Food Safety and Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

R*Lm* and IVT Sampling
for Condensed EIAO

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Overview

- Purpose of FSIS sampling.
- Pathogens and products of concern.
- FSIS **Directive 10,240.5 -** The R*Lm* Sampling Directive.
- FSIS **Directive 10,300.1 -** The IVT Sampling Directive.
- Common Instructions for both R*Lm* and IVT sampling.
  - When establishments change practices.
  - Results and Enforcement.

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Products for R*Lm* or IVT sampling

- Ready-to-eat (RTE) products.
- R*Lm* and IVT sampling is only for Post-Lethality Exposed (PLE), RTE products (9 CFR 430).
- Production lots are typically defined as from clean-up to clean-up.

RTE product (9 CFR 430.1) - A meat or poultry product that is in a form that is **edible without additional preparation to achieve food safety** and may receive additional preparation for palatability or aesthetic, epicurean, gastronomic, or culinary purposes.

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Primary Pathogens of Concern

Listeria monocytogenes (Lm) and ***Salmonella***

- Both can cause human disease.
- Both can be cross contaminated to products from contaminated equipment or the environment.

The presence of ***Lm*** in a RTE product is typically due to post-lethality contamination.

The presence of ***Salmonella*** in a RTE product may be an indicator of a lethality process failure, but it can also be a result of post-lethality contamination.

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Primary Pathogens of Concern

According to the *Listeria* Rule (9 CFR 430.4), establishments produce RTE PLE meat and poultry products must implement control of *Listeria* and its indicator using one of three *Lm* control alternatives.

Risk of 9 CFR 430.4 production alternatives, from most risky to least risky:

Alt 3 (sanitation alone to control *Lm*)
Alt 2b (AMAP + sanitation)
Alt 2a (PLT + sanitation)
Alt 1 (PLT + AMAP + sanitation)

Decreasing Risk

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Purpose

- **Help** verify that the establishment's process is producing safe, wholesome, unadulterated product
- **Help** verify the adequacy of establishment's HACCP plan, Sanitation SOP, and prerequisite programs.
- The **R*Lm*** sampling program is "routine, risk based" and is intended to **help** verify the adequacy of an establishment's *Listeria* control program, per 9 CFR 430.4.
- The **IVT** sampling program is done for cause, e.g., to **help** verify corrective actions, typically following a previous positive.

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Slide 6

CM0 Should you use “establishment’s” instead of “plant’s” in bullets 1-3?
Miller, Carla - FSIS, 2025-05-19T18:12:22.192

EL0 0 Agree!
Li, Echo - FSIS, 2025-05-19T19:29:56.570

Purpose

- *Listeria* contamination is typically intermittent and non-homogenous.
- Sampling is a snapshot in time.
- **All negative results do not confirm that *Lm* is being controlled.**

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Sampling Key Terms

- **Food Contact Surface (FCS)**
  - A surface in the post-lethality processing environment that comes **in direct contact with RTE product** (9 CFR 430.1).
- **Non-Food Contact (Environmental) Surface (NFCS)**
  - An area that **does not contact product.**
- **Product Sample**
  - Products in the final, intact package.

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Lm Sampling: FCS Examples

General	Cooking and Cooling	Employees	Packaging
• Tables • Conveyor Belts • Cutting Boards • Blades of slicers, shredders, dicers, saws. • Chutes and hoppers.	• Racks • Pans • Tubs • Brine for chilling	• Utensils: knives, tongs, thermometers • Aprons (touches product) • Gloves/hands (touches product)	• Low priority • Storage and handling of packaging material • Film wrap • Bags • Soaker pads

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Lm Sampling: Environmental, NFCS Examples

NFCSs

- Drains, floors, floor mats, boots, etc.
- Wheels of equipment, e.g., carts and pallet jacks
- High contact areas, door jambs, knobs, etc.
- Anything associated with moisture or condensation: overhead surfaces, squeegees, drip pans, etc.

Collect near FCSs

- Handles, switches, and control panels
- Bottom and side edges of chairs or tables used by employees
- Equipment that overhangs product
- Sides or undersides of conveyers
- Oven smokehouse exit areas

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NFCS Sampling Sites Examples

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Sampling Key Terms

- **Post-lethality Processing Environment**
  - The area in an establishment into which product is routed after having been subjected to an initial lethality treatment. The product may be exposed in this area as a result of further processing. (9 CFR 430.1)
- **Production Line** (from the *Lm* Compliance Guideline)
  - A line refers to the flow of product during production. This includes all equipment, personnel, and utensils that contact the RTE product. Multiple individual product lines can meet at a piece of equipment (e.g., packaging machine), but can still be considered multiple lines.

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Slide 12

CM0 Is this defined somewhere?
Miller, Carla - FSIS, 2025-05-19T18:18:16.246

EL0 0 Spelled out!
Li, Echo - FSIS, 2025-05-19T19:37:49.525

Reason to perform R*Lm* vs IVT

- **R*Lm***
  - R*Lm* sampling could be performed at the DO's discretion to help inform the Public Health Risk Evaluation (**PHRE)** recommendation for a PLE, RTE processor could be used as apart of an Assessment Plan when an **FSA** is recommended to assess the PLE, RTE processes.
- **IVT**
  - If a "**for cause**" trigger is relevant to RTE production, i.e., a positive pathogen test result in RTE product, and if sampling is desired, then "for cause" IVT sampling is to be performed, not R*Lm* sampling.

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R*Lm* and IVT Directives

- **R*Lm***
  - **Directive 10240.5**, Verification Procedures for Enforcement, Investigations and Analysis Officers for the *Listeria Monocytogenes* Regulation and Routine Risk-Based *Listeria* Monocytogenes Sampling Program
- **IVT**
  - **Directive 10300.1**, Intensified Verification Testing (IVT) Protocol for Sampling of Product, Food Contact Surfaces and Environmental Surfaces for *Listeria Monocytogenes*

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Slide 13

CM0 Spell out?
Miller, Carla - FSIS, 2025-05-19T18:20:13.955

R*Lm* vs. IVT

	R*Lm*	IVT
Collect samples in Units	For R*Lm*s, a unit consists of: • 5 product samples • 10 FCS swab samples • 5 NFCS swab samples	For ***Lm*** IVTs, a unit consists of the same number of samples as R*Lm* unit.
		For ***Salmonella*** IVTs, a unit consists of same number of samples as RLm unit.
How many units?	Based on establishment size: • Large est. : up to 3 units • Small est. : up to 2 units • Very small est. : 1 unit	1 sampling unit for each post-lethality exposed RTE line, maximum 5 units. Sample all lines if the establishment has less than 5 lines.

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R*Lm* vs. IVT

R*Lm*	IVT
Notify the establishment **at least one week before** the R*Lm* sample collection	Notify the establishment **at least 48 hours before** IVT collection
Collect 5 NFCS samples using 5 separate sponges, **analyze** all 5 individual products and NFCS samples **together**	Collect 5 NFCS samples using 5 separate sponges, **analyze** the 5 samples **individually** by laboratory
Only **one sample form** for **all five samples**	**One sample form** for **each** sample
Test for *Lm* **and** non-*Lm Listeria spp.*	Test for *Lm* **or** *Salmonella* **and** non-*Lm Listeria spp.*

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Slide 15

CM0 Are these swab samples?
Miller, Carla - FSIS, 2025-05-19T18:27:44.126

EL0 0 added
Li, Echo - FSIS, 2025-05-19T19:38:52.571

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R*Lm* and IVT: Product Samples

- Collect **5 product samples** in an intact package associated with a particular production lot
- Collect enough intact product so that enough of meat or poultry **per individual product sample** is submitted to the lab for analysis
  - **R*Lm*: At least ¼ pound or four ounces**
  - **IVT: at least ONE pound**
  - Slack-fill or multiple finished packages
  - How to Collect 1-Pound of Product for Multi-Component RTEPROD Sampling
- Collect product samples over the production shift, if possible.
- Samples should be stored under refrigeration before analysis, unless the products are shelf stable products. Samples should be properly labeled to avoid confusion regarding testing results.

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R*Lm* and IVT: FCS Samples

- Some samples can be collected at pre-op, but most samples should be collected **at least 3 hours into operations**, if possible, to allow *Lm* to work its way out of the equipment. If the establishment typically produces RTE product for less than 3 hours, then the samples can be collected less than 3 hours into operations.
- Collect **10 FCS swab samples per unit** from the post-lethality exposed processing area where the sampled product lot was produced

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- Collect samples starting closest to the product areas and then move further out (i.e., collect food contact surfaces first and then environmental samples);

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Slide 17

RF0 Content doesn't all fit on the slide
Rupert, Joshua - FSIS, 2025-05-19T18:02:01.783

EL0 0 fixed
Li, Echo - FSIS, 2025-05-19T21:07:52.041

Slide 18

CM0 Do you need to specify swab sample here and on next slide?
Miller, Carla - FSIS, 2025-05-19T18:40:29.213

R*Lm* and IVT: NFCS Samples

- Collect **5 NFCS swab samples per unit** from the post-lethality exposed processing area where the sampled product lot was produced
- **NOTE:** If the surface is not in direct contact with the product, it is a NFCS.

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RLm and IVT: Brine Sample

Collect **one brine sample per unit or line** (depending on number of brine chillers in use).

Might be FCS or NFCS

- If the casing is **permeable or semi-permeable** then the brine is considered a food contact surface (FCS) sample - use one of the FCS forms (RLMCONT)
- If the casing is **impermeable** then the brine is considered a non-food contact surface sample (NFCS) - requires an extra form (RLMENVR).

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Both RLm and IVT Sampling

- RTE sampled lots are typically defined as all product produced from clean-up to clean-up.
- Samples must be collected during conditions that are representative of routine processing.
- Collect product after the establishment has applied all interventions intended to reduce or control pathogens of concern, per their HACCP program and Hazard Analysis (HA).
- If a treatment is applied only for quality purposes, e.g., to extend shelf life, then product samples may be collected before such a process has been applied.

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Sampling Roles

- Sampling team members and roles:
  - Team Leader/ Sample Collector (EIAO).
  - Sampling Assistant (CSI, PHV, IIC, FLS, etc.).

- **Sample Collector:** FSIS personnel trained in R*Lm* and IVT aseptic sample collection techniques through completing EIAO training.

- **Sampling Assistant:** FSIS personnel who is **not** trained in R*Lm* and IVT aseptic sample collection techniques or did **not** complete EIAO training.

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RLm and IVT: Getting Ready

FSIS personnel may:	Sample Collector	Assistant
• Communicate with the establishment regarding the sampling and document the notification in MOI		
• Schedule sampling with the laboratory and in PHIS		
• Pre-fill sample forms with known information		
• Print out and sign the pre-filled sample forms		
• Check sampling supplier received from lab		
• Freeze ice packs		
• Bring all necessary tools to the establishment		
• Select sampling locations		

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Dey-Engley (D/E) Broth and Pre-hydrated Sponges

- D/E broth can be held at room temperatures for long periods of time, e.g., during sampling.
- Should be refrigerated for long term storage.
- Printed shelf life is dependent on refrigeration.
- Must not be temperature abused.
- The higher the temperature, the faster DE broth degrades.

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Slide 23

CM0 Do these need check mark in the boxes that apply??
Miller, Carla - FSIS, 2025-05-19T18:50:58.220

EL0 0 The yellow color is indication of what applies
Li, Echo - FSIS, 2025-05-19T21:08:59.030

Slide 24

CM0 Spell out Dey-Engley (D/E) on first use
Miller, Carla - FSIS, 2025-05-19T19:00:03.443

RLm and IVT: On Sampling Day

FSIS personnel may:	Sample Collector	Assistant
• Label your sample containers for each unit before entering processing area		
• Follow the establishment's GMP when entering processing area – clean coat, hair net, etc.		
• Choose a staging area for gathering and preparing your sampling supplies		
• Wash your hands and forearms and dry them with a paper towel		
• Clean and sanitize your work surfaces using the establishment's sanitizer per the direction for use		

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Aseptic Sampling

Choose a staging area for gathering and pre-paring your sampling supplies.

Use a wheeled, stainless steel cart and a small tote or caddy to transport your supplies and the sample to and from the sample collection location.

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Slide 26

RF0 I don't think i'd use that caddy picture as it has raw beef sampling supplies.
Rupert, Joshua - FSIS, 2025-05-19T18:19:33.396

Aseptic Sampling

Label your sample containers before collecting the sample.	

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Aseptic Sampling

Wear a clean lab coat and hair net to avoid contamination. Follow your plant's garment requirements when you collect the samples.	

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RLm and IVT: On Sampling Day

FSIS personnel may:	Sample Collector	Assistant
• Place the sampling supplies on the cleaned and sanitized on the work surface		
• Wash and sanitize your hands to the mid-forearm		
• Put on gloves		
• Tear off the top of the bag where indicated. Pull tabs to open bag.		
• Keeping hands outside the bag, guide the handle out the top of the bag. Through the bag, squeeze the excess broth gently out of the sponge.		

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Aseptic Sampling

Wash and sanitize your hands up to the mid-forearm. Dry your hands using disposable paper towels. If a sink is not available at the sample collection location, use a waterless sanitizer. Wash your hands prior to sanitizing the work surface as well. (See *Aseptic Sampling* on IPP Help).	 

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Aseptic Sampling

After cleaning your hands and forearms, clean and sanitize your work surfaces. Use the same sanitizing solution the establishment uses, according to label directions. Allow the surface to air dry completely prior to placing any sampling utensils on it.	

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R*Lm* and IVT: On Sampling Day

FSIS personnel may:	Sample Collector	Assistant
• Using the gloved hand, carefully grasp the handle above the thumb stop and remove the device from the bag.		
• Swab at least a 1' x 1' square of surface area by **pressing down firmly** and flex the handle to ensure the entire sponge head makes full contact with the sample surface. Vigorously scrub back and forth in one direction across the sample surface. FLIP the sampling device over. Change direction 90° and vigorously scrub back and forth across the same surface area. Then swab diagonally, using the same surface side as you just used.		
• Open the bag		

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RLm and IVT: On Sampling Day

FSIS personnel may:	Sample Collector	Assistant
• Insert the sponge portion back into the bag		
• Hold sponge from the outside of the bag		
• Twist off the handle by turning counter-clockwise.		
• Roll down the top of the sample bag at least 3 times and fold wire ties over to securely close.		
• Dispose gloves		
• Verify sampling location with establishment		
• Document sampling information (time and site) on collection logs or sample container		

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Aseptic Sampling

When you collect liquid samples in a jar with a lid, hold the lid in one hand while collecting the sample.

If any product spills on the outside of the jar, cap the jar and wipe it clean with a dry paper towel. Do not use any sanitizer solution to clean the jar.

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Document Sample Sites

- Some pieces of equipment have both FCSs and NFCSs
- Notate exactly where each sample was collected
- **Take good sampling notes**

Activity

Question: Which part is a FCS area?
Answer: Employee hands, the blade, hopper, etc.
Question: Which part is a NFCS?
Answer: The slicer handle.

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Sampling Template Example

Establishment Name/ Number		RLMCONT	Line #	Date
1	Sample Set # sticker	Time	Room	Laboratory Form #
		Description of Sampling Site		
2	Sample Set # sticker	Time	Room	Laboratory Form #
		Description of Sampling site		
3	Sample Set # sticker	Time	Room	Laboratory Form #
		Description of Sampling Site		

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Sampling Template Example

#	Date	Time / Shift	Room / Line	Laboratory Form #	Sampling Site Description	Type of Sample	Sample Seal #	
1	03/13/07	Pre-op	Packing / 1	11000243	Splitter blade	CONT	632831	
2		Pre-op	Cooler / 1	11000244	Probe thermometer # 1	CONT	632832	
3		0900 hr	Packing / 1	11000245	Pack room cutting table	CONT	632833	
4		0900 hr	Packing / 1	11000246	Bagger flaps	CONT	632834	
5		0900 hr	Packing / 1	11000247	Splitter table	CONT	632835	
6		1145 hr	Cooler / 1	11000248	Probe thermometer # 4	CONT	632836	

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R*Lm* and IVT: On Sampling Day

FSIS personnel may:	Sample Collector	Assistant
• Sanitize your hand using sanitizer from the lab.		
• Repeat to collect another sample		
• Collect product samples		
• Return to sample packing area to package samples		
• Complete printed sample forms		
• Package samples and forms, including sealing sample boxes		
• Drop off shipping boxes at shipping center		
• Complete sample forms in PHIS		

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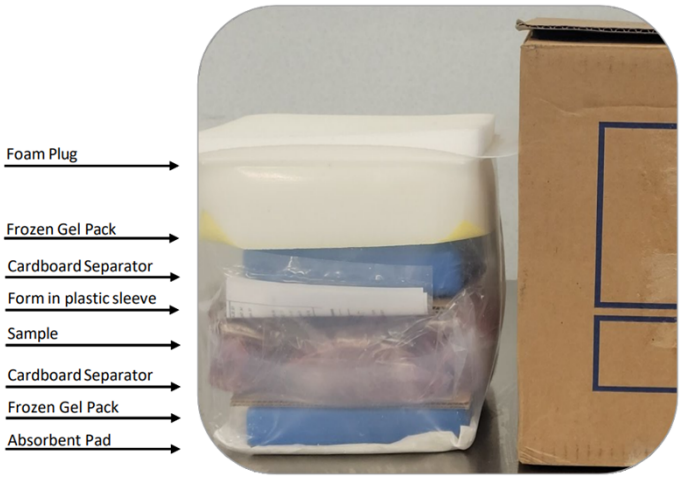
Packing the Samples

Figure 2: How to Pack a Sample for Shipment



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RLm and IVT Sampling, Shipping, or Packaging Issues

- Short of supplies – check sampling supplies upon receipt.
- Improper use of gel packs - Frozen gel packs should be removed from the freezer and placed into shipping containers on the day of shipment.
- Does not follow establishment procedures – review operational SSOPs, GMPs, etc., before sampling.
- Establishment drastically changes operation to the degree it is no longer representative of routine processing.

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Changing Practices During R*Lm* or IVT sampling

Changing practices = implementing changes that are not consistent with their documented food safety system;

- Drastically reducing the typical production time and/or the lot size.
- Temporarily increasing the use of sanitizer.
- Selectively not producing higher risk product (e.g., PLE, RTE line with history of previous *Listeria* positives).
- Not using a line or specific equipment that previously has tested positive (e.g., equipment associated with positive product or FCSs).

Changing practices interferes with FSIS's assessment of the adequacy of their process and food safety system.

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Changing Practices During R*Lm* or IVT sampling

If the establishment changes practices and cannot provide a **supportable rationale;**

- Contact your District Office (DO).
- Do not collect samples if they are not representative of routine processing conditions or practices.

- May recommend that IPP issue a noncompliance report (NR);
  - 416.14 – changes were not incorporated into their SSOP.
  - 417.2(a) - the establishment did not consider or document the changes in its hazard analysis (HA).
  - 417.5(a)(1) - the establishment did not incorporate the supporting documentation from 417.2(a) in its HA.

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RLm and IVT Sampling, Shipping, or Packaging Issues

- Did not collect samples to cover all PLE processing steps.

- IPP are to be aware of the difference between the **sampled lot** and the **implicated lot** in the event of a positive.
  a. The **sampled lot** is product that is represented by the sample FSIS collects and analyzes for *Lm* and *Salmonella*. The establishment is responsible for defining the sampled lot.
  b. The **implicated lot** (or lots) is the product that may be connected to a sampled lot that tested positive through common source material or other root cause findings as described below. The implicated lots are determined by root cause findings and may be defined through investigations by FSIS, other public health agencies, the establishment, or foodborne illness findings.

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EIAO Sampling, Shipping, or Packaging Issues

- Examples of issues with aseptic technique;
  - Finger going below the thumb stop on the sponge stick.
  - Sponge contact with the lip of the bag when removing or replacing sponge.

- Collection related mistakes;
  - Not squeezing the broth out of the sponge before removing it from the Whirl-Pak bag.
  - Not squeezing air out of the Whirl-Pak bag before folding down the top.
  - Thinking NFCS samples must be collected near the line.
  - Thinking samples must be collected at pre-op.

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Slide 44

RF0 Bold font for title? Words are not the same color as previous slides. This applies to the next three slides.
Rupert, Joshua - FSIS, 2025-05-19T18:30:35.658

EIAO Sampling, Shipping, or Packaging Issues

Including samples from more than one line or lot within one unit of samples.

- Each unit should be associated with only one line, one production lot, and one 430 Alternative.
- If not, leads to confusion when positives are found.
- Which production lots are implicated by the positive?

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Issues with Forms, Seals, Bar- Coded Labels

- There must be a corresponding form for all samples within each shipping container.
- Each form must be signed.
- Each shipping container should be sealed to ensure sample integrity, as illustrated in Directive 7355.1 *Use Of Sample Seals For Laboratory Samples*.

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Results and Enforcement

- Check LIMS or PHIS for results.
- Inform the establishment of the results.
- If any **product sample tests positive**, the entire sampled lot is adulterated.
- If an **FCS sample tests positive**, all product which passed over the FCS (the sampled lot) is adulterated.
- Discusses with establishment about corrective actions.

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Results and Enforcement

Actions in response to adulterated product released into commerce;

- If FSIS obtains a **product or FCS sample positive for *Lm*** and the establishment **did not hold or maintain control of the sample lot**, **EIAOs are to immediately contact their DO**.
- The DO will take appropriate administrative action and contact the Recall Management and Technical Analysis Division (RMTAD).
- As appropriate, FSIS will request a recall or detain the product.

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Results and Enforcement – Non-*Lm Listeria* spp.

If **FCS, NFCS, or product test positive for Non- *Lm Listeria* spp.**

- Notice 50-24
- Results reported in PHIS
- Inform the establishment of the results and required actions if applicable
- Verify the establishment take Corrective Actions per 416.15, document NR per 416.15 if needed
- Document the discussion in an MOI
- Product okay to ship
- Indication that sanitation program is ineffective at preventing conditions where contamination with *Lm* may occur and may have failed to prevent direct contamination of product resulting in adulteration.

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Whole Genome Sequencing (WGS) – Directive 10,240.6

WGS is used to determine whether two or more *Lm* isolates are related.

The relatedness of *Lm* isolates is used to determine;

1. If there is evidence of **harborage** or **cross contamination**.
2. If an FSIS isolate is "potentially related to a clinical isolate" and whether the isolate is of interest to FSIS.

This information is distributed by email in WGS reports, typically sent 7-14 days after a confirmed *Lm* positive. WGS not performed for non-*Lm Listeria* spp. results

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Whole Genome Sequencing (WGS) – Directive 10,240.6

Harborage:

- The persistence of *Lm* in a processing environment over time.
- When two or more closely related *Lm* isolates are collected from the same establishment over multiple days, weeks, months, or years.

Cross-contamination:

- The transfer of *Lm* among food, FCS, or environmental, NFC surfaces.
- When two or more closely related *Lm* isolates are collected from the same establishment on the same day from product, FCS, or NFCS environmental samples.

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Whole Genome Sequencing (WGS) – Directive 10,240.6

WGS reports will indicate whether there is potential harborage, cross-contamination, or both, and whether the FSIS isolate is "potentially related to a clinical isolate(s)" and will provide further context as to whether the isolate is of interest.

If DO personnel recommend an enforcement action associated with the R*Lm* or IVT sampling, include the findings of the WGS report in the enforcement letter to further demonstrate an establishment's failure to control *Lm* in the post-lethality environment and to support enforcement. (FSIS Directive 5100.1)

Directive 10,240.6 instructs EIAOs on what to do with this WGS information and defines harborage and cross contamination.

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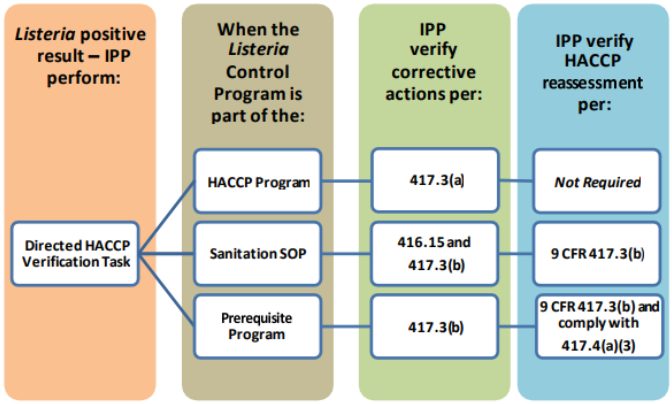
Verify Corrective Actions

- EIAOs are to verify the establishment meets corrective action requirements of 417.3(a), 417.3(b), and/ or 416.15.

Figure 2. Steps for Verifying an Establishment's Corrective Actions



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Questions?

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Slide 54

RF0 This is a great presentation! I tried to go through and italicize Lm to make it consistent. I added some periods too just for consistent use. All other comments are on a per slide basis.
Rupert, Joshua - FSIS, 2025-05-19T18:40:03.699

EL0 0 Thank you! Got it!
Li, Echo - FSIS, 2025-05-19T21:10:39.025