Chapter 3

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

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

3.1 Sampling for *Lm* or an Indicator Organism

According to the *Listeria* Rule, establishments in all three alternatives may use verification testing for *Lm* or an indicator organism (*Listeria* spp. or *Listeria*-like organisms (LLO)) to verify sanitation in their post-lethality processing environment (9 CFR 430.4(c)(1)). Establishments in Alt. 2b and 3 are required to test their **food contact surfaces (FCS)** in order to verify sanitation in the environment (9 CFR 430.4(b)(2)(iii)(A) and (3)(i)(A)). Testing FCSs is encouraged for establishments in Alt. 1 and Alt. 2a. **If a product or FCS tests positive for *Lm*, then the product will be considered adulterated and the product must be reworked or destroyed, and FSIS would typically request that establishments recall such products if they have been released into the marketplace.**

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

3.2 Design of the *Listeria* Control Program

Establishments may control *Lm* through their HACCP plan, Sanitation SOP, or prerequisite program. Establishments that choose to control *Lm* through their Sanitation SOP or prerequisite program may do so through the use of a *Listeria* Control Program. The *Listeria* Control Program can be incorporated as part of the Sanitation SOP or designed to work with the Sanitation SOP and HACCP plan as a prerequisite program. It is expected that the *Listeria* Control Program will be designed based on the relative risk of the product, depending on the alternative. It is also

recommended that establishments take corrective and preventative actions and perform enhanced sampling in response to positives (see Chapter 4).

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

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

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

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

In addition, Alt. 3 deli and hotdog processors are required to perform follow-up sampling and hold and test product after a second positive (9 CFR 430.4(b)(3)(ii)(B)). The *Listeria* Control Program should also include information about the sampling and testing methods that are used to analyze the samples, and actions taken in response to positive test results, including disposition of contaminated product. Also, although not required, if **non food-contact surfaces (NFCS**) and product samples are collected as part of the establishment's routine sampling program, they should be described in the *Listeria* Control Program (Sections 3.3-3. 6 and 4.1-4.3).

Listeria Control Program Considerations

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

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

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

Parts of the *Listeria* Control Program

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

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

NOTE: Recommended timeframes for performing follow-up sampling and intensified sampling are included in Table 4.1.

3.3 Routine Sampling Program

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

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

In the routine sampling program, establishments can test for *Lm*, *Listeria* spp., or *Listeria*-like Organisms (LLO). For more information on testing methods, see Section 3.5. As previously stated, if a product or FCS tests positive for *Lm*, then the product will be considered adulterated and the product must be reworked or destroyed, and FSIS would typically request that establishments recall such products if they have been released into the marketplace. A finding of *Listeria* spp. or LLO on a FCS indicates conditions where *Lm* may be present and grow, but the product is not considered adulterated. There is no requirement that establishments perform a **confirmation test** on samples that test positive for *Listeria* spp. to determine if the sample is positive for *Lm*. **However, because many tests for *Listeria* spp. are screening tests for *Lm*, a positive result could mean that *Lm* is present, just not confirmed by the test.** Therefore, establishments are expected to take corrective actions and to follow up on *Listeria* spp. positives according to their control alternative, so that product does not become adulterated.

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

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

Food Contact Surface (FCS) Sampling

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

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

The expectation for establishments in Alt. 2b and 3 is that all possible FCSs in the post-lethality processing area will be identified. This includes surfaces which may come into contact with food on a regular basis as well as those that may come into contact on an

intermittent basis. FSIS recommends that the establishment list all possible FCS in their *Listeria* Control Program. This will assist the establishment in identifying all areas that could harbor bacterial pathogens such as *Lm*. By including all possible FCS, the establishment could decrease the likelihood that FSIS would find the food safety system inadequate.

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

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

Sample Collection Considerations

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

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

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

Examples of FCSs may include:

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

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

Size of the Sampling Sites

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

3.4 Frequency of Sampling and Explanation of this Frequency

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

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

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

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

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

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

*At least **3-5 samples** per production line should be sampled each time (every 6 months, quarterly, monthly, biweekly or weekly).
**Establishments producing deli or hotdogs under Alt. 3 may decide to collect samples based on HACCP size or production volume.

Frequency Determinations: How to use Table 3.1

The table lists FSIS expectations for minimal sampling frequencies to meet the *Listeria* Rule. Establishments should consider these frequencies when determining their sampling frequency for their routine sampling program. Establishments can keep this table on file as part of the supporting documentation needed to explain why the testing frequency they have selected is sufficient to control *Lm* or an indicator organism according to 9 CFR 430.4(b)(2)(iii) (E) and (3)(i)(E). The table has been updated to provide Alt. 3 deli and hot dog producers with the option of using daily production volume ranges or establishment HACCP size to determine sampling frequencies. Basing the sampling frequency on the production volume provides more risk-based sampling frequencies and is similar to FSIS sampling programs. If the establishment chooses to follow the testing frequency based on daily production volume, it is important that it **modifies the documentation** associated with its sampling programs. It would not be sufficient for the establishment to make modifications to its testing frequency without changing its programs and supporting documentation.

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

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

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

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

a) **Construction activities**,
b) Change in the HACCP plan or addition of a new HACCP plan,
c) Addition of a new product,
d) Roof leaks, condensation, equipment breakdowns, or other events that could change or increase the probability of product contamination,
e) Increased positives from routine sampling, or
f) Increased aerobic plate count (APC) or bioluminescence counts indicating sanitation issues.

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

Sample Frequency Considerations

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

  For example, assuming that if there are 20 production days in a typical production month (excluding weekends), and an establishment produces RTE product 1-2 days a week, then it may be able to justify sampling quarterly rather than monthly.

- **Representative:** Samples should be representative of conditions at the establishment and collected over different shifts and seasons.

- **Sampling Frequency:** Establishments are expected to increase their sampling frequency in the event of a positive or other event (e.g., construction) in the establishment.

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

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

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

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

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

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

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

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

3.5 Sample Collection and Laboratory Testing Methods

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

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

FSIS Expectations for Sampling Methods

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

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

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

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

When to collect samples: Some samples can be collected at pre op, but most samples should be collected at least 3 hours into operations. *Lm* often works its way out of the equipment after 3 hours of operation (Tompkin, 2002). If the establishment typically produces RTE product for less than 3 hours then the samples can be collected less than 3 hours into operations.

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

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

Sample compositing: Generally, FSIS does not recommend compositing of FCSs because it becomes more difficult to trace the source of contamination. However, if compositing is performed, FSIS recommends that no more than 5 samples be composited, and **separate** sponges (or other sampling device) be used to collect each sample. Compositing is more appropriate for NFCSs, as they are less likely to directly contaminate the product.

In addition, FSIS recommends that like or similar surfaces are composited (e.g., cutting board samples with cutting board samples). The individual locations for the composite sample should be noted to assist in determining the site of contamination to facilitate follow-up testing. **If a composited sample tests positive, the establishment should consider all the sites represented by the sample as positive and take corrective actions accordingly.** During follow-up sampling of FCSs, the sites should be **re-sampled individually,** along with additional swabs in the area.

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

FSIS Expectations for Testing Methods

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

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

The following are FSIS's expectations for testing methods:

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

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

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

3) **The method has been validated**. All screening methods should either be used by a regulatory body (e.g., FDA Bacterial Analytical Manual (BAM)), or validated by a recognized independent body (e.g., AOAC, AFNOR, ISO, NordVal, Microval). A validated method from a scientifically robust study using the FSIS *Lm* qualitative method as a reference method, or other validated cultural methods is also acceptable, but would be subject to FSIS review.[5] Test kit developers should refer to FSIS guidance on the design of validation studies for pathogen testing methods, found at: http://www.fsis.usda.gov/PDF/Validation_Studies_Pathogen_Detection_Methods.pdf.

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

[5] Submit request for review of methods to AskFSIS (http://askfsis.custhelp.com)

FSIS Review of Sampling and Testing Methods

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

3.6 Other Routine Sampling

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

Testing Indirect and Non Food Contact Surfaces (NFCS)

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

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

Indirect FCS sites include the following:

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

NFCS sites include the following:

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

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

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

Product Testing

Although product testing is **not** required by the *Listeria* Rule (except under hold and test conditions for Alt. 2b or 3), establishments may decide to test product as part of their *Listeria* Control Program. Product testing can be used as a verification of the effectiveness of establishments' PLTs, AMAs, and sanitation control measures. Also, as most of FSIS testing is of product (ALLRTE and RTE001 testing programs), product testing by the establishment can help to detect product contamination before it is found through FSIS testing.

Product that tests positive for *Lm* would be considered adulterated and the establishment would be expected to recall the product if in commerce and destroy or rework the product with a process that is destructive of *Lm*. A product that is positive for *Listeria*. spp. or LLO is not summarily determined to be adulterated, however, without compelling documentation the establishment may not be able to conclude that the product is not adulterated. In order to support that the product is not adulterated, the establishment should perform further **confirmation testing** of the same sample enrichment to determine if it is positive for *Lm*, or provide compelling evidence why the product is unlikely to be contaminated with *Lm*. It would **not** be sufficient for the establishment to retest another sample or samples from the same lot to demonstrate that it is negative. Many establishments choose to test product quarterly as part of their *Listeria* Control Programs. Product testing protocols are typically designed and validated for a 25-gram analytical portion (i.e., the portion of the collected sample that is actually tested). Before testing larger analytical portions from single or multiple composited samples, ensure that the testing method has been validated for use with the larger portion.

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

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

Production lot

A production lot is the amount of product that may be impacted by a product or FCS positive test result. As previously stated, if the product or FCS sample tests positive for *Lm*, the production lot may be recalled if it has been released into commerce. A production lot is typically defined as all product produced from clean-up to clean-up unless the establishment can support a smaller lot size. If the establishment performs a complete cleaning and sanitizing (following the procedures in its Sanitation SOP) between lots, the lot size could be reduced. Factors that should be taken into account when determining lot size include RTE source materials used, frequency of cleaning and sanitizing, and processing steps.

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

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

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

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

3.7 Glossary

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

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

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

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

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

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

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

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

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

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

3.7 References

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

FSIS Microbiology Laboratory Guidebook, 1998.

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

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

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

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

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

Appendix 3.1: FSIS Sampling Programs

ALLRTE

The ALLRTE sampling program began in January of 2004 and was designed to obtain random samples across all RTE products and across all establishments producing a RTE product, regardless of risk. As in the RTE001 sampling program, products are sampled for *Lm* and *Salmonella*. In the ALLRTE program, however, both post-lethality exposed and non-post-lethality exposed products are tested and samples are randomly selected by FSIS.

Question: If a sample is a cook- in-bag product and not post-lethality exposed, will FSIS collect a sample?

Answer: Yes. Under the ALLRTE sampling program, FSIS collects samples of all RTE products, even if they are non post-lethality exposed (e.g. cook-in-bag). Under the RTE001 program, only post-lethality exposed samples are collected.

Samples are scheduled for the ALLRTE program so that all RTE establishments, regardless of plant size production volume or process design, have an equal chance of being sampled each fiscal year. One two-pound sample of product produced at the establishment is collected and sent to FSIS laboratories for testing.

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

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

Regulations and directives specific to ALLRTE include the following: FSIS Directive 10,240.4, Revision 2 "Verification Procedures for Consumer Safety Inspectors for the *Listeria monocytogenes* (*Lm*) Regulation and *Lm* Sampling Program," February 3, 2009; 9 CFR 430.4 "Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products" published on June 6, 2003 (68 FR 34207) [includes definitions for RTE Alternatives 1, 2, and 3]; and FSIS Directive 10,210.1, "Unified Sampling Form," October 14, 1997.

RTE001

The RTE001 sampling program is a risk-based verification testing program, implemented in January 2005 with the issuance of FSIS Notice 61-04. This sampling program is used primarily to verify that establishments producing post-lethality exposed RTE meat and poultry products, are controlling *Lm* and are in compliance with the requirements of the *Listeria* Rule. In this program, products are sampled for *Lm* and *Salmonella.* Establishments are identified for sampling based on a risk ranking algorithm, which takes into account the control alternative,[6] the production volume, the type of product produced, and the sampling history. FSIS is considering combining the RTE001 and

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

ALLRTE sampling programs so that all RTE samples are collected under one sampling program.

Regulations and directives specific to the RTE001 sampling program include the following: 9 CFR 430.4 "Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products" published on June 6, 2003 (68 FR 34207) [includes definitions for RTE Alternatives 1, 2, and 3]; FSIS Directive 10,240.4, Revision 2, "Verification Procedures For Consumer Safety Inspectors for the *Listeria monocytogenes* (*Lm*) Regulation and *Lm* Sampling Programs," February 3, 2009.

RLm

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

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

For the RLm program, FSIS collects 3 sample units from large establishments (500 or more employees), 2 sample units from small establishments (10-499 employees) and 1 sample unit from very small establishments (< 10 employees). A sample unit consists of 10 food contact surface swabs, 5 environmental swabs (which are composited), and 3 intact product samples. FSIS plans to increase the number of RLm and IVT product samples

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

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

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

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

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

from 3 to 5 samples per unit and composite the 5 RLm product samples. In establishments that use brine chillers, the EIAO is to collect a sample of brine from each line using a brine chiller.

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

Regulations and directives specific to the RLm sampling program include the following: 9 CFR 430.4 "Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products" published on June 6, 2003 (68 FR 34207) [includes definitions for RTE Alternatives 1, 2, and 3]; FSIS Directive 10,240.5, Revision 2, "Verification Procedures for Enforcement, Investigations, and Analysis Officers (EIAOs) for the *Listeria monocytogenes* (*Lm*)Regulation and Routine Risk-Based *Listeria monocytogenes* (RLm) Sampling Program," February 3, 2009.

IVT

In the IVT sampling program, FSIS tests product, food contact surfaces, and environmental surfaces for *Lm.* An IVT is initiated after an establishment has a positive *Lm* result, in either finished product or on a food contact surface. An IVT can also be initiated at the discretion of the District Manager, in response to continuing sanitation non-compliances at the establishments. The IVT is performed after the establishment has taken its corrective and preventative measures in response to FSIS findings. In an IVT**,** FSIS collects samples in units. A unit consists of 10 food contact surface samples, 5 environmental samples, and 3 product samples per post-lethality exposed RTE processing line in operation on the day of sampling. FSIS plans to increase the RLm and IVT product samples from 3 to 5 samples per unit. If the establishment uses a brine chiller, FSIS will also collect 1 brine sample per line from the brine chiller. IVTs are performed with a "for cause" Food Safety Assessment (FSA) to provide an in-depth evaluation of food safety systems at the establishment.

IVTs are scheduled according to the FSA prioritization model, with all establishments with *Lm* positives receiving an IVT. The districts have 30 days in which to schedule the IVT.

Regulations and directives specific to IVT include the following: 9 CFR 430.4 "Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products" published on June 6, 2003 (68 FR 34207) [includes definitions for RTE Alternatives 1, 2, and 3]; FSIS Directive 10,300.1, "Intensified Verification Testing (IVT) Protocol for Sampling of Product, Food Contact Surfaces and Environmental Surfaces for *Listeria monocytogenes*," February 3, 2009; FSIS Directive 10,210.1, "Unified Sampling Form", October 14, 1997.

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

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

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

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

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

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

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

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

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

Appendix 3.2: FSIS Sampling Procedure

I. Sampling Using SpongeSicles® For Food Contact and Non-Food Contact Surface Sampling

Equipment needed:
Sterile gloves
SpongeSicles®
10 ml tubes of Dey Engley or other neutralizing broth
Marker to label the sample bag

1. Wash and sanitize hands to the mid-forearm. Aseptically place a sterile glove on the hand used for swabbing by:

    a. Positioning the glove package so that the L and R (L=left, R=right) are facing the sample collector. When the package is open, the gloves are folded, forming a cuff on the sleeve and lying palm up. Leave them in the package until ready for use;

    b. Holding the glove for the hand that will be used for swabbing by the inside cuff area. Inserting hand into the glove, palm side up, and lifting the glove from the package.

    c. Pulling the glove completely on, touching only the fold cuff with your ungloved hand. Do not touch the sterile outside surface of the glove with your ungloved hand. Unroll the fold of the glove. Do not touch any non-sterile surface (clothes, counter tops, or the outside of the bag containing the SpongeSicle®) with the sterile glove. The other hand can be left ungloved for the manipulation of non-sterile surfaces and materials.

2. Using the ungloved hand, open the bag containing the SpongeSicle® by pulling off the clear perforated strip at the top of the bag;

3. Pull apart the white tabs to open the mouth of the bag;

4. Aseptically pour 9-10 ml of sterile Dey-Engley (D/E) broth into the bag to hydrate the SpongeSicle®, being careful not to contaminate the broth or sponge during the transfer. If the D/E broth is not purple, discard the tube;

5. Press the mouth of the bag back together;

6. Evenly moisten the SpongeSicle® by using hand pressure on the outside of the bag to massage the sponge;

7. Position the SpongeSicle® so that the handle is sticking out of the bag. Press the top of the bag back together around the handle;

8. Through the bag, squeeze the excess broth gently out of the sponge. Do not let your hand go past the thumb stop on the handle;

9. Carefully take the SpongeSicle® out of the bag by grasping the handle and swab the area selected. Be careful to maintain sanitary conditions when sampling and collect the samples aseptically. Do not let your hand go past the thumb stop on the handle

10. Swab at least a 1' X 1' square of food contact or environmental surface area, if possible;

11. Swab the chosen area using firm and even pressure:

    a. Vertically (approximately 10 times); then

    b. Flip the sponge and use the other side to swab horizontally (approximately 10 times); then

    c. Swab diagonally, using the same surface side as you used for horizontal (approximately 10 times).

12. Open the bag and insert the sponge portion of the SpongeSicle® back into the bag;

13. Grip the SpongeSicle® through the bag and bend the handle of the SpongeSicle® back and forth with slight force, while gripping the sponge through the bag. The stick should break easily within the sponge (do not break the handle at the thumb stop). Discard the broken handle. If the handle is sticking out above the sponge, discard the sample. Take a new sample following the same steps in VIII. C. 2-14;

14. Squeeze as much air out of the bag as possible and fold the top of the bag down at least 3 times. Fold in the tabs to lock the fold in place;

15. Label the bag with the date and location of the sample.

16. Ship the sample or deliver it to the laboratory as soon as possible for analysis.

II. Liquid Sampling for Brine

Equipment needed:
Sterile gloves
500 ml sterile pitcher or other sample collection device
1000 ml sterile bottle
90 ml D/E broth
Marker to label the sample

1. Wash and sanitize hands to the mid forearm. Wear sterile gloves on both hands when collecting a sample;

2. Aseptically pull a 500 ml sterile pitcher (beaker with a handle) from its packaging, being careful not to let the pitcher touch any non-sterile surface, including the exterior of the packaging;

3. Open a collection bottle and with the pitcher aseptically transfer 500 ml of the chill water or brine using the gradations on the side of the collection bottle to ensure the proper volume;

4. Aseptically add 90 ml of D/E to each sample collected to neutralize chlorine and other disinfectants;

5. Tightly cap the collection bottle and gently mix by rotating back and forth;

6. Label the bottle with the date and sample location

7. Send or deliver to the laboratory as soon as possible.

Appendix 3.3 Sample Collection and Testing Methods

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

Sample Collection Methods

As part of its *Listeria* Control program, the establishment should provide written instructions for collecting FCS samples, and product and NFCS samples (if performed). The sampling procedure used by FSIS to sample FCSs, NFCSs, and brines during IVT or RLm sampling is provided in Appendix 3.2. Establishments may use this method to sample their FCSs, or adjust the method based on the needs of the establishment. It is important that establishments use appropriate sampling method, to ensure that low levels of *Lm* or *Listeria* spp. are detected in the post-lethality processing environment. FSIS expectations for sample collection methods can be found in Section 3.5. Some establishments use sentinel site programs to collect samples of FCS, NFCS, and product. An example can be requested at the following link: http://www.tysonfoods.com/Business-to-Business/FoodSafety/~/link.aspx?_id=8B6CFFCFC897444E81DC3C6D06D175D5&_z=z.

Laboratory Methods

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

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

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

Establishments may choose to test for *Lm*, *Listeria* species (*Listeria* spp.), or *Listeria*-like organisms (LLO). While *Listeria* spp. and LLO are appropriate indicators for *Lm,* most establishments choose to test for *Listeria* spp., because it is more closely related to *Lm*. In many cases, laboratory tests for *Listeria* spp. are the same initial tests that are used to screen for *Lm.*

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

- Tests for *Listeria* spp. include immunoassays (e.g., lateral flow immunoassays, enzyme linked assay) and nucleic acid based assays (e.g., polymerase chain reaction (PCR), reverse-transcriptase PCR, DNA hybridization).

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

- If the establishment tests for Aerobic Plate Count (APC), Total Plate Counts (TPC),Total Viable Count (TVC) or bioluminescence-based testing for organic contamination as an indicator for sanitation, they may use the results to indicate where increased *Listeria* testing may be needed. *Howeve*r, these tests cannot be used to meet s*ampling* requirements for *Lm*, *Listeria* spp. or LLO. For more information on use of these tests for to verifying sanitation see .

Confirmation Methods

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

1) Culture-based Confirmation

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

2) Non-Culture-based Confirmation

Non-cultural methodology does not involve a cultural isolation step, and consists of a single test (e.g., a PCR-based test). **This type of confirmatory test is always performed on the same sample and enrichment broth as the screening test**. The non-cultural test should identify a different set of characteristics than the screening test (in other words, the same test used for screening, or a similar test, may not be re-used to "confirm" the screening result). The non-cultural confirmation test should provide high sensitivity and enhanced

specificity (ability to detect true negative results) compared to the screening test and it should be demonstrated and documented to perform acceptably under the conditions of use, which includes the enrichment conditions for the screening test (e.g., enrichment time, temperature, enrichment broth). Acceptable performance is determined by validation, preferably through an independent organization (e.g., the Association of Analytical Chemists (AOAC), Association Française de Normalization (AFNOR), ISO, or NordVal).

Recording Testing Results

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

The records should include the following:

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

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

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

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

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

observations at an establishment) and should not be used by itself to demonstrate a definitive link between the product and the illness during outbreak investigations.

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

1. Cross contamination is suggested if an identical or highly similar PFGE pattern is found in product and surface samples collected during the same production day. If an identical pattern is found on product and a surface, the surface is more likely to be the source, unless under-processing of RTE product is suspected.

2. Harborage or ongoing contamination of the post-lethality environment is suggested if an identical or highly similar pattern is found in product and surface samples collected over multiple days, weeks, or months.

3. Food-borne exposure is suggested if the identical PFGE pattern is found in FSIS and case-patient samples, especially if the pattern is rare.

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