

Chapter 2

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

2.1 [Post Lethality Treatments \(PLT\)](#)

2.2 [Antimicrobial Agents \(AMA\) and Antimicrobial Processes \(AMP\)](#)

[Table 2.1: Growth Limits for *Lm*](#)

2.3 [Sanitation](#)

2.4 [Expected Levels of Control](#)

[Table 2.2: Expected Control Levels for Post-lethality Treatments and Antimicrobial Agents or Processes under Alternatives 1 & 2](#)

2.5 [Training](#)

2.6 [New Technology and New Ingredient Review](#)

2.7 [Glossary](#)

2.8 [References](#)

Attachments

2.1 [Post-lethality Treatments](#)

2.2 [Antimicrobial Agents or Processes](#)

Appendices

2.1 [Validation](#)

2.2 [Sanitation](#)

2.3 [Training](#)

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](#) (AMA) or [antimicrobial process](#) (AMP)) 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

Examples of Post-lethality Treatments (PLT)

PLT for *Lm* may include:

- 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*.

(9 CFR 430.4(c)(7)). Expected levels of control for PLTs and AMAs and AMPs are provided in [Table 2.1](#). 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 AMA 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 0.85** will not support the growth of *Lm* and 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 this 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* to an undetectable level. 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 AMAs and AMPs, see [Appendix 2.1](#).

Question: Could cure (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.

2.2 Antimicrobial Agents (AMA) and Antimicrobial Processes (AMP)

According to the *Listeria* Rule, AMAs and AMPs must suppress or limit the growth of *Lm* throughout the shelf-life of the product. AMAs can include lactates and diacetates added in the formulation of the product and growth inhibitors added in the immediate packaging material. AMAs and AMPs must be included in the establishment's HACCP plan, [Sanitation Standard Operating Procedure \(Sanitation SOP\)](#), or [prerequisite program](#) and the establishment must validate that the AMA or AMP is effective as used.

It is FSIS's expectation that AMAs or AMPs are designed to allow no more than 2-logs of growth of *Lm* over the shelf-life of the product. If the AMA or AMP 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 AMA or AMP 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 AMA or AMP 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 AMAs are provided in [Table 2.2](#). For further information on validation of AMAs and AMPs, see [Appendix 2.1](#).

Question: Could cure (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.

1. Antimicrobial Agents (AMA)

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 antimicrobials 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 AMAs and AMPs and [Appendix 2.1](#) for more information.

According to the *Listeria* Rule, the AMA or AMP 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. A report

of the National Advisory Committee for Microbiological Criteria for Foods (NACMCF) that gives guidance on how establishments can develop safety-based consume-by date-labels for refrigerated RTE foods can be found at:

http://www.fsis.usda.gov/ohs/nacmcf/2004/NACMCF_Safety-based_Date_Labels_082704.pdf.

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, antimicrobial agents 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.

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 inhibitors in the immediate packaging material.

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: 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.

Example 2: Vinegar as an AMA

Acidulants or added vinegars can be considered as AMAs. 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. FSIS will consider starter cultures used in dry or semi-dry fermented sausages or vinegar-based pickles as 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/limits growth of *Lm*.

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*. If the process is also listericidal during the shelf-life of the product, it could also serve as a post-lethality treatment, as long as at least 1-log reduction of *Lm* is demonstrated. A_w below 0.85 may result in a decrease of *Lm* in certain products; however establishments would need to support the effectiveness of drying as a PLT in their particular product and process, prior to distribution into commerce.

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 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.

Example: Other AMPs

Some AMAs or AMP may have increased effectiveness in controlling growth of *Lm* when added in combination with other AMA or AMP. 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. For more examples of AMAs and AMPs, see [Attachment 2.2](#).

Ensuring the Effectiveness of AMAs and AMPs

According to the *Listeria* Rule, establishments must document that the AMA or AMP 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 AMA or AMP 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 AMA or AMP. 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 AMAs 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 AMAs. 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 2.3](#) 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 very well 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.

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 AMAs or AMPs 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, AMA or AMP 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.

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 AMA or AMP 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 AMAs and AMPs, 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 a 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 AMA or AMP would not be considered eligible as an AMA or AMP 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 program is critical to the success of any *Listeria* control program. A *Listeria* control program will only be effective if employees understand the program, understand their roles, and are able to perform the duties required of them in the program.

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 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:

<http://www.fsis.usda.gov/Regulations & Policies/Labeling FDA MOU/index.asp>

The FSIS Innovations (New Technology) Staff 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 this office 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:

<http://www.fsis.usda.gov/Regulations & Policies/New Technologies/index.asp>

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" found at <http://www.gpo.gov/fdsys/pkg/CFR-2012-title9-vol2/pdf/CFR-2012-title9-vol2-sec424-21.pdf>

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. It is available at: <http://www.fsis.usda.gov/About FSIS/labeling & consumer protection/index.asp>.

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).

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).

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).

2.8 References

A. Post-lethality Treatments and Antimicrobial Agents

Bedie, B. K., J. Samelis, J.N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith . 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* postprocessing contamination on frankfurters stored at 4° C in vacuum packages. J. Food Protect. 64:1949-1955

Buege, D.R., Ingham, S.C. and J.A. Losinski (University of Wisconsin-Madison), “Evaluation of Del Ozone’s Delzone® Sanitation System as a Post-Lethality Treatment to Control *Listeria monocytogenes* Contamination on Ready-To-Eat Meat Products”, Confidential Report to Del Ozone, April 16, 2004.

Butts, J. 2003. Seek & destroy: Identifying and controlling *Listeria monocytogenes* growth niches. Food Safety Mag. 9(2):24-9, 58.

Gande, N., and Muriana, P. M. 2002. Pre-package surface pasteurization of ready-to-eat meats with radiant heat oven for reduction of *Listeria monocytogenes*. Accepted for publication, Journal of Food Protection.

Glass, K. G., D. A. Granberg, A. L. Smith, A. M. McNamara, M. Hardin, J. Mattias, K. Ladwig, and E. A. Johnson. 2002. Inhibition of *Listeria monocytogenes* by sodium diacetate and sodium lactate on wieners and cooked bratwurst. J. Food Protect. 65: 116-123.

Guenther, S., D. Huwyler, S. Richard, and M.J. Loessner. 2009. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Appl. Environ. Microbiol. 75:93-100

International Commission on Microbiological Specifications for Foods (ICMSF) 1996. Microorganisms in Foods 5 – Microbiological Characteristics of Food Pathogens, p. 148. (Blackie Academic & Professional, NY)

Janes, M. E., S. N. Kooshesh and M.G. Johnson. 2002. Control of *Listeria monocytogenes* on the surface of refrigerated, ready-to-eat chicken coated with edible zein films containing nisin and calcium propionate. J. Food Sci. 67(No. 7): 2754-2757.

Marsden, J.L., M.N. Hajmeer, H. Thippareddi, and R.K. Phebus. 2000. Evaluation of Spray Application of Acidified Sodium Chlorite on Frankfurters and Its effect on Reduction of *Listeria monocytogenes*. Alcide Corporation. Unpublished.

Muriana, P.M. and W. Quimby, C.A. Davidson, and J. Grooms. 2002. Postpackage pasteurization of ready-to-eat deli meats by submersion heating for reduction of *Listeria monocytogenes*. J. Food Protect. 65:963-969.

Murphy, R.Y., L. K. Duncan, K.H. Driscoll, B.L. Beard, M. E. Berrang and J.A. Marcy. 2003. Determination of thermal lethality of *Listeria monocytogenes* in fully cooked chicken breast fillets and strips during post cook in-package pasteurization J. Food Protect 66:578-583.

Murphy, R.Y., L. K. Duncan, E. R. Johnson, M.D. Davis, R. E. Wolfe, and H. G. Brown. 2001. Thermal lethality of *Salmonella senftenberg* and *Listeria innocua* in fully cooked and packaged chicken breast strips via steam pasteurization. J. Food Protect. 64:2083-2087.

Murphy, R.Y., L. K. Duncan, K.H. Driscoll, and J.A. Marcy. 2003. Lethality of *Salmonella* and *Listeria innocua* in fully cooked chicken breast meat products during postcook in-package pasteurization. J. Food Protect. 66:242-248.

Murphy, R.Y., L.K. Duncan, K.H. Driscoll, J.A. Marcy, and B.L. Beard. 2003. Thermal inactivation of *Listeria monocytogenes* on ready-to-eat turkey breast meat products during post-cook in-package pasteurization via hot water. J. Food Protect. (accepted).

Porto, A.C.S., B. D. G. M. Franco, E.S. Sant'anna, J. E. Call, A. Piva, and J. B. Luchansky. 2002. Viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, commercially prepared with and without 2.0 or 3.0% added potassium lactate, during extended storage at 4 and 10° C. J. Food Prot. 65:308-315.

PURAC America, Inc. Opti.Form *Listeria* Control Model. 2003. Personal Communication

Raghubeer, E.V. and E.D. Ting. 2003. The Effects of high hydrostatic pressure (HPP) on *Listeria monocytogenes* in RTE meat products. Avure Technologies, Inc. Submitted for publication.

Samelis, J. G.K. Bedie, J.N. Sofos, K.E. Belk, J.A. Scanga, and G.C. Smith. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4° C in vacuum packages. J. Food Protect. 65: 299-307.

Scott, V.N., K.M.J. Swanson, T.A. Freier, W. P. Pruett, Jr., W.H. Sveum, P.A. Hall, L.A. Smoot, and D.G. Brown. 2005. Guidelines for conducting *Listeria monocytogenes* challenge testing of foods. Food Prot. Trends 25: 818-825.

Seman, D.L., A. C. Borger, J. D. Meyer, P. A. Hall, and A.L. Milkowski. 2002. Modeling the growth of *Listeria monocytogenes* in cured, ready-to-eat processed meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture control. *J. Food Protect* 65:651-658.

Viskase Corporation. NOJAX® AL. 2003. Personal Communication.

B. Sanitation Guidelines

AMI. 1988. Interim guideline: microbial control during production of ready-to-eat meat products.

AMI. March, 2008. AMI Fact Sheet. Sanitary Equipment Design

AMI Foundation. April 26, 2005. Food Safety Interventions and Food Attribution Workshop: Minimum Requirements For Effective Food Safety Interventions to Reduce *Listeria monocytogenes* Contamination of Ready to Eat Meat Products

Anonymous. 2003. Sanitation systems and solutions. *Food Safety* 9(1):30-40, 45, 48-9.

Anonymous. 1999. Guidelines for developing good manufacturing practices (GMPs), standard operating procedures (SOPs), and environmental sampling/testing recommendations (ESTRs). Ready-to-Eat Products.

Belessi, CE, Gounadaki. AS, Psomas, AN, Skandamis PN. 2011. Efficiency of different sanitation methods on *Listeria monocytogenes* biofilms formed under various environmental conditions. *Int. J. Food Microbiology*. 145 Suppl 1:S46-52.

Conference for Food Protection: Voluntary Guidelines of Sanitation Practices, Standard Operating Procedures, and Good Retail Practices To Minimize Contamination and Growth of *Listeria monocytogenes* Within Food Establishments – developed by the 2004-2006 *Listeria monocytogenes* Intervention Committee

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.

Ednie, D. L, R. Wilson and M. Lang. 1998. Comparison of two sanitation monitoring methods in an animal research facility. *Comtem. Top. Lab. Anim. Sci.* 37(6):71-4.

Food and Drug Administration (FDA). February, 2008. Guidance for Industry : Control of *Listeria monocytogenes* in Refrigerated or Frozen Ready-To-Eat Foods ; Draft Guidance

Grau, F. H. 1996. Smallgoods and *Listeria* . *Food Australia*. 48 (2): 81-83.

Huss, H. H., L. V. Jorgensen and B. F. Vogel. 2000. Control options for *Listeria monocytogenes* in seafoods. *Int. J. Food Microbiol.* 62:267-74.

International Commission on Microbiological Specifications for Foods (ICMSF). Microorganisms in Foods 7. Microbiological Testing in food Safety Management. 2002. Kluwer Academic/Plenum Publishers New York, New York.

Joint Task Force on Control of Microbial Pathogens. 1999. Interim guidelines: microbial control during production of ready-to-eat meat and poultry products.

Kohn, B. A., K. Costello and A. B. Philips. 1997. HACCP verification procedures made easier by quantitative *Listeria* testing. Dairy Food Environ. Sanit. 17(2):76-80.

Krysinski, E. P., L. J. Brown, and T. J. Marchisello. 1992. Effect of cleaners and sanitizers on *Listeria monocytogenes* attached to product contact surfaces. J. Food Protect. 55:(4):246-251.

Mead, P. 1999. Multistate Outbreak of Listeriosis Traced to Processed Meats. August 1998-March 1999. Unpublished.

Moore, G. and C. Griffith. 2002. A comparison of surface sampling methods for detecting coliforms on food contact surfaces. Food Microbiol. 19:65-73.

National Advisory Committee on the Microbiological Criteria for Foods. 1991. Int. J. Food Microbiol. 14(3/4):232-37.

Penn State U. College of Agricultural Sciences. Agricultural Research and Cooperative Extension. 2006. Control of *Listeria monocytogenes* in Retail Establishments.

Penn State U. Cutter, C. Henning, W. September, 2001. Controlling *Listeria monocytogenes* in Small and Very Small Meat and Poultry Plants.

Perl, P. 2000. Outbreak. In Washington Post, January 16, 2000. pp. 8-13, 20-27.

Seeger, K. and M. W. Griffiths. 1994. Adenosine triphosphate bioluminescence for hygiene monitoring in health care institutions. J. Food Prot. 57(6):509-12.

Silliker Laboratories. 1996. Smart sanitation: principles and practices for effectively cleaning your plant. Video.

Suslow, T. and L. Harris. Guidelines for Controlling *Listeria monocytogenes* in Small- to Medium-Scale Packing and Fresh Cut Operations. 2000. University of California Publication 8015.

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.

Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the Food-Processing Environment. J. Food Prot. 65(4):709-25.

University of Florida. Institute of Food and Agricultural Sciences. Florida Cooperative Extension Service. March 2011. Hand Hygiene and Hand Sanitizers

University of Maryland and Cooperative Extension System. April 26, 2010. Industry Guidelines to Prevent Contamination from *Listeria monocytogenes*

Attachment 2.1: Post-Lethality Treatments

NOTE: Mention of trade marks 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-package strips, and 454 gm-package 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.) 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/ingredients/Meat_poultry_and_fish/Preservation/Food-safety/Listeria.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 are 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 shows:

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 + Sodium diacetate	1.8 0.25	120 days no growth; 35-50 days growth 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 secs, 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 are 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™ 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 AMAs, AMPs, and PLTs will be covered. Further recommendations can be found in the complete Validation guidance [http://www.fsis.usda.gov/PDF/HACCP_Systems_Validation_Draft_Guidance_0412.pdf].

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 AMA, AMP, PLT, or other treatment. These include:

- Published processing guidelines

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.

- Regulatory performance standards
- 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 program 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 program.

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, 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 AMAs and AMPs, 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 fully 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 Articles from a Peer-Reviewed Journal

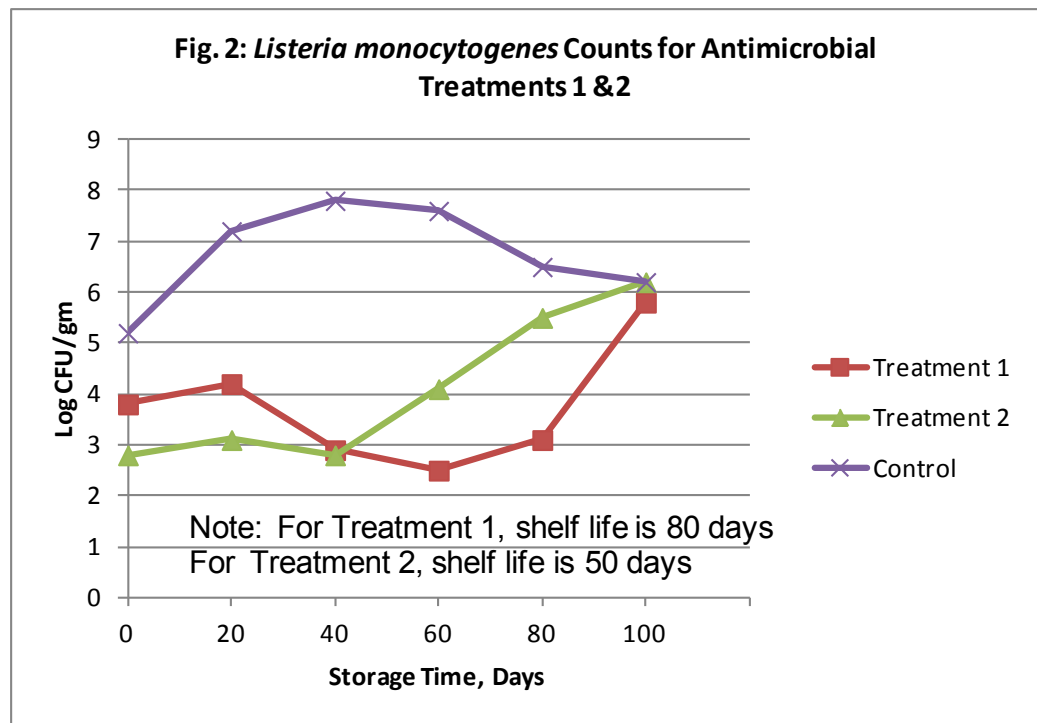
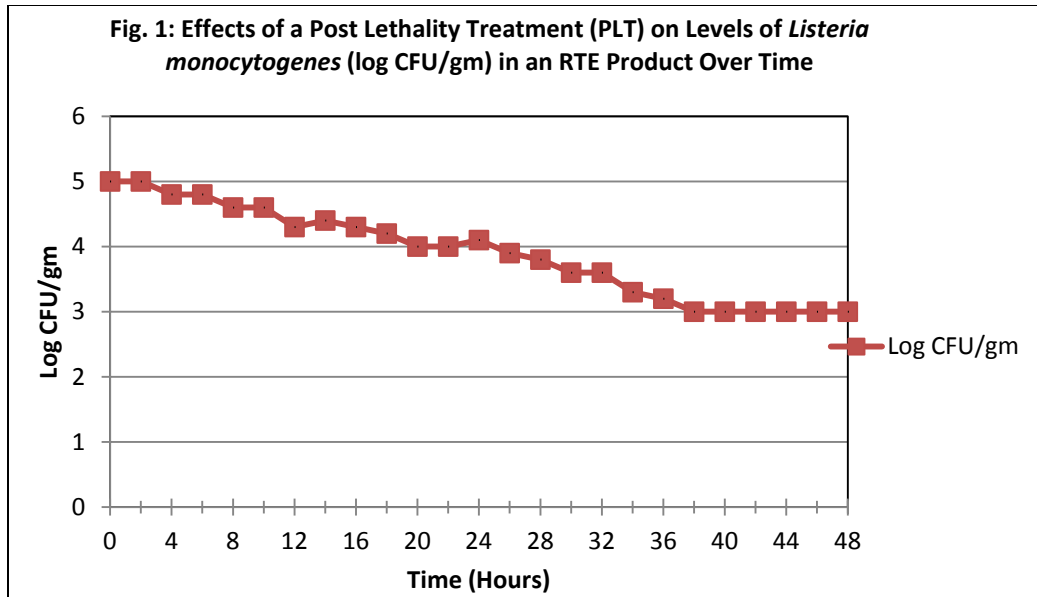
A scientific article from a peer-reviewed journal that describes a process and the results of use of the process can provide adequate supporting documentation. However, the study should relate closely to the establishment's process with regards to species, product characteristics, and equipment. The establishment should use the critical operational parameters cited in the journal article that achieve the required or expected lethality or stabilization if the establishment does not intend to perform additional research to validate its process. In addition, for biological hazards such as *Lm*, 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 whether the process design has been adequately validated.

There are a number of published journal articles 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](http://www.fsis.usda.gov/pdf/nacmcf_jfp_inoculated_pack.pdf) in 2010 [http://www.fsis.usda.gov/pdf/nacmcf_jfp_inoculated_pack.pdf].

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.

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.

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, A_w , 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 than the log reduction to be demonstrated. The inoculum should be composed of a cocktail of 5-6 *Listeria* strains, including some strains known to be relatively resistant to the 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; <http://www.atcc.org/Home.cfm>) or the National Collection of Type Cultures (NCTC; <http://cphl.phls.org.uk/divisions/cdmssd/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 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 AMA or AMP 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 AMA or AMP 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 shelf-life study is one that measures the increase or decrease in the number of the target organism or pathogen during storage. For an AMA or AMP, 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 AMA to determine that the agent is effective in 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 Establishing Safety-Based Consume-by Date Labels for Refrigerated RTE Foods (NACMCF, 2004), found at:

http://www.fsis.usda.gov/ophs/nacmcf/2004/NACMCF_Safety-based_Date_Labels_082704.pdf.

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.

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.

Implementing process specifications in a similar enough way in the establishment's process means that changes among the critical operational parameters used in the scientific support and those used in the actual process will not affect the efficacy of the AMA, AMP, PLT, or other treatment. 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 (the first element of validation), is following the parameters in the scientific support, and can 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 – SSOPs	<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 http://www.sanitarydesign.org/pdf/Sanitary%20Design%20Fact%20Sheet.pdf.</p> <p>Tompkin, R.B. 2004. Environmental Sampling – A tool to verify the effectiveness of preventative 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. 2006. Compliance Guidelines to Control <i>Listeria monocytogenes</i> in Post-lethality Exposed Ready-to-eat Meat and Poultry Products. http://www.fsis.usda.gov/oppde/rdad/FRPubs/97-013F/LM_Rule_Compliance_Guidelines_May_2006.pdf</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. Postpackage 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.	<p>In plant monitoring records for 90 day period demonstrating time and temperature can be consistently achieved.</p> <p>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.</p>

*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 unit, 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, 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 #2.
- 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 required.

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.

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.

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

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. Handwashing

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

FSIS resources can be ordered from the following FSIS website:

http://www.fsis.usda.gov/Science/HACCP_Resources_Order_Form/index.asp

FSIS Resources:

1. *Listeria* Guidelines for Industry. Booklet
2. [FSIS *Listeria monocytogenes* Workbook](http://www.fsis.usda.gov/Science/Workshop_SmallPlants_Lm/index.asp), Fall 2003.
http://www.fsis.usda.gov/Science/Workshop_SmallPlants_Lm/index.asp
3. HACCP-10: Generic HACCP Model for Heat-Treated, Shelf-Stable Meat and Poultry Products <http://www.fsis.usda.gov/OPPDE/nis/outreach/models/HACCP-10.pdf>
4. HACCP-12: Generic HACCP Model for Fully Cooked, Not Shelf-Stable Meat and Poultry Products <http://www.fsis.usda.gov/OPPDE/nis/outreach/models/HACCP-12.pdf>

5. HACCP-15: Generic HACCP Model for Not Heat-Treated, Shelf-Stable Meat and Poultry Products <http://www.fsis.usda.gov/OPPDE/nis/outreach/models/HACCP-15.pdf>.

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.