillus* spp., among others.

Non Food Contact Surface (NFCS): An area that does not contact product. NFCS samples may be collected from any area where RTE product is held in the establishment (e.g., coolers, freezers, loading docks, and trucks). NFCS samples may also be collected in areas associated with post-lethality processing, such as equipment storage and wash rooms, spice rooms, and ingredient rooms.

Production Line: A line refers to the flow of product during production. This includes all of the equipment, personnel, and utensils that contact the RTE product. Multiple individual product lines can meet at a piece of equipment (e.g., packaging machine), but they are still considered to be different lines.

Pulsed-field gel electrophoresis (PFGE): a laboratory method used for subtyping bacterial isolates below the level of species using bacterial deoxyribonucleic acid (DNA). PFGE patterns consist of DNA fragments of varying sizes resolved by passage through an agarose gel. PFGE patterns can be compared to determine their degree of relatedness.

Screen test: A preliminary test to determine if a sample contains organisms that share certain characteristics (growth parameters, sensitivity to antibiotics, similar genetic make-up) as the target organism. Many tests for *Listeria* spp. are screening tests for *Lm*. In order to definitively define the organism as *Lm*, further confirmatory tests would be needed.

3.8 References

Tompkin, R.B. 2002. Control of *Listeria monocytogenes* in the Processing Environment. Journal of Food Protection. 65 :709-725.

Tompkin, R. B., V. N. Scott, D. T. Bernard, W. H. Sveum, and K. S. Gombas. 1999. Guidelines to Prevent Post Processing Contamination from *Listeria monocytogenes*. Dairy, Food and Environmental Sanitation. 19 (8): 551-562.

FSIS Microbiology Laboratory Guidebook, 1998.

Attachment 3.1: Possible Food Contact Surface and Non Food-Contact Sites

This table provides examples of possible FCS and NFCS sites for use in developing *Listeria* Control Programs. The list is not all-inclusive. Careful efforts should be made to determine all possible food contact sites in an establishment's environment.

Table of Possible Food Contact and Non Food-Contact Sampling Sites

Food Contact	Non Food Contact
Aprons*	Air blower, filter
Baggers	Boots
Band saws	Carts
Belts	Ceilings
Blades	Coat racks
Brine*	Condensation
Chiller shelving	Control buttons
Chutes	Cooling units
Coats*	Doors
Conveyors	Drains
Cutting boards	Equipment framework
Equipment surfaces	Equipment sides
Equipment shields*	Exposed insulation
Gloves*	Fans
Grinders	Flaps
Guiding bars	Floor mats
Hopper surface	Floor/wall junctions
Knives	Floors
Mixers	Forklifts
Packaging machines	Gaps between close-fitting parts
Packaging materials	Gaskets
Paddles	Hoses
Peelers	Legs (hollow)
Plastic wrap	Lifters
Plates	Machinery
Product carts	Maintenance Tools
Racks	Mops
Saw table	Motor housing units
Scales	Overhead pipes
Scoops	Pallets
Scrapers	Platforms
Sealers	Refrigeration units
Shredder	Roller bars (hollow)
Slicers	Rough welds
Smoke sticks	Sinks
Tables	Spiral Freezer
Thermometers	Squeegees
Tongs	Standing water
Trays	Stands
Trees	Trash cans
Tubs	Walkways
Utensils	Walls
Wipers	Wheels of carts

*Could be considered either a food contact or a non food-contact surface, depending on if the surface comes in direct contact with the product.

Appendix 3.1: FSIS RTE Sampling Program

As of August 1, 2013, FSIS combined its random ALLRTE and risk-based RTE001 product sampling projects into a single project, called RTEPROD. The RTEPROD sampling project uses two project codes: RTEPROD_RAND for product samples selected randomly, and RTEPROD_RISK for post-lethality-exposed product samples selected based on risk. The RTEPROD sampling project is expected to increase response rates and conserve laboratory resources.

Under the **RTEPROD_RAND** project code, both post-lethality exposed and non-post-lethality exposed products are tested, and samples are randomly selected by FSIS. FSIS conducts testing on non post-lethality exposed products (e.g., cook-in bag products) to verify that adequate lethality has been achieved in the products, and that they are not contaminated with *Lm* and *Salmonella*, in accordance with 9 CFR 417.8(g).

Samples are scheduled for the RTEPROD_RAND project code so that all RTE establishments, regardless of plant size, production volume, or process design, have an equal chance of being sampled each fiscal year.

The **RTEPROD_RISK** project code is used primarily to verify that establishments producing post-lethality exposed RTE meat and poultry products are controlling *Lm* and are in compliance with the requirements of the *Listeria* Rule. Establishments are identified for sampling based on a risk-ranking algorithm, which takes into account the control alternative,⁶ the production volume, the type of product produced, and the sampling history.

Under both project codes (RTEPROD_RAND and RTEPROD_RISK), a two pound sample of the product in its final finished package is collected, and the sample is tested for *Lm* and *Salmonella* spp.

Regulations and directives specific to the RTEPROD sampling program include the following: 9 CFR 430.4 “Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products” published on June 6, 2003 (68 FR 34207); FSIS [Directive 10,240.4, Revision 3](#), “Verification Activities for

Question: If an establishment delivered product from a sampled lot to a customer but retrieved all of it before the report of the FSIS sample result, will the product be deemed to have been shipped?

Answer: Yes, once an establishment completes its pre-shipment record review, the product is considered “eligible for shipment” or “shipped.” Upon report of a positive result, establishments are expected to prevent product from entering commerce in accordance with paragraphs 9 CFR 417.3(a)(4) or (b)(3) of the regulations and to process it in a manner that will make it no longer adulterated.

Question: Why does FSIS require a 2 pound sample of jerky and other RTE products?

Answer: The amount of product requested depends on the type and number of tests that are performed. The Agency tests for more than one pathogen in a sample and enumerates the samples. Therefore, at least 2 pounds of product are required for most analyses. One pound of product is required for the RLM project, because only *Lm* is analyzed.

⁶ For Alternative 1, the establishment uses a post-lethality treatment for its product and an antimicrobial agent or process that suppresses or limits growth of *Lm*. For Alternative 2, the establishment uses a post-lethality treatment for product or an antimicrobial agent or process that suppresses or limits the growth of *Lm*. For Alternative 3, the establishment uses a sanitation project that controls *Lm* contamination in the processing environment and on the product.

the *Listeria monocytogenes* (*Lm*) Regulation and Ready-to-Eat (RTE) Sampling Program,” (January 10, 2014).

RLm

The RLm sampling project, implemented in April 2006, is a routine risk-based sampling project which consists of food contact, environmental and product samples that are taken during the production of RTE meat and poultry products that are exposed to the post-lethality environment. All samples are analyzed for *Lm* and are to be taken during the same day of production. In conducting the RLm project, it is anticipated that FSIS will be able to assess the compliance of establishments with regulation 9 CFR 430.1 regarding the control of *Lm* in post-lethality exposed RTE production areas and to help ensure that RTE products are safe for consumption at the end of the production process.

RLm samples are scheduled using a Food Safety Assessment (FSA) prioritization model which takes into account levels of inspection (LOI),⁷ control alternative, and type of product produced. Starting in August 2009, RLms sampling was increased so that establishments producing post-lethality exposed RTE product are sampled at least once every four years under this project.

For the RLm project, FSIS collects 3 sample units from large establishments (500 or more employees), 2 sample units from small establishments (10-499 employees) and 1 sample unit from very small establishments (< 10 employees). A sample unit consists of 10 food contact surface swabs, 5 environmental swabs (which are composited), and 5 intact product samples. FSIS laboratories composite the 5 product samples per unit. In establishments that use brine chillers, the EIAO is to collect a sample of brine from each line using a brine chiller.

RLm sampling is performed in conjunction with a routine FSA, which provides an in-depth evaluation of the effectiveness of the food-safety practices employed by an establishment. The ability to use the product, contact and environmental sampling information collected from the establishments, can help identify possible risk factors that could be associated with positive results.

Regulations and directives specific to the RLm sampling project include the following: 9 CFR 430.4 “Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products” published on June 6, 2003 (68 FR 34207) [includes definitions for RTE Alternatives 1, 2, and 3]; and

Question: If a product or food contact surface sample tests positive for a pathogen, what is the status of product(s) produced on days subsequent to the day the sample was collected?

Answer: In general, FSIS does not consider product that is produced on days subsequent to the day of sampling and that is coded differently from the sampled lot to be represented by the sample. Under most circumstances, the product is not subject to retention, detention, or voluntary recall. A positive sample does call into question the adequacy of an establishment’s process for producing safe product, and the establishment should take corrective actions to address the positive result.

⁷ The three LOI are defined as follows: LOI 3—Establishments with strong indications that they are not maintaining effective food safety process controls. LOI 2—Establishments with some indication that they may not be maintaining effective food safety process controls. LOI 1—Establishments that consistently demonstrate they are maintaining effective food safety process controls.

FSIS Directive 10,240.5, Revision 3, Verification Procedures for Enforcement, Investigations, and Analysis Officers (EIAOs) for the *Listeria monocytogenes* (*Lm*) Regulation and Routine Risk-Based *Listeria monocytogenes* (RLm) Sampling Project” published on March 28, 2013.

IVT

In the IVT sampling project, FSIS tests product, food contact surfaces, and environmental surfaces for *Lm*. An IVT is initiated after an establishment has a positive *Lm* result, in either finished product or on a food contact surface. An IVT can also be initiated at the discretion of the District Manager, in response to continuing sanitation non-compliances at the establishments. The IVT is performed after the establishment has taken its corrective and preventive measures in response to FSIS findings. In an IVT, FSIS collects samples in units. A unit consists of 10 food contact surface samples, 5 environmental samples, and 5 product samples per post-lethality exposed RTE processing line in operation on the day of sampling. If the establishment uses a brine chiller, FSIS will also collect 1 brine sample per line from the brine chiller. IVTs are performed with a “for cause” Food Safety Assessment (FSA) to provide an in-depth evaluation of food safety systems at the establishment.

IVTs are scheduled according to the FSA prioritization model, with all establishments with *Lm* positives receiving an IVT. IVTs may also be performed in response to repetitive occurrences of non compliances because of sanitation issues or to verify corrective actions before closing-out an enforcement action. The districts have 30 days in which to schedule the IVT after a product or FCS *Lm* positive.

Regulations and directives specific to IVT include the following: 9 CFR 430.4 “Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products” published on June 6, 2003 (68 FR 34207) [includes definitions for RTE Alternatives 1, 2, and 3]; FSIS Directive 10,300.1, Revision 1, “Intensified Verification Testing (IVT) Protocol for Sampling of Product, Food Contact Surfaces and Environmental Surfaces for *Listeria monocytogenes*,” published on March 28, 2013; and FSIS Directive 10,210.1, “Unified Sampling Form”, October 14, 1997.

Question: If a RTE product tested by FSIS is found positive for *Lm*, is the establishment required to take corrective actions and reassess their HACCP plan?

Answer: If *Lm* control is addressed as a CCP in the HACCP plan (e.g. PLT) the establishment must meet the requirements of 9 CFR 417.3(a), which requires that corrective actions are taken but does not require reassessment of the HACCP plan.

If *Lm* is addressed in the Sanitation SOPs, then the establishment must implement the corrective actions in 9 CFR 417.3(b), which includes reassessment of the HACCP plan. In addition, they must implement the corrective action requirements for the Sanitation SOPs in 9 CFR 416.15, which includes appropriate re-evaluation or modification of the Sanitation SOP.

If *Lm* is addressed in a prerequisite project (e.g., *Listeria* Control Program) that is used to support the decision that *Lm* is not a hazard reasonably likely to occur in the product, then the establishment must implement the corrective actions in 9 CFR 417.3(b) and comply with 417.4(a)(3). These regulations state that when there is a change in the process (e.g., a positive result) that could impact the hazard analysis, a reassessment must be performed.

Question: If a RTE product tested by FSIS is found positive for *Lm*, is the HACCP system automatically considered inadequate?

Answer: According to 417.6, the HACCP system may be found inadequate if among other things, the establishment fails to take corrective actions. In determining whether the HACCP plan is inadequate, the Agency will consider whether: 1) some or all products produced under the same or a substantially similar HACCP plan are affected, 2) there have been other incidents of product contamination with the pathogen, 3) if corrective actions have been effective, and 4) if incidents of product contamination have been persistent or recurring. FSIS will review all of this information and consider the entire situation before making a determination of HACCP plan inadequacy.

Question: Can an establishment use the results of FSIS verification sampling instead of taking their own product or FCS sample if an FSIS sample is taken at the time the company is scheduled to take their own sample?

Answer: Yes, if FSIS verification sampling occurs within the same time frame as that defined in the establishment's *Listeria* Control Program, and the same types of samples are collected. For example, if an establishment samples its product once a quarter as part of the verification activities in their HACCP plan and FSIS takes a product sample in that same quarter, then the company can use the FSIS results as part of the verification for their HACCP plan. Likewise, if an establishment samples FCSs once a month, and FSIS samples FCSs during that month, the establishment can use the results from the FCS sampling as part of their own project.

However, establishments **may not** use the results of FSIS product samples in lieu of taking their own FCS samples, because the sample types are different, and the FCSs samples are used to verify the sanitation in the establishment's environment.

Appendix 3.2: FSIS Sampling Procedure**I. Sampling Using SpongeSicles® For Food Contact and Non-Food Contact Surface Sampling**

Equipment needed:

Sterile gloves

SpongeSicles®

10 ml tubes of Dey Engley or other neutralizing broth

Marker to label the sample bag

1. Wash and sanitize hands to the mid-forearm.
2. Using ungloved hands open the bag containing the SpongeSicle® by pulling off the clear perforated strip at the top of the bag;
3. Pull apart the white tabs to open the mouth of the bag;
4. Aseptically pour 9-10 ml of sterile Dey-Engley (D/E) broth into the bag to hydrate the SpongeSicle®, being careful not to contaminate the broth or sponge during the transfer. If the D/E broth is not purple, discard the tube;
5. Press the mouth of the bag back together;
6. Evenly moisten the SpongeSicle® by using hand pressure on the outside of the bag to massage the sponge;
7. Position the SpongeSicle® so that the handle is sticking out of the bag. Press the top of the bag back together around the handle;
8. Through the bag, squeeze the excess broth gently out of the sponge. Do not let your hand go past the thumb stop on the handle;
9. Aseptically place a sterile glove on the hand used for swabbing by:
 - a. Positioning the glove package so that the L and R (L=left, R=right) are facing the sample collector. When the package is open, the gloves are folded, forming a cuff on the sleeve and lying palm up. Leave them in the package until ready for use;
 - b. Holding the glove for the hand that will be used for swabbing by the inside cuff area. Inserting hand into the glove, palm side up, and lifting the glove from the package.
 - c. Pulling the glove completely on, touching only the fold cuff with your ungloved hand. Do not touch the sterile outside surface of the glove with your ungloved hand. Unroll the fold of the glove. Do not touch any non-sterile surface (clothes, counter tops, or the outside of the bag containing the SpongeSicle®) with the sterile glove. The other hand can be left ungloved for the manipulation of non-sterile surfaces and materials.
10. Using the gloved hand, carefully take the SpongeSicle® out of the bag by grasping the handle and swab the area selected. Be careful to maintain sanitary conditions when

sampling and collect the samples aseptically. Do not let your hand go past the thumb stop on the handle

11. Swab at least a 1' X 1' square of food contact or environmental surface area, if possible;
12. Swab the chosen area using firm and even pressure:
 - a. Vertically (approximately 10 times); then
 - b. Flip the sponge and use the other side to swab horizontally (approximately 10 times); then
 - c. Swab diagonally, using the same surface side as you used for horizontal (approximately 10 times).
13. Open the bag using the ungloved hand, and insert the sponge portion of the SpongeSicle[®] back into the bag;
14. Grip the SpongeSicle[®] through the bag and bend the handle of the SpongeSicle[®] back and forth with slight force, while gripping the sponge through the bag. The stick should break easily within the sponge (do not break the handle at the thumb stop). Discard the broken handle. If the handle is sticking out above the sponge, discard the sample. Take a new sample following steps 2-13.
15. Squeeze as much air out of the bag as possible and fold the top of the bag down at least 3 times. Fold in the tabs to lock the fold in place;
16. Label the bag with the date and location of the sample.
17. Ship the sample or deliver it to the laboratory as soon as possible for analysis.

II. Liquid Sampling for Brine

Equipment needed:

Sterile gloves

500 ml sterile pitcher or other sample collection device

1000 ml sterile bottle

90 ml D/E broth

Marker to label the sample

1. Wash and sanitize hands to the mid forearm. Wear sterile gloves on both hands when collecting a sample;
2. Aseptically pull a 500 ml sterile pitcher (beaker with a handle) from its packaging, being careful not to let the pitcher touch any non-sterile surface, including the exterior of the packaging;
3. Open a collection bottle and with the pitcher aseptically transfer 500 ml of the chill water or brine using the gradations on the side of the collection bottle to ensure the proper volume;

4. Aseptically add 90 ml of D/E to each sample collected to neutralize chlorine and other disinfectants;
5. Tightly cap the collection bottle and gently mix by rotating back and forth;
6. Label the bottle with the date and sample location
7. Send or deliver to the laboratory as soon as possible.

Appendix 3.3 Sample Collection and Testing Methods

According to the *Listeria* Rule, establishments in all three alternatives may use verification testing to verify the effectiveness of their sanitation programs (9 CFR 430.4(c)(1)). Using proper sample collection technique is important to ensure that samples provide the best measure of sanitary conditions at the establishment. **It is also important that results are accurate and reliable so they can be used to support the decision made in the hazard analysis that *Lm* is not reasonably likely to occur in the product.**

Sample Collection Methods

As part of its *Listeria* Control Program, the establishment should provide written instructions for collecting FCS samples, and product and NFCS samples (if performed). The sampling procedure used by FSIS to sample FCSs, NFCSs, and brines during IVT or RLm sampling is provided in [Appendix 3.2](#). Establishments may use this method to sample their FCSs, or adjust the method based on the needs of the establishment. Some establishments use sentinel site programs to collect samples of FCS, NFCS, and product. An example can be requested at the following link: <http://www.tysonfoods.com/Safe-Food/Sentinel-Site-Program.aspx>

The box on in Section 3.5 describes FSIS recommendations for establishment sampling methods. As stated in the box, establishments may choose to composite food contact surface and environmental samples to save laboratory resources. Although compositing food contact surface samples is permissible, establishments should be aware that some loss of information occurs when the samples are composited. For example, if the sample tests positive, the establishment will no longer know what particular site tested positive and would be expected to take corrective actions in all of the sites represented by the composited sample. Therefore, FSIS recommends that establishments collect samples from like areas (e.g., drains in a processing room) in one set of composited samples. Likewise, all of the samples in a composited group could be from the same production line. In that case, a positive result would indicate contamination in a particular area, and establishments could tailor their corrective actions accordingly.

Laboratory Methods

Laboratory methods should be fit for the intended purpose, meaning that the test should effectively detect low levels of potentially injured *Lm* or indicator organisms on food contact or environmental surfaces, including brines, if appropriate. Testing can be performed either in-house or by a third party laboratory, but the methods used should be reliable and accurate. In either case, it is important that the testing protocol be validated for the purpose, that the procedure is carefully followed (including time and temperature of enrichment and incubation steps), and fresh (non expired) media and testing kits be used. **If a third-party laboratory is used, the establishment should be familiar with the method used by the laboratory, have the method on file at the establishment, and know whether it meets FSIS expectations for testing methods.** FSIS will ultimately hold the establishment responsible for any 3rd party laboratory results; therefore, if an establishment is unsure whether a testing methodology meets FSIS expectations, it can submit a question through [AskFSIS](#). Guidance for laboratory methods can be found in [Section 3.5](#). Information on selecting a third-party lab can be found in the [FSIS Establishment Guidance for the Selection of a Commercial or Private Microbiological Testing Laboratory](#).

NOTE: In house labs or third-party labs can be used to analyze the samples, but the sampling methods should be reliable and accurate.

Testing for *Lm*, *Listeria* spp., or *Listeria*-like organisms

Establishments may choose to test for *Lm*, *Listeria* species (*Listeria* spp.), or *Listeria*-like organisms (LLO). While *Listeria* spp. and LLO are appropriate indicators for *Lm*, most establishments choose to test for *Listeria* spp., because it is more closely related to *Lm*.

Establishments that test for LLO should take the same actions specified in this guideline for *Listeria* spp. In many cases, laboratory tests for *Listeria* spp. are the same initial tests that are used to screen for *Lm*.

NOTE: If an establishment testing FCS for *Listeria* spp. or LLO receives a positive test result, **there is no requirement that the positives are confirmed for *Lm***. However, establishments are required to take corrective actions according to their Alternative.

- Tests for *Listeria* spp. include immunoassays (e.g., lateral flow immunoassays, enzyme linked assay) and nucleic acid based assays (e.g., polymerase chain reaction (PCR), reverse-transcriptase PCR, DNA hybridization).
- LLO tests usually employ traditional *Listeria* culture enrichment and isolation media to screen for bacteria that have biochemical characteristics typical for but not necessarily exclusive to *Listeria* spp. Many LLO methods are based on the ability of *Listeria* species to hydrolyze esculin or other compounds, resulting in a color change to the broth or solid media (usually to dark brown or black).
- If the establishment tests for Aerobic Plate Count (APC), Total Plate Counts (TPC), Total Viable Count (TVC) or bioluminescence-based testing for organic contamination as an indicator for sanitation, they may use the results to indicate where increased *Listeria* testing may be needed. However, these tests cannot be used to meet sampling requirements for *Lm*, *Listeria* spp. or LLO. For more information on use of these tests to verify sanitation see Appendix 2.2.

Compositing Samples and Pooling Enrichments

A composited sample is defined as combining sponges from multiple sample sites into a single enrichment media for outgrowth prior to analysis. This practice saves on the cost of media and analytic testing supplies. FSIS recommendations for compositing samples can be found on page 95. Pooling enrichments is when individual sampling sponges (or other sampling devices) are enriched separately and are combined into a single pool after incubation for analysis. Pooling can save on the cost of analytic testing supplies. However both of these practices can reduce the establishment's ability to quickly identify the source of a *Listeria* positive result.

Pooling enrichments can make it difficult to isolate the source of the positive from the original enrichments. Typically if the result from an analysis of pooled enrichments is positive, the original enrichments are tested to identify the source of the *Listeria*. **FSIS does not recommend going back to the original enrichments after a positive result in a pooled sample unless the follow criteria are met:**

- 1) The enrichment times and temperatures are sufficient for the outgrowth of *Listeria* to levels that would ensure that pooling does not dilute the single sample to levels below the limit of detection of the test. Additional validation of the test method may be required to support this.
- 2) The time between taking the original enrichment for pooling and taking the individual enrichments for 'isolation' is within validated test kit specifications or can be validated not to significantly affect the detection of the analyte.
- 3) The enrichments have been handled appropriately during the intervening period (e.g. refrigerated and kept sterile), or a validated re-enrichment procedure is used for subsequent analyses. Re-enrichment is defined as the transfer of an older enrichment to a new enrichment broth with subsequent incubation to ensure sufficient levels of bacteria are present for analysis. Test kit manufacturers may supply guidance on this issue otherwise, a validation study may be needed to demonstrate the effectiveness of the re-enrichment procedure.

If the screening of the original enrichments returns negative results for all samples, then all the original samples should be considered positive since the original positive result cannot be localized to a single enrichment. Additionally the establishment may want to review the SOP for handling enrichments that may have contributed to a lack of a positive result.

Similarly, as described in Section 3.5, if a composited sample tests positive, the establishment should consider all sites represented by the sample as positive. It would not be appropriate to retest individual sites and consider those sites negative, unless the retesting is being performed as a separate, follow-up sampling activity to evaluate the effectiveness of corrective actions.

If these criteria are met, then FSIS recommends that no more than 5 enrichments be pooled, and that enrichments from like or similar surfaces are pooled (e.g., cutting board samples with cutting board samples). The individual locations for the pooled sample should be selected and documented to assist in determining the site of contamination to facilitate follow-up testing.

Confirmation Methods

As stated previously, establishments are **not** required to confirm samples that are positive for *Listeria* spp. or LLO. However, if they do choose to confirm the samples, the establishment should follow the recommendations below:

- 1) Culture-based Confirmation

Cultural methodology involves enrichment in one or more culture broths, subsequent isolation of a pure culture on solid media, and finally confirmation of culture identity through multiple interdependent and sequential biochemical and genetic *tests*. **The cultural method should always be performed on the same sample and enrichment broth as the screening test.** Common appropriate enrichment-based culture isolation and confirmation methods include the FSIS [Microbiology Laboratory Guidebook](#) (MLG) Chapter 8 methods, the FDA BAM culture method and ISO 11290-1. Non-enrichment-based “direct plating” methods intended for detection of higher levels of Lm, including ISO 11290-2, are not appropriate for detecting low levels of Lm contamination. The cultural method should detect

the same group of organisms as the FSIS MLG method. The laboratory procedure should indicate the specific steps taken to **confirm** the presence of the target microorganism.

2) Non-Culture-based Confirmation

Non-cultural methodology does not involve a cultural isolation step, and consists of a single test (e.g., a PCR-based test). **This type of confirmatory test is always performed on the same sample and enrichment broth as the screening test.** The non-cultural test should identify a different set of characteristics than the screening test (in other words, the same test used for screening, or a similar test, may not be re-used to "confirm" the screening result). The non-cultural confirmation test should provide high sensitivity and enhanced specificity (ability to detect true negative results) compared to the screening test and it should be demonstrated and documented to perform acceptably under the conditions of use, which includes the enrichment conditions for the screening test (e.g., enrichment time, temperature, enrichment broth). Acceptable performance is determined by validation, preferably through an independent organization (e.g., the Association of Analytical Chemists (AOAC), Association Française de Normalization (AFNOR), ISO, or NordVal).

Recording Testing Results

Establishments are expected to maintain records of FCS sampling results and other sampling they may perform (product and NFCS) testing. According to the *Listeria* Rule, establishments must make the verification results that demonstrate the effectiveness of the measures it employs, whether under its HACCP plan, Sanitation SOP, or other prerequisite program, available on request to FSIS (9 CFR 430(c)(7)).

The records should include the following:

- 1) Sample collection and analysis date,
- 2) Testing result (positive or negative),
- 3) Analysis that was performed (*Lm*, *Listeria* spp., or LLO),
- 4) Testing method (AOAC number or method name),
- 5) Technician or laboratory who performed the analysis,
- 6) Sampling site or product type analyzed.

Records can be in electronic or paper format and should be maintained as described in 9 CFR 417.5.

NOTE: It would not be appropriate for an establishment to rely on an "inconclusive" or "incomplete" laboratory result. If possible, further analysis should be performed on the original sample or enrichment to determine the testing result. In the absence of further information, the establishment should consider the sample to be positive.

Use of Pulsed Field Gel Electrophoresis (PFGE) Data by FSIS

When a sample collected by FSIS tests positive for *Lm*, the isolate is analyzed using [Pulsed Field Gel Electrophoresis \(PFGE\)](#). FSIS plans to start providing PFGE data to establishments on a routine basis, so that they can determine if harborage or cross contamination is occurring in the environment or if there are matches to clinical isolates (see below). PFGE is a laboratory method used for subtyping bacterial isolates below the level of species using bacterial deoxyribonucleic acid (DNA). PFGE patterns consist of DNA fragments of varying sizes resolved by passage through an agarose gel. PFGE patterns are compared to determine their

degree of relatedness. Establishments that test for *Lm* may consider using PFGE to analyze their own testing data to determine whether harborage or cross contamination is occurring in their environment.

Electronic images of PFGE patterns from FSIS and other public health organizations like the Food and Drug Administration (FDA) are uploaded to a central database (PulseNet database) maintained by the Centers for Disease Control and Prevention (CDC), where database managers evaluate and assign IDs to uploaded patterns. FSIS compares the pattern to others from the same establishment (plant comparison), to recently uploaded patterns from listeriosis cases (hotlist comparison), and to all PFGE patterns uploaded to PulseNet (pattern comparison). Because PFGE can't detect small changes in DNA, investigators focus on patterns that are indistinguishable or closely similar (1 or 2 band difference). Isolates with indistinguishable or closely similar PFGE patterns may have shared a recent ancestor and may have originated from a common source, such as a contaminated food product. PFGE data is used to supplement information gathered from other sources (epidemiological investigation, observations at an establishment) and should not be used by itself to demonstrate a definitive link between the product and the illness during outbreak investigations.

Lm PFGE pattern data can be interpreted in the following way:

1. Cross contamination is suggested if an identical or highly similar PFGE pattern is found in product and surface samples collected during the same production day. If an identical pattern is found on product and a surface, the surface is more likely to be the source, unless under-processing of RTE product is suspected.
2. Harborage or ongoing contamination of the post-lethality environment is suggested if an identical or highly similar pattern is found in product and surface samples collected over multiple days, weeks, or months.
3. Food-borne exposure is suggested if the identical PFGE pattern is found in FSIS and case-patient samples, especially if the pattern is rare.

Information associated with samples with indistinguishable PFGE patterns is reviewed by the FSIS Office of Public Health and Science (OPHS) staff, and may be shared with Agency staff conducting establishment-based investigations (IVT or FSA) and food-borne illnesses investigations. The PFGE data is used to supplement concurrent investigations and does not alter the regulatory implications of microbiological test results.

Chapter 4

FSIS *Listeria* Guideline: Enhanced Sampling Program

4.1 [Follow-up Sampling](#)

[Table 4.1: Timeframe for Follow-up Sampling, Intensified Sampling, and Hold and Test Performed in Response to Positive Food Contact Surface Results](#)

4.2 [Intensified Sampling](#)

4.3 [Hold and Test](#)

4.4 [Reprocessing *Lm* Contaminated Product](#)

4.5 [Determining *Listeria* Trends](#)

4.6 [Glossary](#)

4.7 [References](#)

Appendices

4.1 [Sampling Scenarios by Alternative](#)

4.2 [Hold and Test Scenario](#)

4.3 [Listeria Trends Examples](#)

4.4 [Findings from Food Safety Assessments \(FSAs\)](#)

This chapter provides information on developing an **[Enhanced Sampling Program](#)** as part of the *Listeria* Control Program. The Enhanced Sampling Program includes **[follow-up](#)** and **[intensified sampling](#)** performed in response to a food-contact surface (FCS) result from the routine-sampling program. Sections on developing **[Hold and Test](#)** Programs and determining **[Listeria trends](#)** are also included in this chapter.

4.1 Follow-up Sampling

According to the *Listeria* Rule, establishments in Alt. 3 (deli and hotdog producers) are required to conduct follow-up testing (sampling) in response to FCS positive sampling results (9 CFR 430.4(b)(3)(ii)(A)). If follow-up testing (sampling) yields a second FCS positive result, then products must be held and tested using a sampling plan that will ensure that products are not adulterated with *Lm* before they are released into commerce (9 CFR 430.4(b)(3)(ii)(B)).

In response to a positive FCS result, establishments in Alt. 1, 2, and 3 (non-deli or hotdog producers) are required to perform **[corrective actions](#)** (9 CFR 416.15(a) and (b) and 417.3(a) and (b)). FSIS recommends that they also perform follow-up sampling in response to a positive FCS result. By making efforts to find and address the source of contamination in the environment, establishments can take proactive steps to avoid *Lm* contamination of products. [Appendix 4.1](#)

Question: An establishment produces hotdog and deli products using Alt.3 and has 3 production lines in the post-lethality processing area. The establishment receives a positive result for *Lm* or indicator organism on line 1 FCS. Does the establishment need to sample FCSs only from line 1 or from all the 3 lines for the follow-up testing?

Answer: The follow-up sampling is verification that the corrective actions taken by the establishment are effective. If the establishment can support that line 1 is using equipment, personnel and processing area that is separate and independent of the other lines (i.e., not used by other lines) and has supporting documentation that there is no history of cross-contamination among the three lines, then follow-up testing and corrective actions should be conducted on line 1.

provides step-by-step guidance for sampling in each Alternative. The establishment’s follow-up testing program can be included as part of its *Listeria* Control Program in its Enhanced Sampling Program.

In the *Listeria* Control Program, the establishment should specify the number of samples it will collect during follow-up sampling. FSIS recommends that **3-5 samples** are collected from the site of the original FCS positive and the surrounding area. According to the *Listeria* Rule, establishments in Alt.3 deli and hotdog producers must conduct **follow-up sampling that includes the specific FCS site that tested positive, as well as such additional tests in the FCS area as are necessary to ensure the effectiveness of the corrective actions.** These may include other FCSs that are upstream from the original positive. It would be useful for the establishment to record the rationale for selecting follow-up sampling sites. For example, if a slicer tests positive, the establishment may choose to sample the conveyor or other equipment leading up to the slicer. Follow-up sampling could also include other FCSs on the same piece of equipment that were not previously tested (e.g., slicer blade or plate) or employees’ gloves that come in contact with the product as it is placed on the slicer.

The establishment should also include a brief description of corrective and preventive actions that will be taken in response to positive results (details can be included in the Sanitation SOP) and response to positive results (next steps). As stated previously, establishments in Alt. 3 (deli and hotdog producers) are required to hold and test product in response to a second positive test (obtained during follow-up testing) for *Lm* or an indicator organism. After the 2nd consecutive positive, the establishment should also enter into intensified sampling mode to find the source of positives (see [Section 4.2](#)). It is also recommended that establishments in the other alternatives enter into intensified sampling mode after the 2nd positive (although hold and test is not required at this point). Recommendations for follow-up testing, intensified testing, and hold and test are provided in Table 4.1. Sampling scenarios by alternative can also be found in [Appendix 4.1](#).

Table 4.1 Timeframe for Follow-up Sampling, Intensified Sampling, and Hold and Test Performed in Response to Positive Food Contact Surface Results

Alternative	After the 1 st positive	After the 2 nd positive	After the 3 rd Positive	After Multiple Positives
Alternative 1	Follow-up sampling	Intensified sampling		Hold and test recommended
Alternative 2 , Choice 1 (2a)	Follow-up sampling	Intensified sampling		Hold and test recommended
Alternative 2, Choice 2 (2b)	Follow-up sampling	Intensified sampling	Hold and test required* (recommended after 3 rd positive)	
Alternative 3	Follow-up sampling	Intensified sampling	Hold and test required* (recommended after 3 rd positive)	
Alternative 3 (deli or hotdog)	Follow-up sampling required	Intensified sampling Hold and test required after 2 nd positive.		

*Establishments in Alt. 2b and 3 (non-deli or hotdog producers) are **required** to identify when they will hold and test product. FSIS recommends that they do so after the 3rd consecutive

positive. Establishments in Alt 3 (deli and hotdog producers) are **required** to hold and test product after the 2nd consecutive positive.

4.2 Intensified Sampling

FSIS recommends that all establishments enter into **intensified sampling mode** after a 2nd FCS positive. Intensified sampling mode includes:

- Intensified samples collected from FCSs, indirect and NFCSs, and product, and
- Escalated intensified cleaning and sanitation (details included in the establishment's Sanitation SOP).

Intensified sampling may include the collection of FCS, NFCS, and product samples, and is performed to find sources of harborage and [cross contamination](#) in the post-lethality processing environment. [Harborage](#) is defined as the persistence of *Lm* in the establishment over time. Once a harborage point is formed, *Lm* may transfer through cross contamination onto FCSs or the product. Examples of conditions that may lead to cross contamination include condensation dripping onto product or FCSs, aerosolization from the drains, splashing from the floors, or product brushing against doors, walls, or pallets. For more examples of cross contamination and harborage, see [Appendix 2.2](#)).

Procedures for intensified sampling can be included in the establishment's *Listeria* Control Program. During intensified sampling, at least 3-5 samples should be collected per site that was found positive during follow-up sampling. Efforts should also be taken to find and address sources of harborage, track cross contamination in the establishment, and to find and address *Listeria* trends (for more information on *Listeria* trends, see [Section 4.5](#)).

Intensified sanitation efforts should be used in conjunction with intensified sampling after the 1st positive result to address sources of contamination. Intensified sanitation includes sanitation measures that are performed in addition to normal sanitation procedures and are escalated in response to continuing findings of positives. Intensified sanitation can include increasing the frequency of cleaning and sanitizing for certain pieces of equipment, breaking down the equipment into its parts for further cleaning, repairing or replacing broken equipment, and construction, if needed. For more descriptions of intensified sanitation, see [Appendix 2.2](#).

As part of its *Listeria* Control Program, the establishment should also include a response to positive results found during intensified testing. **The finding of three consecutive positive samples for *Listeria* spp. from the same sampling site indicates a serious contamination issue, and increases the risk that product could be contaminated with *Lm*.** The establishment should be taking preventive steps such as:

- Increasing its routine sampling for *Listeria*,
- Collecting intensified samples to find sources of harborage and cross contamination,
- Holding and testing product (Alt. 2b and 3 non-deli or hotdog producers),
- Reassessing its **Sanitation SOPs** to determine if sanitation issues could be leading to positive results,
- Assessing the effectiveness of its PLT or AMAPs to address the increased likelihood of positives,

- Determining whether *Listeria* trends exist (see [Section 4.5](#)), and
- Reassessing its **HACCP plan**,⁸ to determine if the actions it is taking are effective in controlling *Listeria*.

NOTE: The finding of three consecutive positive samples from the same sampling site indicates a serious contamination issue, and increases the risk that product could be contaminated with *Lm*.

4.3 Hold and Test

According to the *Listeria* Rule, establishments in Alt. 3 (deli and hotdog producers) are required to hold product after a 2nd consecutive food contact surface positive for *Lm* or an indicator organism until the establishment corrects the problem indicated by the test result (9 CFR 430.4(b)(3)(ii)(B)). Further, in order to release product into commerce, the establishment must sample and test the lots of product using a method that will provide a level of statistical confidence that the product is not adulterated (for more information see International Commission on Microbiological Specifications for Foods (ICMSF) Sampling Plans for *Lm* below). Alternatively, the establishment may rework or condemn the product (9 CFR 430.4(b)(3)(ii)(C)). Establishments in Alt. 3 (non-deli or hotdog producers) and Alt. 2b are required to identify when they will hold and test product ((9 CFR 430.4(b)(2)(iii)(B) and (3)(i)(B)) and FSIS recommends that they do so after the **3rd positive** (see [Table 4.1](#)). As stated in the note above, the finding of three consecutive positive samples increases the risk that the product could be contaminated with *Lm*. If the establishment does not hold and test the product after the 3rd positive, it should provide other support demonstrating that the product is not likely to be contaminated. In addition, FSIS recommends that establishments in Alt. 1 and 2a hold and test product after multiple positives for *Lm* or an indicator organism.

Establishments can include their hold and test procedures in their *Listeria* Control Program. Products can be tested for either *Lm* or *Listeria* spp.; however, if a product tests positive for *Listeria* spp. an establishment may be asked to provide further evidence (such as confirmatory testing results) to demonstrate that the product is not contaminated with *Lm* (see Section 3.6).

Establishments should hold the entire product lot (and subsequent day's lots) until control is regained. For more information on defining product lots, see [Section 3.5](#). Control is considered regained after 3 consecutive days of negative FCS results are obtained, and all other NFCS and product samples are negative. **If product tests positive for *Lm* during hold and test (see [Appendix 4.2](#)), then the product lot represented by the sample is considered adulterated.**

NOTE: Control is regained after **3 consecutive days of negative** FCS results are obtained, demonstrating that corrective actions are sufficient to address the contamination issue.

A hold and test scenario is provided in [Appendix 4.2](#) that provides a day-by-day description of hold and test procedures. Establishments should also describe product disposition in response to positives (procedures for reworked or condemning the product).

Hold and test can only be used as a means to release product in situations where an FCS tests positive for *Listeria* spp. If FCSs or product tests positive for *Lm* the product is

⁸ Reassessment of the SSOP or HACCP plan is required in response to an FSIS *Lm* positive according to 9 CFR 417.3(b) and 416.15 (b) (see the Q&As in Appendix 3.1 for more information).

considered adulterated. In that case, testing product would not be an appropriate means of determining product safety, because even the best-designed testing program cannot detect all *Lm* that may be present. Therefore, product testing cannot be used as a means for the establishment to release adulterated product into the marketplace.

NOTE: If a FCS or product tests positive for *Lm*, the product is considered adulterated. Product testing can't be used as a means to demonstrate that the product is safe. The product must be reworked or condemned, and FSIS would typically request that establishments recall such products if they have been released into the marketplace.

International Commission on Microbiological Specifications for Foods (ICMSF) Sampling Plans for *Lm*

According to the *Listeria* Rule, establishments in Alt. 3 producing deli or hotdog products must sample and test lots for *Lm* or an indicator organism using a sampling method and frequency that will provide a statistical level of confidence that ensures that each lot is not adulterated with *Lm* (9 CFR 430.4(b)(3)(ii)(C)). In order to meet this requirement, FSIS recommends that establishments use the International Commission on Microbiological Specifications for Foods (ICMSF) Tables. Additionally, FSIS recommends that establishments in other alternatives use these tables if they hold and test product.

ICMSF categorizes microbial hazards according to risk:

- 1) Moderate
- 2) Serious
- 3) Severe

NOTE: ICMSF ranks *Lm* as either a serious hazard in foods for the general population or a severe hazard in foods for restricted populations (high risk groups e.g., hospital and nursing home patients).

ICMSF describes 15 different cases of sampling plans, with sampling plan stringency based on degree of risk and the effect on risk of the conditions of use. Cases 10, 11, and 12 would apply to the serious category and cases 13, 14, or 15 would apply to the severe category of microbial hazards. ICMSF considers cases 13, 14, and 15 to apply to foods intended specifically for highly susceptible individuals (e.g., patients in hospitals and nursing homes) because a large proportion of the individuals would be potentially susceptible to foodborne illness; thus, increasing the stringency of the sampling plans is appropriate. **FSIS also expects establishments that produce product for the school lunch program to use cases intended specifically for high-risk populations, because of the potential for increased risk in that population.**

Question: Should the ICMSF sampling plan be used for regular sampling or only for hold and test sampling?

Answer: For routine sampling, the establishment can use whatever sampling plan it justifies as appropriate to demonstrate that the product is safe. For hold and test sampling for *Lm*, a statistically-based sampling plan should be used. The ICMSF table provides examples of statistically-based sampling plans that are commonly used for demonstrating lot acceptance.

For cases 10 or 13, conditions of use reduce risk (e.g., the numbers of *Lm* will decrease). For cases 11 and 14, conditions cause no change in the hazard (e.g., the organism cannot grow), and for cases 12 and 15, conditions may increase the risk (e.g., foods in which *Lm* can grow are subjected to conditions that allow growth). Sampling plans for the cases are given in the table below, where n is the number of samples and c=0 means that none of the “n” samples can be positive for *Lm*. The table also provides the sampling plan performance, assuming a log-normal distribution with a standard deviation of 0.8; lots having the calculated mean concentrations or greater will be rejected with at least 95% confidence. Each of these plans achieves assurance that *Lm* is present at <1 CFU in the sample size. FSIS recommends analyzing a 25 g sample. **If the risk of the population is unknown, FSIS recommends that establishments use cases 13-15.**

NOTE: Product samples should be analyzed separately and not composited. However, if compositing is to be done, composites of 25-g portions should not exceed a total of 125 g in order to maintain the sensitivity of the method of analysis, and a validated method should be used.

Conditions reduce concern	Conditions cause no change in concern	Conditions increase concern
Case 10 n=5, c=0 Mean Concentration 1 cfu/32g	Case 11 n=10, c=0 Mean Concentration 1 cfu/83g	Case 12 n=20, c=0 Mean Concentration 1 cfu/185g
Case 13 n=15, c=0 Mean Concentration 1 cfu/135g	Case 14 n=30, c=0 Mean Concentration 1 cfu/278g	Case 15 n=60, c=0 Mean Concentration 1 cfu/526g

When RTE products must be sampled (hold and test) under the *Listeria* Rule, the number of samples (randomly selected) would be as specified for these cases based on the risk of the product and the intended consumers. Since deli and hotdog products are ranked as the top causes of foodborne illness, the establishment producing these products should select these products to be sampled first. Sampling starts after the establishment has conducted corrective actions that are specifically designed to find the most likely cause of the contamination and controls are put in place to prevent recurrence.

Case 10 n=5, c=0	Case 11 n=10, c=0	Case 12 n=20, c=0
Products with continued decline in population due to antimicrobial or other formulation considerations such as pH and A_w . Products in Alternative 1	Products that limit growth (< 1 log) due to antimicrobial or other formulation considerations such as pH and A_w . Products in Alternative 2	Products that support growth and that will be stored refrigerated for an extended period of time. Products in Alternative 3
Case 13 n=15, c=0	Case 14 n=30, c=0	Case 15 n=60, c=0

As for case 10, but where products are produced for a hospital or nursing home or for another higher risk population	As for case 11, but where products are produced for a hospital or nursing home or for another higher risk population	As for case 12, but where products are produced for a hospital or nursing home or for another higher risk population
Products in Alternative 1 intended for a hospital, nursing home or for another higher risk population	Products in Alternative 2 intended for a hospital, nursing home or for another higher risk population	Products in Alternative 3 intended for a hospital, nursing home or for another higher risk population.

The number of samples recommended should be collected in one day and all affected products should be held during the testing period. Testing can be for *Listeria* spp. or *Lm*. Any positive results from this follow-up testing (using the ICMSF approach) should lead to more significant investigations of the cause of the contamination. 3. Products that test positive for *Listeria* spp. may be considered adulterated if the establishment cannot support that the product is not adulterated, or if insanitary conditions exist, see Section 3.6. The establishment should conduct rigorous corrective and preventive actions and other sanitation activities.

Establishments may send a letter or certification when they ship tested products to nursing homes, hospital, and other institutions with susceptible populations. Such a letter should indicate that product has been sampled and tested according to ICMSF recommendations. Establishments supplying nursing homes, hospitals and other institutions with susceptible populations are expected to implement whatever additional controls and verification procedures are necessary to ensure that product is not adulterated.

4.4 Reprocessing *Lm* Contaminated Product

Product that tests positive for *Lm* or an indicator organism, or passes over an FCS that tests positive for *Lm*, or is suspected to be positive because of sanitation or processing issues at the establishment, may be reprocessed. A process that has been validated to achieve at least a 5-log *Lm* reduction would be accepted by FSIS to reprocess the product. In order to reprocess the product, the establishment may use a processing treatment such as re-cooking and re-cooling the product (see below), applying a PLT (as described in [Section 2.1](#)), or other supportable process. An example of a PLT which has been found to achieve a 5-log *Lm* reduction is HPP. If an establishment chooses to use HPP to reprocess *Lm*-positive product, then the establishment should have scientific support that demonstrates that the process achieves at least a 5-log reduction of *Lm* in their particular product (see [Appendix 2.1](#) for more information on validation).

NOTE: FSIS will consider PLTs achieving at least a 5-log reduction of *Lm* sufficient for reprocessing contaminated product.

In addition, establishments may use both [Appendix A](#) and [Appendix B](#) of the final rule, “[Performance Standards for the Production of Certain Meat and Poultry Products](#)” [FSIS Guidance on Safe Cooking of Non-Intact Meat Chops, Roasts, and Steaks](#) and the [Time-Temperature Tables for Cooking Ready-to-Eat Poultry Products](#), or other supportable processes to reprocess *Lm*-positive product. When using these guidance documents, establishments should ensure that adequate humidity is maintained during heating according to Appendix A and that *C. perfringens* and *C. botulinum* growth is controlled according to Appendix B, or other

scientific support. Although Appendix A and B, the FSIS Guidance on Safe Cooking of Non-intact Meat Chops, Roasts, and Steaks, and the Time-Temperature Tables for Cooking Ready-to-Eat Poultry Products, are designed to achieve reductions in *Salmonella*, establishments are not expected to validate that these processes also achieve reductions in *Lm* because *Salmonella* is considered an indicator of lethality for *Lm* (see [Appendix 2.1](#)).

4.5 Determining *Listeria* Trends

As described previously, establishments are expected to take corrective and preventive actions in response to positives based on their alternative. One way that establishments can ensure that their corrective actions are effective is to track sampling results. Repeated *Listeria* spp. positives on FCSs, NFCSs, or product indicate positive ***Listeria* trends** in the establishment. The finding of *Listeria* trends could indicate that the establishment's *Listeria* Control Program is not effective in controlling the presence of *Lm* in the establishment's post-lethality processing environment. In response to a finding of *Listeria* trends, the establishment should perform intensified testing and sanitation, and conduct a comprehensive investigation to determine the source and the cause of the contamination (the steps in a comprehensive investigation can be found below the section on identifying and addressing *Listeria* trends). One way to track and address *Listeria* trends is through a sentinel site program.

NOTE: Repeated *Listeria* spp. positives on FCS, NFCS, or product (***Listeria* trends**) could indicate that the establishment's *Listeria* Control Program is not effective in controlling the presence of *Lm* in the establishment's processing environment.

Identifying and Addressing *Listeria* Trends

Establishments should track their sampling results over time, to identify *Listeria* trends. *Listeria* trends can consist of increases in positive samples over a particular time period (e.g., weekly, biweekly, monthly, quarterly, or 6 months) or increases in positives in particular sites or areas (see [Appendix 4.3](#) for specific examples). By tracking their percent positive sampling results, establishments can determine if the percentage of positives in the establishments is increasing, indicating that changes in their cleaning protocols or sanitation procedures should be made.

Listeria trends may also exist if positives are seen in a particular area over time. In the example provided in the tracking sheet in [Appendix 4.3](#), positives were found on a freezer fan, wall, floor, and conveyor belt over a six month period. Although the establishment addressed each individual positive by routine cleaning and sanitizing (and the sampling site subsequently tested negative), positives still continued to occur in other areas of the freezer. The *Listeria* trend was not addressed until cleaning and sanitizing were escalated and repairs made to the freezer. Although every finding of *Listeria* trends may not require extreme steps such as equipment repairs or replacement, it is important for establishments to track their results in order to address harborage points. For more information on cleaning and sanitizing steps that can be taken to address positive results, see [Appendix 2.2](#).

Positive product results for either *Listeria* spp. or *Lm* over time could also indicate a *Listeria* trend. FSIS uses results from its product and RLM and IVT sampling to track trends over time, by comparing pulsed-field gel electrophoresis (PFGE) patterns. These results can be used to demonstrate possible harborage and cross contamination in the establishment (see [Appendix 3.3](#)). FSIS may use this data to take regulatory action against the establishment. By

monitoring and addressing *Listeria* trends, establishments can take a proactive role in demonstrating that they have controlled contamination in their processing environment.

When *Listeria* trends are identified, establishments should take corrective actions to address the trend. Corrective actions should include **intensified sampling** (as described in [Section 4.2](#)) and **intensified sanitation**. Along with intensified sampling and sanitation, establishments should perform a **comprehensive investigation** to find the source of the problem (see explanation below). **Preventive actions**, such as increasing sanitation frequency, intensified sanitation in particular areas or equipment, repairing or replacing equipment, increasing testing frequency, and reassessing the Sanitation SOP and HACCP program, should be taken.

NOTE: Continued findings of *Lm* in an establishment's products or contact surfaces could lead to foodborne illness and regulatory action (including suspension of inspection by FSIS). Therefore, it is important to ensure that trends are addressed **before** the product becomes contaminated.

Parts of a Comprehensive Investigation

In response to findings of *Listeria* trends, establishments should conduct a comprehensive investigation into the source of positives, which includes:

- a. Review the cleaning and sanitizing procedures, including the types of cleaning agents.
- b. Review traffic control patterns, equipment layout and adherence to employee hygiene procedures.
- c. Locate possible niches that may represent harborages.
 - i. Repeated, non-consecutive positives usually indicate the presence of a niche or harborage site for *Lm*.
 - ii. Increase testing of the positive site including individual pieces of equipment to locate the source of the contamination.
 - iii. Test up stream in the production area from the initial positives to find the source of contamination
 - iv. Collect at least 3-5 samples per sampling event until negatives are found.

In conjunction with the comprehensive investigation, the establishment should take preventive actions, including examining and reviewing the HACCP plan, Sanitation SOP, or prerequisite project where the sanitation and testing projects are included. As part of this review, the establishment should evaluate these projects to determine if there are any design or execution flaws, and modify them as necessary.

4.6 Glossary

Comprehensive Investigation: An investigation performed by the establishment to address *Listeria* trends. As part of this investigation, the establishment should review cleaning and sanitizing procedures, traffic control patterns, and identify sources of harborage.

Corrective Actions: Procedures to be followed when a deviation occurs. These include actions the establishment will take to ensure that the cause of the deviation is identified and eliminated, the critical control point (CCP) will be under control after the corrective action is taken, measures to prevent recurrence are established; and no product that is injurious to health or otherwise adulterated enters commerce (9 CFR 417.3(a)).

Cross Contamination: Movement of a microorganism (e.g., *Lm*) from one site to another. Cross contamination may occur in the post-lethality processing area when *Lm* moves from a harborage area, such as a drain, onto equipment and product.

Enhanced Sampling Program: Includes follow-up and intensified sampling, performed in response to a positive FCS result from routine sampling program. Samples should be collected in addition to those collected as part of the routine sampling program.

Follow-up Sampling: Collection of a 2nd FCS sample performed in response to a 1st FCS positive result. Follow-up samples should be collected from the specific site of the original positive sample, as well as additional samples of the surrounding FCS areas as necessary to ensure the effectiveness of corrective actions (required for Alt. 3 deli and hotdog processors).

Harborage: Persistence of *Lm* in a processing establishment over time. Harborage areas are areas where bacteria may survive and multiply, and are often NFCSs that may be cleaned less frequently than FCSs.

Hold and test: Product samples that are held and tested by the establishment in response to a 2nd FCS positive result (required for Alt. 3 deli and hotdog producers), according to the *Listeria* Rule. In addition, establishments are required to hold or maintain control of RTE products that FSIS has tested for *Lm*, and RTE products that have passed over food contact surfaces that FSIS has tested for *Lm*. Establishments may move such products off-site provided they maintain control of them (e.g., through company seals).

Intensified Sampling: Sampling performed in response to a 2nd FCS positive testing result. Intensified sampling may include the collection of FCS, NFCS, and product samples, and is performed in order to find sources of harborage and cross contamination in the post-lethality processing environment.

Intensified Sanitation: Intensified sanitation includes sanitation measures that are performed in addition to normal sanitation procedures and are escalated in response to continuing findings of positives.

***Listeria* Trends:** Repetitive positive FCS, NFCS, or product samples that are not addressed by routine cleaning and sanitation. *Listeria* trends should be addressed by intensified sanitation and investigative sampling to find sources of harborage and cross contamination.

Preventive Actions: Actions taken in response to positive results to prevent further positives from occurring. These may include increased sanitation in particular areas or equipment,

increased testing frequency, and review and revision of the HACCP program and Sanitation SOPs.

4.7 References

International Commission on Microbiological Specifications for Foods (ICMSF). *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management*. Kluwer Academic/Plenum Publishers, NY. 2002).

Appendix 4.1: Sampling Scenarios by Alternative

The following sections provide steps that establishments can take, depending on their alternative, once a positive is found. For a description of requirements by alternative, see [Attachment 1.1](#).

a) Alternative 1

- i) **Recommended:** Conduct tests of food contact surfaces (FCS) for *Lm* or *Listeria* spp.) **at least twice a year**.
- ii) Sample at least a 12"x12" area for each surface, if possible.
- iii) Record the test results.
- iv) If the test results are positive for *Lm* or *Listeria* spp.:
 - (1) Take corrective action (as specified in the HACCP plan, Sanitation SOP, or prerequisite program), which should include intensified cleaning and sanitizing.
 - (2) If the FCS test is positive for *Lm*, the product is considered adulterated. If the FCS is positive for *Listeria* spp., the product is not summarily considered adulterated, but corrective actions should be taken.
 - (3) Record the corrective actions taken.
 - (4) Collect follow-up samples from the FCS and surrounding areas (**recommended**).
 - (5) Repeat corrective action and testing until samples are negative for *Lm* or *Listeria* spp.
 - (6) Initiate intensified sampling after the 2nd consecutive positive.
 - (7) If FCSs continue to test positive, hold and test product (**recommended**).
- v) If the product tests positive for *Lm*,
 - (1) Recall the product, if already shipped, and
 - (2) Destroy the product, or
 - (3) Re-work the product with a process that is destructive of *Lm*.

b) Alternative 2, choice 1 (Alt. 2a)

- i) **Recommended:** Conduct tests of FCSs for *Lm* or *Listeria* spp. **at least quarterly**.
- ii) Sample at least a 12"x12" area for each surface, if possible.
- iii) Record the test results.
- iv) If the test results are positive for *Lm* or *Listeria* spp.:
 - (1) Take corrective action (as specified in the HACCP plan, Sanitation SOP, or prerequisite program)
 - (2) If the FCS test is positive for *Lm*, the product would be considered adulterated. If the FCS is positive for *Listeria* spp., the product is not summarily considered adulterated, but corrective actions should be taken.
 - (3) Record the corrective actions taken.
 - (4) Collect follow-up samples from the FCS and surrounding areas (**recommended**).
 - (5) Repeat corrective action and testing until samples are negative for *Lm* or *Listeria* spp.
 - (6) Initiate intensified sampling after the 2nd consecutive positive.
 - (7) If FCSs continue to test positive, hold and test product (**recommended**).
- v) If the product tests positive for *Lm*,
 - (1) Recall the product, if already shipped, and
 - (2) Destroy the product, or
 - (3) Re-work the product with a process that is destructive of *Lm*.

c) Alternative 2, choice 2 (Alt. 2b)

- i) **Required:** Conduct tests of FCSs for *Lm* or *Listeria* spp. **recommended frequency: at least quarterly.**
- ii) Sample at least a 12"x12" area, if possible.
- iii) Record the test results.
- iv) If the test results are positive for *Lm* or *Listeria* spp:
 - (1) Take corrective action (as specified in the HACCP plan, Sanitation SOP, or prerequisite program).
 - (2) If the FCS test is positive for *Lm*, the product is considered adulterated. If the FCS is positive for *Listeria* spp., the product is not summarily considered adulterated, but corrective actions should be taken.
 - (3) Record the corrective actions taken.
 - (a) Collect follow-up samples from the FCS and surrounding areas **(recommended)**.
 - (4) Repeat corrective action and testing until samples are negative for *Lm* or *Listeria* spp.
 - (5) Initiate intensified sampling after the 2nd consecutive positive.
- v) Holding and testing of product is **required*** (recommended after the 3rd positive).
- vi) If the product tests positive for *Lm*,
 - (1) Recall the product, if already shipped, and
 - (2) Destroy the product, or
 - (3) Re-work the product with a process that is destructive of *Lm*.

*The establishment is **required** to identify when they will hold and test product. FSIS recommends that it hold and test product after the third consecutive positive result.

d) Alternative 3 (non-deli or hotdog products)

- i) **Required:** Conduct tests of FCS for *Lm* or *Listeria* spp. **Recommended frequency: once a month**
- ii) Sample at least a 12"x12" area for each surface, if possible.
- iii) Record the test results.
- iv) If the test results are positive for *Lm* or *Listeria* spp.:
 - (1) Take corrective action (as specified in the HACCP plan, Sanitation SOP or prerequisite program).
 - (2) If the FCS test is positive for *Lm*, the product is considered adulterated. If the FCS is positive for *Listeria* spp., the product is not summarily considered adulterated, but corrective actions should be taken.
 - (3) Record the corrective actions taken.
 - (4) Collect follow-up samples from the FCS and surrounding areas **(recommended)**.
 - (5) Repeat corrective action and testing until samples are negative for *Lm* or *Listeria* spp.
- v) Initiate intensified sampling after the 2nd consecutive positive.
- vi) Hold and test of product is **required*** (recommended after the 3rd positive).
- vii) If the product tests positive for *Lm*,
 - (1) Recall the product, if already shipped, and
 - (2) Destroy the product, or
 - (3) Re-work the product with a process that is destructive of *Lm*.

*The establishment is required to identify when they will hold and test product. FSIS recommends that it hold and test product after the **3rd consecutive positive result**.

e) Alternative 3 (deli and hotdog products)

- i) **Required:** Conduct tests of FCSs for *Lm* or *Listeria* spp. **Recommended frequency:**
 - (1) Large Establishments: **four times per month per line**
 - (2) Small Establishments: **two times per month per line**
 - (3) Very Small Establishments: **once per month per line**
- ii) Sample at least a 12"x12" area, if possible.
- iii) Record the test results.
- iv) If the test results are positive for *Lm* or *Listeria* spp.:
 - (1) Take corrective action (as specified in the HACCP plan, Sanitation SOP, or prerequisite program), which should include intensified cleaning and sanitizing. If the FCS test is positive for *Lm*, the product is considered adulterated. If the FCS is positive for *Listeria* spp., the product is not summarily considered adulterated, but corrective actions should be taken.
 - (2) Record the corrective actions taken.
 - (3) Collect follow-up samples from the FCS and the surrounding area (**required**).
 - (4) Repeat corrective action and testing until samples are negative for *Lm* or *Listeria* spp.
- v) Initiate intensified sampling after the 2nd consecutive positive.
 - (1) Take corrective action (as specified in the HACCP plan, Sanitation SOP or prerequisite program), which should include intensified cleaning and sanitizing.
 - (2) If the FCS test is positive for *Lm*, the product in the sampled lot would be considered adulterated. If the FCS is positive for *Listeria* spp., the product is not summarily considered adulterated, but corrective actions should be taken.
 - (3) Record the corrective actions taken.
 - (4) Hold the product (see hold-and-test scenario below in [Appendix 4.2](#)).
 - (5) **Test** product for *Lm* at a rate that provides a level of **statistical confidence** that the product is not adulterated (**required after the 2nd consecutive positive result**).
 - (6) Conduct follow-up testing of the FCS each day until there are 3 consecutive negative test results for *Lm* or *Listeria* spp.
 - (7) At the same time, continue to **hold** each day's production lot until the test results for the FCS are negative.
 - (8) If the test results for the product are positive for *Lm*,
 - (a) Destroy the product, or
 - (b) Re-work the product with a process that is destructive to *Lm*.

Appendix 4.2: Hold and Test Scenario

Hold-and-Test Scenario for Deli and Hotdog Products in Alternative 3

Assuming it takes to 3 days to obtain a test result for *Listeria* spp.:

Day 1 – Take food contact surface (FCS) samples

Day 4 –If FCS samples (from Day 1) are **negative** for *Listeria* spp.

- ✓ Continue production, as the corrective action appears to have resolved the problem and test FCSs as scheduled.

If the FCS samples are **positive** (from Day 1) for *Listeria* spp.:

- ✓ Take corrective action (as specified in the HACCP plan, Sanitation SOP, or prerequisite program), which should include an intensified cleaning and sanitizing.
- ✓ Collect follow-up samples of FCS—target the most likely source of contamination, and also perform additional tests in the surrounding FCS area.
- ✓ Continue production.

Day 7 – If the follow-up FCS sample (from Day 4) is **negative** for *Listeria* spp.:

- ✓ Continue production, as the corrective action appears to have resolved the problem and test the FCSs as scheduled.

If the follow-up FCS sample (from Day 4) is **positive** for *Listeria* spp.:

- ✓ Take Corrective Action (as specified in the HACCP plan, Sanitation SOP or prerequisite program), which should include an intensified cleaning and sanitizing.
- ✓ Test the FCS-- target most likely source of contamination, and also take additional tests in the surrounding FCS area.
- ✓ Collect intensified samples of FCS, NFCS, and product.
- ✓ Hold and test Day 7 product lot (for *Lm*).
- ✓ Continue production, hold product from the day's production.

Day 8 –

- ✓ Test the FCS— target the most likely source of contamination, and also perform additional tests in the surrounding FCS area.
- ✓ Continue intensified sampling of FCS, NFCS, and product.
- ✓ Hold product from this day's production.

Day 9 –

- ✓ Test the FCSs-- target the most likely source of contamination, and also perform additional tests in the surrounding FCS area.
- ✓ Continue intensified sampling of FCS, NFCS, and product.
- ✓ Hold product from this day's production.

Day 10 –If the FCS sample (day 7 sample) is **negative** for *Listeria* spp.:

- ✓ Continue production and hold product from days 7, 8, 9 and 10 until the results from Day 7 product testing and Days 8, 9, and 10 FCS testing are available and found to be negative, unless there is compelling justification that affected products are not adulterated.
- ✓ Resume the FCS testing according to the frequency stated in the HACCP plan, Sanitation SOP, or prerequisite program.

If the FCS sample (day 7 sample) is **positive** for *Listeria* spp.:

- ✓ Hold and test product from day 10 production.
 - ✓ Test product from days 7, 8, 9, and 10 for *Lm*.
 - ✓ Take corrective action.
 - ✓ Intensive cleaning and sanitizing.
- ✓ Take FCS sample-- target the most likely source of contamination, and also perform additional tests in the surrounding FCS area.

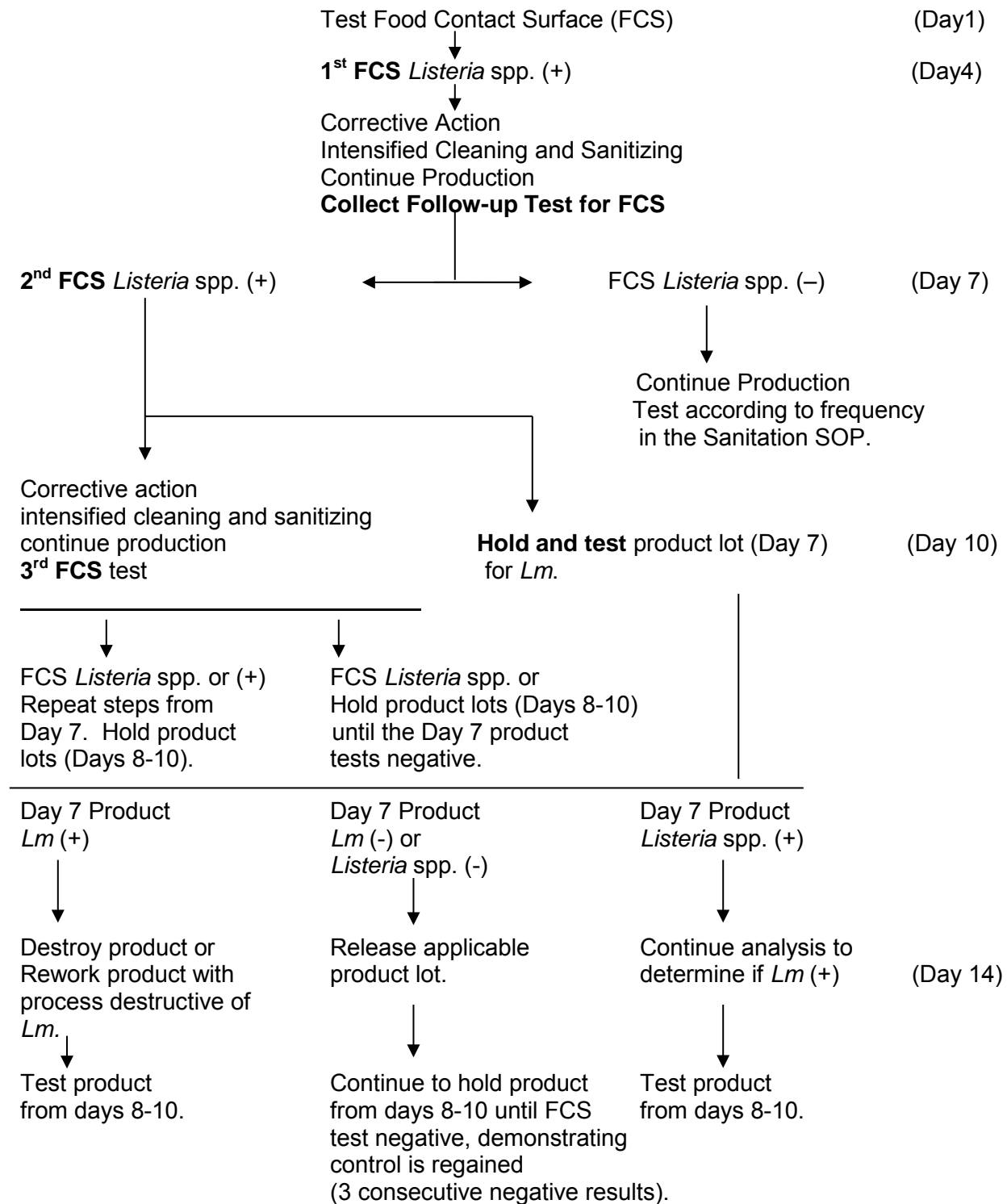
Day 14 – If the Day 7 product is **positive** for *Lm*, destroy product, or rework product with a process that is destructive of *Lm*. Recall product if already in commerce. If product is positive for *Listeria* spp., verify by testing that products (Days 7, 8, 9, 10), which may have been exposed to insanitary conditions are not adulterated. Product that is positive for *Listeria* spp. may be considered adulterated if it was produced under insanitary conditions, see Section 3.6.

Question: An establishment that produces Alt. 3 deli and hotdog products tests FCSs on a Monday. The test comes back positive on Thursday. How would this affect the product produced on Monday, Tuesday, Wednesday, and Thursday?

Answer: If the test is positive for *Listeria* spp., the result would not affect product produced on Monday through Thursday. However, on Thursday, the establishment must initiate corrective actions, intensified cleaning and sanitizing, and verify the effectiveness of the corrective actions by follow-up testing of the FCSs.

If the test is positive for *Lm*, product that comes into direct contact with a FCS that tests positive for *Lm* is considered adulterated and FSIS would typically request that establishments recall such products if they have been released into the marketplace. That product must be destroyed or reworked with a process that is destructive of *Lm*. The establishment must have supporting documentation explaining why products produced on Tuesday, Wednesday and Thursday would not be contaminated with *Lm*. On Thursday, when it receives the positive result, the establishment must take corrective actions, conduct intensified cleaning and sanitizing, and test FCSs for *Lm* or indicator organisms to verify the effectiveness of the corrective actions.

Hold-And-Test Scenario Flowchart for Alt.3 (deli or hotdog producers)



FCS: food contact surface

Listeria spp.: *Listeria* species (test results available after 2 or 3 days).

Lm: *Listeria monocytogenes* (test results available after 6 or 7 days)

Enforcement Strategy for Alternative 3 Deli and Hotdog Products

Under the *Listeria* Rule, an establishment with deli and hotdog products in Alternative 3 must provide for testing of FCSs. If a FCS tests positive for *Lm* or *Listeria* spp., the establishment must conduct follow-up testing to verify that its corrective actions are effective. If during the follow-up testing another positive FCS occurs for *Listeria* spp., the establishment must hold the applicable product lot. If the product is positive for *Lm*, destroy or rework with a process destructive of *Lm*, and test the FCS until the establishment corrects the problem as indicated by the test result. In addition, the establishment must test held product lots for *Lm* using a sampling plan that will provide a statistical level of confidence. The flowchart above shows a hold and test scenario that establishments under hold and test can use. The days described are approximate, depending on the typical amount of time needed to obtain a positive test result (see key at bottom of the flowchart). Establishments can adjust the flowchart based on their own process and time frame for sample results. The following section describes the likely action and reaction of inspection personnel during a hold and test situation.

Day 1 and 4

The testing program and the test results for FCSs and NFCs should be made available to inspection program personnel (IPP). In case of a FCS testing positive for *Listeria* spp., IPP will verify that the establishment is performing the corrective actions as specified in the HACCP plan, Sanitation SOP or prerequisite programs, including any intensified cleaning and sanitizing. For deli and hotdog products in Alternative 3, IPP will verify that the establishment is conducting follow-up testing for FCSs to determine the effectiveness of the corrective actions, targeting the most likely source of contamination, performing additional tests in surrounding FCS area, and recording the results of all these.

Day 7

Results of the follow-up FCS tests are available on this day. If the FCS tests are negative, then the establishment continues with its normal production and Sanitation SOP. If the follow-up FCS tests are positive for *Listeria* spp., IPP will verify that the establishment is following its corrective action for a second FCS positive, including intensified cleaning and sanitizing. For deli and hotdog products in Alt. 3, inspection personnel will verify whether the establishment is holding the product produced that day and testing the product lot for *Lm*, and whether the establishment is conducting follow-up testing of FCS during each production day, and holding all products until a negative follow-up FCS test is obtained. Products produced on days 8, 9, and 10 are held until the follow-up FCS test available after about 3 days is found negative. The *Listeria* Rule states that products must be held until the problem is corrected, as indicated by testing. For establishments in Alt. 3 producing deli and hotdog products, inspection personnel can cite the establishment if these procedures are not followed.

Days 8, 9, and 10

The presence of *Listeria* spp. on a FCS or on RTE product is associated with the potential for an insanitary condition to exist. FSIS expects an establishment to develop a compelling justification for concluding that product produced on days in which insanitary conditions may have existed is not adulterated. Thus, FSIS would further expect that the establishment, on days 8-10, would conduct verification testing on the FCSs to demonstrate that the potential insanitary condition was adequately redressed via the corrective and preventive actions. In addition, to further develop a compelling justification to support the establishment's decision, FSIS would expect a prudent establishment to also compile data on product testing to confirm and verify that the corrective and preventive actions were effective in preventing product from becoming adulterated.

Day 10

If the Day 7 FCS Test is Positive, IPP will verify that if the follow-up FCS test taken on Day 7 is positive, then the day's production lots of deli and hotdog products in Alt. 3 are held and tested for *Lm* or *Listeria* spp, and the same procedures are followed as in the second FCS (+) test as in Day 7.

If the FCS samples taken on day 7 are found positive for *Listeria* spp. on day 10, the establishment should hold and test product produced on days 8, 9, and 10 unless the establishment has supporting documentation to justify that product produced on days 8, 9, and 10 would not be contaminated with *Lm*. The sampling plan must provide a level of confidence that each product is not contaminated with *Lm*. Because of 3 consecutive positive FCS samples, the establishment should conduct intensive cleaning and sanitizing and reevaluate its Sanitation SOP.

If the FCS sample is positive for *Lm*, affected product lots are considered adulterated. The establishment should also hold and test products produced on days 8, 9, and 10 because an FCS positive for *Lm* shows that the corrective action may not have been effective in removing the contamination and products produced on succeeding days may also be contaminated.

If the Day 7 FCS Test is Negative

If the FCS samples taken on day 7 are found negative for *Listeria* spp. on day 10, the establishment should wait for the results of the FCS tests conducted on days 8, 9, and 10 as detailed above, and results of the Day 7 product test before releasing these products. **Control is considered regained after 3 days of negative results.**

Day 14

If day 7 product was found positive for *Lm* on day 14, affected product lots produced on day 7 are considered adulterated. The establishment must destroy the product lots or rework them with a process destructive of *Lm*. The establishment should continue holding product lots produced on days 8, 9, and 10 until results of products tests are available, unless the establishment has supporting documentation for why product produced on days 8, 9, and 10 would not be contaminated with *Lm*. Establishment should also hold and test product produced before day 7 and recall them if already in commerce or provide compelling evidence that product produced before day 7 was not adulterated.

For a product sample that tests positive for *Lm*, inspection personnel will verify that the product lots affected are disposed properly, i.e., destroyed or reworked with a process that is destructive to *Lm*. Establishments should have supporting documentation that products lots produced before Day 7 are not contaminated with *Lm*, so that these lots will not be included as adulterated.

A product that is positive for *Listeria* spp. may be considered adulterated if it was produced under insanitary conditions, see Section 3.6.

Appendix 4.3: *Listeria* Trends Examples

The following are some scenarios describing how establishments can track and address *Listeria* trends.

Establishment A

Establishment A makes RTE salads, including potato salad, chicken salad, and ham salad for delicatessens in grocery stores. The establishment manufactures product in two 8-hour shifts, 6 days a week. The third shift is reserved for sanitation. It has identified three tiers in its sampling program: NFCS sampling, FCS sampling, and finished product testing.

It has identified 30 NFCS sampling sites, including the walls next to the preparation tables, the exterior of the mixing kettles, the mixer shaft, and the drains under the preparation tables. Each week it randomly picks 15 of the 30 sites for testing for *Listeria* spp.; these 15 sites are tested twice a week ("routine monitoring") before production. Results are tracked as total number of positives over time and also by site. When a positive is detected at any site, it is given extra attention during the next sanitation. If the number of positives exceeds 10% (e.g., if there are 3 positives out of 30) during the week (two test periods, rolling window) or if the same NFCS site comes up positive more than one time in a month, these sites are given extra attention during the next sanitation shift, and the areas are re-swabbed daily until there are three consecutive days of negatives. Once this has occurred, the establishment reverts to routine monitoring. If the problem is not corrected within 5 days, the establishment enters "trouble shooting" mode, which includes more stringent decontamination procedures, such as disassembly and sanitizing, fogging with sanitizers, changing sanitizers, double sanitizing, and heat treatments.

Establishment A also conducts routine random FCS testing and it has identified 20 FCSs, including tables, conveyor belts, and slicer blades. Each week, 10 of these are randomly selected and tested for *Listeria* spp., twice per week at the end of production and before cleaning. If a positive is detected, the site is given extra attention during the next sanitation shift and a follow-up sample is collected. The site is tested daily for 5 days. If the site is positive during this 5-day period, the line is shut down and, if appropriate, torn apart, taking trouble-shooting swabs during the disassembly. The product contact surface and surrounding areas receive extra sanitation and the line is re-assembled. FCS swabs are then taken every two hours during production and all products are placed on hold. If any swab tests positive, product from the 2-hour time period and from each period on either side is tested for *Lm*. Product that is negative is released. Product that tests positive is destroyed, since re-processing is not an option for this product.

The establishment conducts random product testing of one salad product each month by taking one package every two hours from an 8-hour shift and compositing product from two packages. The product is tested for *Lm*. Product found to be positive for *Lm* is destroyed and intensified sampling of FCSs for *Listeria* spp. is conducted daily for a week. If positive FCS results are found, the establishment undertakes investigations to determine the cause of the problem. The *Listeria* Control Program is also reviewed and revised, as appropriate.

Establishment B

Establishment B produces fully cooked, breaded chicken products. The establishment manufactures product on three separate lines in two 8-hour shifts, 6 days a week. The third shift is reserved for sanitation. The establishment's NFCS monitoring component of its *Lm* Control

Program targets the area where product exits the fryer, is chilled, and then packaged. There are two parts to this establishment's program: product contact surface testing and non-product contact surface testing.

The establishment monitors 20 NFCs on a weekly basis for *Listeria* spp. (routine monitoring). For each line, 5 swabs are composited, resulting in 4 tests per line for a shift. If a positive is detected, the establishment investigates by re-swabbing and testing the swabs individually, as well as by taking additional swabs in the area. If there are no additional positives, the establishment considers the initial positive to be an isolated incident and returns to routine monitoring. If additional positives are detected, the establishment institutes corrective actions, which may include a review of the current *Listeria* Control Program, revising GMPs, changing sanitizers, enhanced sanitation in clean areas, and employee retraining. The establishment then monitors twice a week (enhanced monitoring) until there are 4 consecutive negative periods, at which point the establishment returns to routine monitoring.

The establishment also monitors 15 FCSs on each line during each shift of production every other week. If the swabs are all negative, it continues routine monitoring. If there is a positive result, the establishment investigates by collecting a follow-up sample of the area, as well as by taking additional swabs in the surrounding area. In addition, it institutes corrective actions, which may include intensified cleaning and changing sanitizers. The establishment then takes swabs to confirm that the actions taken have been effective. If there are no positives, the establishment returns to routine monitoring. If there are any positives, the establishment escalates its corrective actions, which may include intensified testing, breaking down pieces of equipment and sanitizing, and heating pieces of equipment. It would also evaluate the need to conduct finished product testing based on all the existing evidence.

Example Table for Tracking Microbiological Sampling Trends

This table provides an example spread sheet that establishments may use to track testing results and corrective actions for *Listeria* spp. over time. Tracking this information will assist establishments in identifying trends and determining whether they are taking the appropriate corrective actions in response to positives and in reaction to trends. In the scenario below, a positive testing result was found on the freezer fan and addressed by the establishment. No trend was identified because it was the first positive found in that area. However, positives continued to be found in the same general area (Line 4 freezer) leading up to a food contact surface (FCS) positive on the belt exiting the freezer, despite progressively intensified corrective actions taken by the establishment. Negative results seen after the establishment identified a trend and took corrective action (including 3 negatives on the belt exiting the freezer) indicate that the trend was addressed. Corrective actions listed below are only examples and should not be considered the only methods to address *Listeria* spp. contamination. Regulatory testing for FCSs and non-regulatory testing of NFCS are shown within the table. **NOTE:** Establishments are **NOT** required to perform NFCS testing or follow-up testing in response to NFCS positives.

Sampling Results for *Listeria* spp. in an Alternative 3 Deli and Hotdog Small Volume Establishment

Date	Line #	FCS or NFCS	Surface	Shift	Results	Follow-up Test Date	Follow-up Test Result	Intensified testing	Corrective Action	Trend Identified?
9-Jan	4	FCS	QA utensil	2	neg.					
30-Jan	5	FCS	conveyor belt	pre-op	neg.					
9-Feb	1	FCS	conveyor belt	1	neg.					
12-Feb	3	FCS	eagle scale	pre-op	neg.					
19-Feb	1	FCS	plastic film	2	neg.					
19-Feb	5	NFCS	freezer structure	pre-op	neg.					
19-Feb	4	NFCS	Freezer fan	pre-op	positive	24-Feb	positive	3 days of Tests; (-) results	Removed product, re-cleaned freezer and freezer fan	None
23-Feb	2	FCS	Freezer belt	2	neg.					
6-Mar	1	NFCS	Roller belts	pre-op	neg.					
6-Mar	4	NFCS	Hose	2	neg.					
10-Mar	4	FCS	product slide to freezer	pre-op	neg.					
10-Mar	4	NFCS	freezer air handler	pre-op	neg.					
18-Mar	5	FCS	return belt	1	neg.					
18-Mar	3	NFCS	wall	1	neg.					
18-Mar	4	NFCS	stand	2	neg.					
23-Mar	2	NFCS	drain	2	neg.					

Date	Line #	FCS or NFCS	Surface	Shift	Results	Follow-up Test Date	Follow-up Result	Intensified testing	Corrective Action	Trend Identified?
23-Mar	4	NFCS	freezer wall	1	positive	28-Mar	positive	3 days of tests; (-) results	Increase cleaning frequency for freezer, scrub freezer floors and walls.	Second positive in freezer area may indicate possible harborage, addressed by increased cleaning
3-Apr	6	FCS	tub	post-op	neg.					
3-Apr	4	NFCS	freezer floor	post-op	positive	8-Apr	positive	3 days of tests; (-) results	Intensified Cleaning of Freezer, consulted freezer manufacturer,	Third positive in area addressed by intensified cleaning.
3-Apr	1	NFCS	freezer structure	post-op	neg.					
6-Apr	4	NFCS	freezer floor	post-op	neg.					
21-Apr	3	FCS	conveyor belt	post-op	neg.					
21-Apr	4	NFCS	freezer wall	pre-op	neg.					
15-May	2	FCS	conveyor belt	1	neg.					
18-May	4	FCS	line personnel	pre-op	neg.					
15-Jun	1	FCS	product table	pre-op	neg.					
15-Jun	2	FCS	product scoop	1	neg.					
7-Jul	4	FCS	belt exiting the freezer	2	positive	13-Jul	positive	3 days of tests; (-) results; hold and test product (-) results	Stopped production. Repaired refrigerant leak. Intensified cleaning of freezer. Production resumed after repairs and cleaning.	Freezer is identified as a harborage point and addressed by repairs and cleaning.
14 Jul	1	NFCS	drain	1	neg.					
14 Jul	2	NFCS	wall	2	neg.					

Date	Line #	FCS or NFCS	Surface	Shift	Results	Follow-up Test Date	Follow-up Test Result	Intensified testing	Corrective Action	Trend Identified?
30-Jul	4	FCS	knife blade	1	neg.					
3-Aug	1	FCS	spiral slide	2	neg.					
14-Aug	4	NFCS	freezer wall	pre-op	neg.					
14-Aug	4	NFCS	product entrance facing freezer	pre-op	neg.					
20-Aug	4	FCS	belt exiting the freezer	2	neg.					
26-Aug	10	FCS	product rack	1	neg.					
26-Aug	4	NFCS	freezer floor	post-op	neg.					
12-Sep	2	FCS	line personnel	2	neg.					
26-Sep	9	NFCS	condemn tub	pre-op	neg.					
26-Sep	7	FCS	product tray	pre-op	neg.					
28-Sep	4	FCS	belt exiting the freezer	2	neg.					
1-Oct	4	NFCS	freezer air handler	1	neg					
1-Oct	4	NFCS	freezer wall	pre-op	neg					
1-Oct	6	FCS	employee gloves	1	neg					
12-Oct	4	NFCS	freezer floor	2	neg					
12-Oct	4	FCS	belt exiting the freezer	1	neg					

<p>Key FCS = Food contact surface NFCS = Non food contact surface</p>
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Appendix 4.4: Findings from Food Safety Assessments (FSAs)

In 2009, FSIS began performing routine Food Safety Assessments (FSAs) in RTE establishments at a frequency of once every 4 years. These FSAs are performed along with routine risk-based *Lm* (RLm) sampling. FSIS also performs “for cause” FSAs along with Intensified Verification Testing (IVT). The purpose of the FSA is to evaluate the food safety systems (including the HACCP plan and Sanitation SOP) at the establishment to determine if they are effective in controlling the safety of the product. FSAs are performed according to [FSIS Directive 5100.1 “Enforcement, Investigations, and Analysis Officer \(EIAO\) Food Safety Assessment Methodology.”](#)

FSIS reviewed the findings from “for cause” FSAs performed in response to IVTs on a quarterly basis. FSIS is planning to review the findings in routine FSAs performed during RLms and to include the results of those analyses in future issuances of this guidance. The findings from these reviews are used to help develop new policy and revise current policy to ensure that establishments are meeting the requirements of the *Listeria* Rule. By summarizing the findings FSA reports, FSIS can provide information to RTE establishments so that they can focus their attention on areas where further improvements in their food-safety systems may be needed. During the FSA review, it was found that several of the establishments had deficiencies in their Sanitation and HACCP design and record keeping systems.

These problems included the following:

- **The establishment failed to follow written *Listeria* programs.**

According to the *Listeria* Rule, establishments in Alt. 2b and 3 are required to indicate their sampling frequency and explain why the frequency they have identified is sufficient to control *Lm* or an indicator organism. As described in the *Listeria* Guideline, establishments can document the sampling frequency they have identified as part of the *Listeria* Control Program ([Section 3.1](#)). Once the establishment has established a frequency as part of its program, it would need to follow the sampling frequency. If an establishment does not follow the sampling frequency by not collecting a sample during the timeframe specified in their program, it would be found to be non-compliant, unless it can provide other supporting documentation demonstrating that its process is safe.

- **The establishment did not perform monitoring at frequencies specified in the HACCP plan.**

In some cases, establishments identified a certain frequency for monitoring the CCPs associated with RTE products (e.g., measuring lethality temperatures) and did not monitor the temperature at the specified frequency. By failing to monitor the CCPs at the specified frequency, the establishment could miss processing deviations that could occur, leading to under processing or other safety issues in the product.

- **The establishments did not document corrective actions sufficiently.**

If a deviation occurs from a critical limit, establishments are required to take corrective actions to bring the process under control (9 CFR 417.3). These corrective actions must include measures to prevent recurrence of the deviations. In some cases, the corrective actions written by the establishments did not provide sufficient explanation to demonstrate how future deviations would be prevented.

- **The establishment did not provide supporting documentation for their PLT and AMAPs.**

In some cases, establishments did not support that their PLTs achieved at least a 1-log reduction of *Lm* in the product or that the AMAP allowed no more than 2-logs growth of *Lm* over the shelf-life of the product. The *Listeria* Guideline provides specific guidance establishments can use to ensure that the supporting documentation for the PLT and AMAP is sufficient and reflects the critical operational parameters of their process (see [Appendix 2.1](#)).

- **The establishment failed to maintain sanitary operations and failed to maintain equipment and utensils in a sanitary manner.**

In some cases, positive results were found during the RLM or IVT, indicating that sanitary operations were not maintained or that equipment and utensils were not maintained in a sanitary manner. In one case, condensation was dripping directly on exposed-RTE product. The *Listeria* Guideline provides information establishments can use to ensure that sanitary operations are maintained (see [Appendix 2.2](#)). In addition, establishments can use verification testing to ensure that their food-contact surfaces are sanitary and free of *Lm*. By collecting samples of non food contact surfaces, establishments can find potential harborage points and address them before the product becomes contaminated. Establishments are required, according to 9 CFR 416.2 (b), to ensure that the facility and the equipment are sanitary and in good repair, so that potential sources of cross contamination, such as condensation, are minimized.

- **The establishment did not identify the location and the sites that will be sampled for testing of food contact surfaces in the post-lethality processing environment and provide an explanation of why the testing frequency was sufficient to ensure that effective control of *Lm* or of indicator organisms is maintained.**

If an establishment chooses either Alt. 2b or 3, it must test FCSs in the post-lethality processing environment, identify the frequency for testing, and provide an explanation of why the testing frequency is sufficient to ensure the effective control of *Lm* or indicator organisms (9 CFR 430.4(b)(2)(iii)(A), (C), and (E) and 430.4(b)(3)(i)(A), (C), and (E)). The FSIS expectation is that establishments in Alt. 2b or 3 will identify all possible FCS for testing. The *Listeria* Guideline provides information on site selection and a list of possible FCSs and NFCSs the establishment could sample (see [Appendix 3.1](#)).

Recommended minimum testing frequencies are also provided in the *Listeria* Guideline (see [Section 3.3](#)). Establishments can use the recommended frequencies or select their own frequency; however they would need to provide support that the level of testing is sufficient to demonstrate that *Lm* is controlled in the product. Establishments should increase their sampling frequency due to repeated positive results, construction, or sanitation issues.

- **The establishment did not address hazards reasonably likely to occur in the production process.**

Some establishments did not list all of the steps in the processing of their product in their flow chart, as required by 9 CFR 417.2(a)(2). In some cases, the establishment did not

consider possible hazards from ingredients (such as spices) added after the lethality treatment. In other cases, the establishment did not have supporting documentation on file, such as letters of guarantee or certificates of analysis (COA) demonstrating that each lot of ingredients it added to product were safe and would not cause the product to become adulterated. Information on ensuring the safety of ingredients in RTE product can be found in the [FSIS RTE *Salmonella* Guideline](#). Information on avoiding sources of environmental contamination can be found in the *Listeria* Guideline (see [Appendix 2.2](#)).

By reviewing the examples provided above and addressing deficiencies in their food-safety programs, establishments can help ensure that they meet the requirements of the *Listeria* Rule. In addition, by reviewing their programs to ensure that possible weaknesses are addressed, establishments can produce safe products and help protect public health.

Appendix 4.5: FSIS Response to Comments

FSIS received 2 comments in response to the September 2012 “FSIS Compliance Guideline: Controlling *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Meat and Poultry Products” (FSIS *Listeria* Guideline). Comment summaries and Agency responses follow.

Comment: One commenter questioned why the FSIS *Listeria* Guideline was revised using only FSIS data and input. The commenter stated that the meat and poultry industry likely would have been able to provide additional assistance or guidance in advance of the draft being issued to field personnel. According to the commenter, revising the guideline after it is already been adopted may lead to confusion among the industry or FSIS field personnel. The commenter also stated that collaboration of industry and Agency efforts has led to a decrease in positives from the FSIS RTE sampling program.

Response: As FSIS has done with all guidance documents it has issued in recent years, FSIS sought industry input by seeking comment on the guidance. This practice has allowed FSIS to incorporate comments and feedback from industry and other interested parties. FSIS now adds a message to all draft compliance guidelines stating that the guideline represents FSIS’s current thinking on the topic and should be considered usable as of its issuance. FSIS will routinely update guidance documents to reflect the most current information available to FSIS and its stakeholders. FSIS agrees that the success of the *Listeria* regulation has been the affect of efforts from industry and FSIS to control contamination of RTE products. This information has been added to the Introduction section of the revised FSIS *Listeria* guideline.

Comment: One commenter stated that the Constituent Update article, issued on September 21, 2012, announcing the availability of the guidance and the instructions in FSIS Notice 59-12, implied that the guidelines should be considered requirements rather than recommendations to industry. Similarly, the commenter stated that instructions in FSIS Notice 59-12 stating that EIAOs are to review the information in the guideline as part of their preparation for performing FSAs, implies that that the guidelines are requirements.

Response: As FSIS has stated in explaining the purpose of the FSIS *Listeria* Guideline, “This document provides **guidance** to assist establishments in meeting FSIS regulations. Guidance represents **best practice** recommendations by FSIS based on the best scientific and practical considerations and does not represent **requirements** that must be met.” While it is true that the guidance can be used to assist establishments in strengthening their food-safety programs, the guidance does not represent requirements. The purpose section of the guideline has been revised to clarify that establishments may choose to adopt different procedures than those outlined in the guideline, but they would need to support why those procedures are effective in controlling hazards from *Lm* in RTE products. By using the recommendations in the guideline, establishments would not need to provide further support for their procedures. In FSIS Notice 59-12, FSIS instructed EIAOs not to recommend that IPP issue NRs based on establishments using previous versions of the guidelines. The purpose of this instruction is to ensure that EIAOs do not interpret the guidance as requirements, and that NRs should not be issued to establishments that fail to adopt the most recent version of the guidelines. However, EIAOs may still recommend the issuance of NRs if the establishment fails to support the efficacy of its food safety system to control pathogens.

Comment: One commenter requested more information on compositing samples, including information on the pros and cons of compositing samples for both environmental and

food contact surfaces. The commenter also suggested that FSIS include provisions for the use of surrogate or indicator organisms for testing purposes in the compliance guideline.

Response: FSIS added new information regarding compositing of samples and laboratory methods for analyzing the samples in Appendix 3.3, Sample Collection and Testing Methods. In addition, this section includes information on the pros and cons of compositing the samples. As stated in Appendix 3.3, establishments may choose to test for *Lm*, *Listeria* spp., or *Listeria*-like organisms (LLO). *Listeria* spp. and LLO are examples of indicator organisms for which establishments may test in lieu of testing for *Lm*. A finding of *Listeria* spp. or LLO on a food contact surface could indicate conditions where *Lm* could survive. FSIS has also provided examples of the use of non-pathogenic *Escherichia coli* (*E. coli*) cultures as surrogate indicator organisms in validation studies in the following Q&A: [Use of Non-pathogenic *Escherichia coli* \(*E. coli*\) Cultures as Surrogate Indicator Organisms in Validation Studies](#). As FSIS continues to revise its guidelines and to issue new guidelines, it plans to provide more information about surrogate and indicator organisms that can be used for in-plant validation studies.

Comment: One commenter suggested that FSIS provide assistance to small and very small establishments concerning the changes in the guidelines by performing outreach in the form of webinars, resource documents, regional meetings, and any other form of education the Agency can provide to those establishments.

Response: FSIS agrees that it would be worthwhile to conduct outreach to small and very small establishments regarding the changes in the guidelines. FSIS is planning to present a series of webinars describing the changes. Establishments may also submit specific questions through [AskFSIS](#), and subject matter experts will respond to their questions.

Comment: One commenter asked about the use of different values in the examples and tables, the definition of “cook-in bag” product, and the use of Safe Handling Instructions (SHI) on RTE product.

Response: With regard to the values in Table 2.1 and the values in the examples, the values in the table would be effective if used alone to control *Lm* growth. However, the values in Example 1 in Section 2.1 are different than the values in Table 2.1 because of the “hurdle effect,” which is the synergistic effect of parameters to control *Listeria* growth. FSIS revised the examples in the guideline to more clearly describe the impact of multiple factors on pathogen growth and provided a new section on the hurdle effect. In addition, FSIS revised Appendix 1.1, Product types, to further clarify that cook-in bag products are not considered deli products because they are not subject to the *Listeria* Rule. FSIS also revised Attachment 1.2, Chart of RTE vs. NRTE Products: Resource 1, to clarify when SHI are required and recommended.