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

RLm and IVT Sampling

Overview

- Purpose of FSIS sampling.
- Pathogens and products of concern.
- FSIS **Directive 10,240.5**, Rev. 3 – The RLm Sampling Directive.
- FSIS **Directive 10,300.1**, Rev. 1- The IVT Sampling Directive.
- Common Instructions for both RLm and IVT sampling.
  - When establishments change practices.
  - Results and Enforcement.

Sampling Purposes

- **Help** verify that the plant's process is producing safe, wholesome, unadulterated product
- **Help** verify the adequacy of a plant's HACCP plan, Sanitation SOP, and prerequisite programs.
- The RLm sampling program is "routine, risk based" and is intended to **help** verify the adequacy of a plant'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.

Products of Concern for RLm or IVT sampling

- Ready-to-eat (RTE) products.
- RLm sampling is only for 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.

Primary Pathogens of Concern

Listeria monocytogenes (Lm) and *Salmonella* spp.

- Both can cause human disease.
- Both can be cross contaminated to products from contaminated plant 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.

FSIS Directive 10,240.5

Verification Procedures for EIAOs for the *Lm* Regulation and Routine, Risk-Based *Lm* (R*Lm*) Sampling Program.

"*The RLm Directive.*"

FSIS Directive 10,240.5 – 2

- Provides instructions for collecting RLm samples.
- An updated version of 10,240.5 was published on 11/02/2022.
- Significant changes include;
  - Prioritizing line sampling based on the risk of the 430-production alternative (future slide).
  - Sequence of establishments eligible for RLm sampling on the monthly spreadsheet is now sequenced based on risk, instead of a 4-year FSA cycle (next presentation).
  - The option for DOs to use RLm sampling to inform a PHRE vs. using R*Lm* sample results to inform an FSA.

FSIS Directive 10,240.5 – 3

RLm testing is;

- **R**outine, risk-based, sampling for ***Lm*** (abbreviated **RLm**).
- Intended for producers of PLE, RTE products only.
- Samples always tested for *Lm*. (RLm Sal testing is not an option.)
- Used to **help** verify compliance with 9 CFR 430.
- RLm results help inform an FSA outcome or;
- New option - use RLm sampling to **help** inform a PHRE outcome.

However;

- 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**.

FSIS Directive 10,240.5 - 4

Rlm sampling uses IVT methodology;

- Consists of product, FCS, and NFCS swab samples.
- Multiple samples collected as a part of sampling "units".
- Each unit has a defined composition, e.g., 5 product, 10 FCS, and 5 NFCS samples per unit.

Examples of significant differences with IVT sampling:

- RLm sampling is “routine, risk-based”. IVT sampling “for cause”.
- Different method for determining the number of units to collect.
- IVT samples are never composited.
- IVT samples may be tested for Lm or Sal (rare).
- Establishment notification timeframe is different.

FSIS Directive 10,240.5 - 5

Prior to RLm scheduling, the EIAO is to:

- Contact in plant inspection personnel (IPP) to gather information (typically a part of the PHRE).
- Ask about risk factors and any IPP concerns.
- Identify eligible products.
- Determine overall RTE production steps and processes.
- Is brine utilized to cool the PLE, RTE products?
- Identify possible day for sampling.
- Determine number of sample units to collect.

FSIS Directive 10,240.5 - 6

The number of RLm units to collect is based on establishment size;

- Large establishments = up to a maximum of 3 units
- Small establishments = up to a maximum of 2 units
- Very small establishments = maximum of 1 unit

Each unit must always be associated with one line, one production lot, and one production alternative.

Establishment size is based on establishment categories in the HACCP preamble (61 FR 38806);

- large establishments – 500 or more employees
- small establishments – 10 or more employees but fewer than 500
- very small establishments – fewer than 10 employees or annual sales of less than $2.5 million.

FSIS Directive 10,240.5 – 7

- Each RLm unit is composed of;
  - 5 **prod**uct samples, **c**omposited by the labs- one RLM**PRODC** form.
  - 10 food **cont**act surface (FCS) swab samples, not composited – 10 RLM**CONT** forms.
  - 5 **env**ironmental, nonfood contact surface (NFCS) swab samples, **c**omposited by the labs - one RLM**ENVC** form.

- **Brine** samples might be FCS or NFCS.
  - Permeable casing ➡ FCS – use one of the FCS forms (RLMCONT).
  - Impermeable casing ➡ NFCS - brine NOT composited with NFCS swab samples. Requires an extra form (RLMENVR).

FSIS Directive 10,240.5 – 8

Production Line (from the Lm CG): 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.

Prioritize sampling of lines based on risk;

- 9 CFR 430 Lm control Alternative.
- Sampling history (both FSIS and establishment sampling).
- Other risk factors such as recent or ongoing construction, condensation issues, use of high-pressure hoses in PLE, RTE area, etc.

FSIS Directive 10,240.5 – 9

According to the Listeria rule (9 CFR 430.4) *Lm* contamination of post-lethality exposed (PLE) products must be controlled 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

FSIS Directive 10,240.5 – 10

- Notify the establishment 1 week prior to Rlm collection date.
- Once at the establishment, hold entrance meeting;
  - ✓ Inform them of the sampling and how it will be conducted – may use entrance letter in Directive 10,300, attachment 1.
  - ✓ Must hold the sampled lot(s), pending the results of FSIS testing.
  - ✓ Confirm that they will produce PLE, RTE product and won't change routine production practices (because you will be sampling).
- Conduct walk-through.
- Prepare for sampling, e.g., stage supplies, etc.

FSIS Directive 10,300.1

Intensified Verification Testing (IVT) Protocol For Sampling of Product, Food Contact Surfaces, and Environmental Surfaces for *Listeria monocytogenes* (*Lm*) or Salmonella spp.

"*The IVT Directive.*"

FSIS Directive 10,300.1 – 1

Provides instructions on collecting product, FCS, and NFCS samples (that are never composited).

IVT sampling is always performed “for cause”, at the discretion of the District Office.

IVT sampling is usually, but not always, done in conjunction with an FSA.

FSIS Directive 10,300.1 – 2

Examples of for cause reasons (in Directive 5100.4) that could lead to IVT sampling;

- A pathogen positive from an FSIS RTE sampling program (*Lm* or *Salmonella* spp.).
- Product has been associated with human illness.
- To verify corrective actions before closing out an enforcement action.
- A RTE positive from another government entity.

FSIS Directive 10,300.1 – 3

The number of IVT units determined by the number of RTE lines;

- 1 IVT unit per line, up to 5 units maximum.
- Vs. RLms where units is based on establishment size.

IVT samples are never composited, thus an extra form for NFCS brine samples is **not** needed.

One unit for ***Lm*** IVTs consist of:

- 5 intact product samples, 5 forms (INTPROD).
- 10 FCS samples, 10 forms (INTCONT)
- 5 NFCS samples, 5 forms (INTENV).

FSIS Directive 10,300.1 – 4

Number of units is determined by number of lines, 1 unit per line, up to 5 units max. However;

One unit for ***Salmonella*** IVTs consists of:

- 5 intact, product samples - 5 INTPROD forms.
- 5 food contact swab samples – 5 INTCON forms
- 8 non-food contact (environmental) samples – 8 INTENV forms.

FSIS Directive 10,300.1 – 5

Notify the establishment 48 hours before the IVT (time to hold the product, but not time to change practices). Document this in a Memorandum of Interview (MOI) in PHIS.

At establishment;

- Conduct an entrance meeting (see IVT entrance letter).
  - ✓ Inform them of the sampling and how it will be conducted.
  - ✓ Must hold all sampled production lots, pending lab testing results.
  - ✓ Confirm that they will be producing RTE product and won't change routine production practices.
- Conduct walk through.
- Prepare for sampling, e.g., stage supplies, etc.

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.

Both RLm and IVT sampling – 1

- Instructions for establishments that change practices.
- Results and enforcement actions.

Planning the RLm and IVT

Steps to Sampling

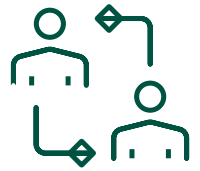
- Plan ahead and stay organized!
- Contact the in-plant inspection team.
- Schedule the date for sampling and order supplies
- Notify plant management.
- Assemble a team.
- Conduct Entrance Meeting with the establishment.
- Take walk through, consider where and what to sample.
- Organize sampling supplies.
- Collect/ Submit samples.

Contact IPP – prescheduling planning.

Example questions for IPP/FLS;

- What is the establishment size?
- How many RTE lines do they utilize?
- What RLm or IVT eligible products are produced?
- What are their RTE production processes, e.g., is brine or a PLT utilized, are they freezing, drying, fermenting, etc.?
- What are the production schedules of those products?
- How are the products packaged, e.g., size?
- Is the establishment Kosher? (DE broth is not Kosher.)
- What is the FSIS and establishment sampling history?
- What issues have they had? What concerns do you have?
- Consider FedEx. Pick up or drop off? When and where?

Scheduling a Sampling Date with FSIS Labs



At least 2 weeks prior to the week of sampling, submit a proposed collection date by sending an email to the correct address in Outlook:

For Rlms:

manually enter **RLMSampleScheduling@usda.gov**

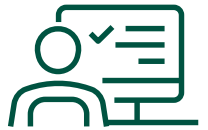
or search using "**FSIS - RLm Sample Scheduling**"

For IVTs:

manually enter **IVTSampleScheduling@usda.gov**

or search using "**FSIS - IVT Sample Scheduling**"

Ordering Supplies



- Ordering supplies and requesting a sampling date are done simultaneously.

- Attach a questionnaire to your email.

- Use prompts to ensure all necessary information is included;
  - Proposed collection date and shift.
  - Number of sample units required.
  - Designated field laboratory.
  - Establishment number.
  - Your contact information (cell).

Ordering Supplies – 2

Prompts continued;

- Physical location to send supplies;
  - Can be office, home, or the establishment
  - **Supplies must be checked on day of receipt**.
  - Refrigerate pre-moistened swabs and/or broth upon delivery for long term storage.
    - Can be held at room temperatures for short term
    - Must ensure no temperature abuse.

- Requests for special supplies;
  - Consider product package size ahead of scheduling. Are more or larger shipping containers needed?
  - Is a kosher broth needed?
  - Are brine sampling supplies needed?

Receiving the Supplies

- Supplies are typically mailed within a few days of the lab's emailed confirmation of a sampling date.
- If supplies are not received, inquire by resubmitting the lab confirmation response and a copy of the questionnaire back to the appropriate mailbox.
- If you receive the supplies, but did not receive everything you need, reply to the lab's confirmation response ASAP to tell them what you're missing.

Notifying Plant Management

- For IVTs
  - provide 48 hours advance notice or enough time to hold product, but not enough time to change practices.

- For RLms
  - provide one-week advance notice.

Assembling the Team

- Identify team members. May include FLS or FSIS in-plant personnel.

- Assign roles to team members.

- Possible team member roles;
  - Team Leader/ Sample Collector (EIAO).
  - Sample Collector Assistant (CSI, PHV, IIC, FLS, etc.).
  - Forms Assistant – complete and double check forms, pack, and prepare samples for shipment.

Entrance Meeting at the Establishment

Review the “Entrance Letter to Establishment Management” with plant personnel;

- RTE product, food contact, and environmental samples will be collected.
- Production lot(s) must be held.
- Equipment does not need to be rinsed after each sample is taken because Dey Engley *(DE) broth is considered GRAS.*
- Most negative results are published within 3 days.
- A RTE product lot is usually defined as all product produced from clean-up to clean-up.

Entrance Meeting at the Establishment – 2

- Reconfirm that the establishment will be producing post-lethality exposed, RTE product on the day that the IVT or RLm sampling has been scheduled.

- Ask the establishment if there have been any changes to its documented production, sanitation, or food safety practices.

- Ask where records are held (e.g., HACCP and sampling records).

At the Establishment

- Take a “walk through” with plant management to familiarize and help develop a sampling plan.
- Be knowledgeable of the establishment’s GMPs and operational SSOPs so you don’t break their rules.
- Review the establishment’s Listeria sampling program and previous results.
    - What sites have previously tested positive?
    - Are samples being collected as required?
    - Are all possible FCS sampling sites represented?
    - Are samples and supplies being handled, stored, and shipped appropriately?

Establishment Walk Through

- Identify RTE areas and determine which products are processed in each area;
  - Processing
  - Cooking
  - Packaging
  - Coolers and/ or freezers
  - Related RTE storage areas
    - packaging supply storage
    - Ingredient storage
    - finished product storage coolers or freezers
- Scope out possible Listeria harborage areas, e.g., hard to clean areas with a source of moisture.

Determine Where You Might Sample

- Identify possible sampling sites.
- Think through a general sampling plan, i.e., where and when.
- Allow for flexibility.
- Final sample site selection should be based on your knowledge, experience, **and** your observations.
  - Areas that are difficult to clean and sanitize, e.g., behind shields, cracks, crevices, floor-wall junctions, drains, etc.
  - Surfaces with a lot of employee contact.
  - Employee behavior at time of sampling.
  - Movement of people, equipment, and supplies at time of sampling.
  - Moisture, condensation, drips, etc., at time of sampling.

Pre-Sampling Preparation

- Final double check to ensure that you have everything needed for sampling.
- Begin “staging” sampling supplies and templates.
- Organize using your sampling template sheets.
- Determine how you will store the samples before shipping, i.e., secure and refrigerated.
- Double check Fed-ex logistics.
- Discuss team members roles/responsibilities.
- Possibly practice aseptic technique and swabbing with your team.

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		

RLm Sampling Est. 0000 / ABC Food Corp
RLMCONT Line 1 / Packing

#	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	

RLm Sampling Est. 0000 / ABC Food Corp RLMENVC Line 1 / Packing

#	Date	Time / Shift	Room / Line	Laboratory Form #	Sampling Site Description	Type of Sample	Bar code label #	
21	03/13/07	Pre-op	Packing / 1	11000263	Packing room drain scraper	RLmENVC	632891	
22		0900 hr	Packing / 1	11000264	Packing room wash hose	RLmENVC	632892	
23		0900 hr	Packing / 1	11000265	Packing room electric switch	RLmENVC	632893	
24		1150 hr	Packing / 1	11000266	Packing room refriger. unit	RLmENVC	632894	
25		1330 hr	Packing / 1	11000267	Packing room door	RLmENVC	632895	

RLm Sampling Est. 0000 / ABC Food Corp RLMPROD Line 2 / Slicing

#	Date	Time / Shift	Room / Line	Laboratory Form #	Sampling Site Description	Lot Number	Bar code label #	
35	03/13/07	1130 / 1st	Slicing / 2	11000275	Sliced Roast Beef	4384	632903	
36	03/13/07	1145 / 1st	Slicing / 2	11000276	Sliced Roast Beef	4384	632904	

Sample Collection

Changing Practices During RLm 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.

Changing Practices During RLm or IVT sampling – 2

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.

Changing Practices During RLm or IVT sampling – 3

If an EIAO is prevented from collecting samples (no supportable rationale):

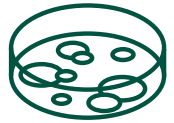
- In the case of an RLm, the DO may schedule an IVT with a “for cause” FSA - less advance notification.
- In the case of an IVT, if the EIAO cannot collect samples in order to determine that the product is not adulterated, the DO may instruct IPP to reject equipment in accordance with Rules of Practice - 9 CFR 500.2(a)(3).
- The DO may issue a NOIE or NOS when insanitary conditions are found or where the food safety system is inadequate, in accordance with 9 CFR 500.4(a) or (b) or 9 CFR 500.3(a)(4).
- OFO has final say in all regulatory enforcement decisions.

Changing Practices During RLm or IVT sampling – 4

If a risky line is not operating - samples can potentially be collected with a justifiable reason for doing so, e.g., a risky or previously positive line is shut down because FSIS is going to sample it.

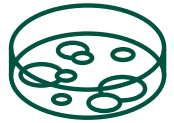
1. Determine if the establishment cleaned and sanitized the line.
2. Document that the line is not in operation.
3. Collect FCS and NFCS samples associated with the line.
4. Ensure that the receiving lab knows that no product will be samples submitted with the unit of samples prior to receipt.

Collecting Samples



- Some samples may be collected at pre-op., but most should be collected during production, during breaks if possible.
- Establishment must be producing FSIS amenable product. Must be post-lethality exposed for RLm sampling.
- With justification, you may sample a line that is not currently being used.
  - Notate this on sampling sheets.
  - Inform the lab before samples arrive that no product samples will be submitted with the unit.

Collecting Samples – 2

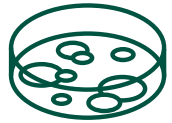


- Each unit of samples should all be associated with a single line, a single lot, and a single production Alternative.

- Can collect samples anywhere FSIS, RTE products or equipment are processed or held (coolers, hallways, production rooms).

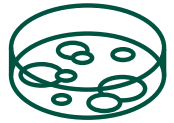
- If the number of FCSs is very limited, then sample those same sites at different times throughout the day of production.
  - Each sample is just a snapshot in time.
  - The timing of your sample collection may be important.

Collect the Samples – 3



- Generally, collect samples closest to the product first and move out.
- Generally, collect NFCS samples from areas closest to processing lines, but you can possibly sample in other areas (drains, coolers, freezers, storage).
- Can sample hands, gloves, aprons, etc.
  - May be FCS or NFCS, depending your observations.
- Briefly notate and justify sampling locations for future reference.

Brine Samples



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

- If the casing is **permeable or semi-permeable** then the brine is considered a food contact surface (FCS) sample.

- If the casing is **impermeable** then the brine is considered a non-food-contact surface sample (NFCS).

Sampling Sites

Lm Sampling: Food Contact Surface (FCS)

- **FCS:** An area in the post-lethality processing environment that comes in **direct contact** with post-lethality exposed RTE product.

FCSs	FCSs: Cooking and Cooling	FCSs: Employees	FCSs: 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

Lm Sampling: Food Contact Surface (FCS) – 2

- 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 an FCS area?

Answer: Employee hands, the blade, hopper, etc.

Question: Which part is a NFCS?

Answer: The slicer handle.

Environmental, Non-Food Contact Surface (NFCS) Sampling

- **NFCS:** A surface that has no direct contact with exposed product.
- EIAOs may collect NFCS samples from any area where FSIS RTE product is processed, held, or stored.
- This includes other areas associated with post-lethality exposed, RTE processing, such as storage areas for spices, packaging, ingredients, etc.

Environmental, Non-Food Contact Surface (NFCS) Sampling -2

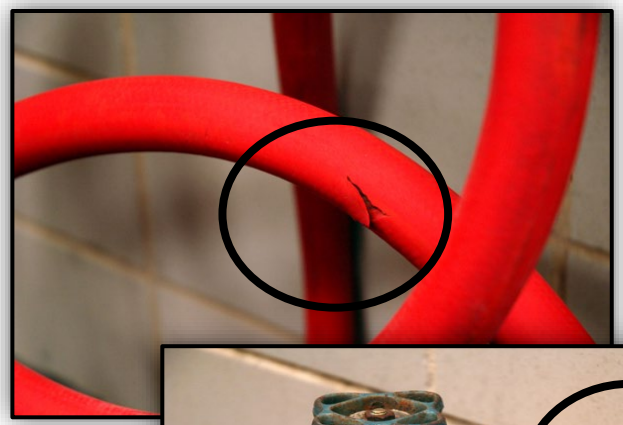
NFCSs

- 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.

NFCS Sampling Sites



Past Outbreak Related Positive RTE FCSs

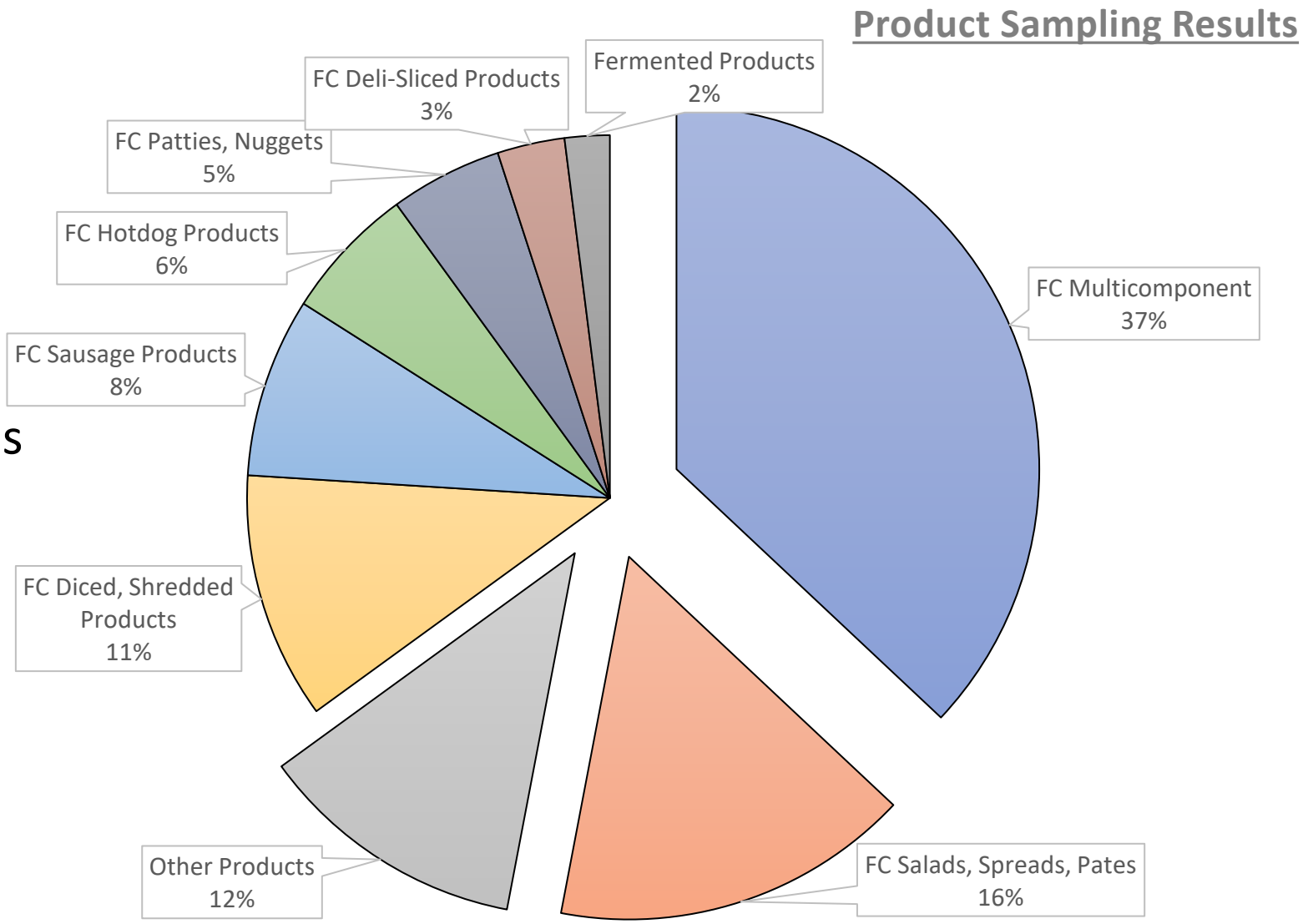
SAMPLE	RATIONALE
Tray for deboned ribs	Extensive hand contact
Surface of portion scale	Extensive hand contact
Belt entering beef slicer (post op)	Index Sample
Top belt entering slicer (post op)	Index Sample
Slicer blades (post op)	Index Sample
Weighing bowl for sliced beef	Index Sample

Past Outbreak Related Positive NFCSs

SAMPLE	RATIONALE
Wet spots at floor/wall junction	Possible harborage
Underside of rib deboning table	Sanitation overlooked
Condensation	Possible harborage
Freezer door jamb	Common contact point
Electrical Switches	Common contact point
Drains	Possible harborage
Table that trays pass over	Sanitation overlooked
Door jamb gap between door and wall	Possible harborage

IVT Product Results (2005-2014)

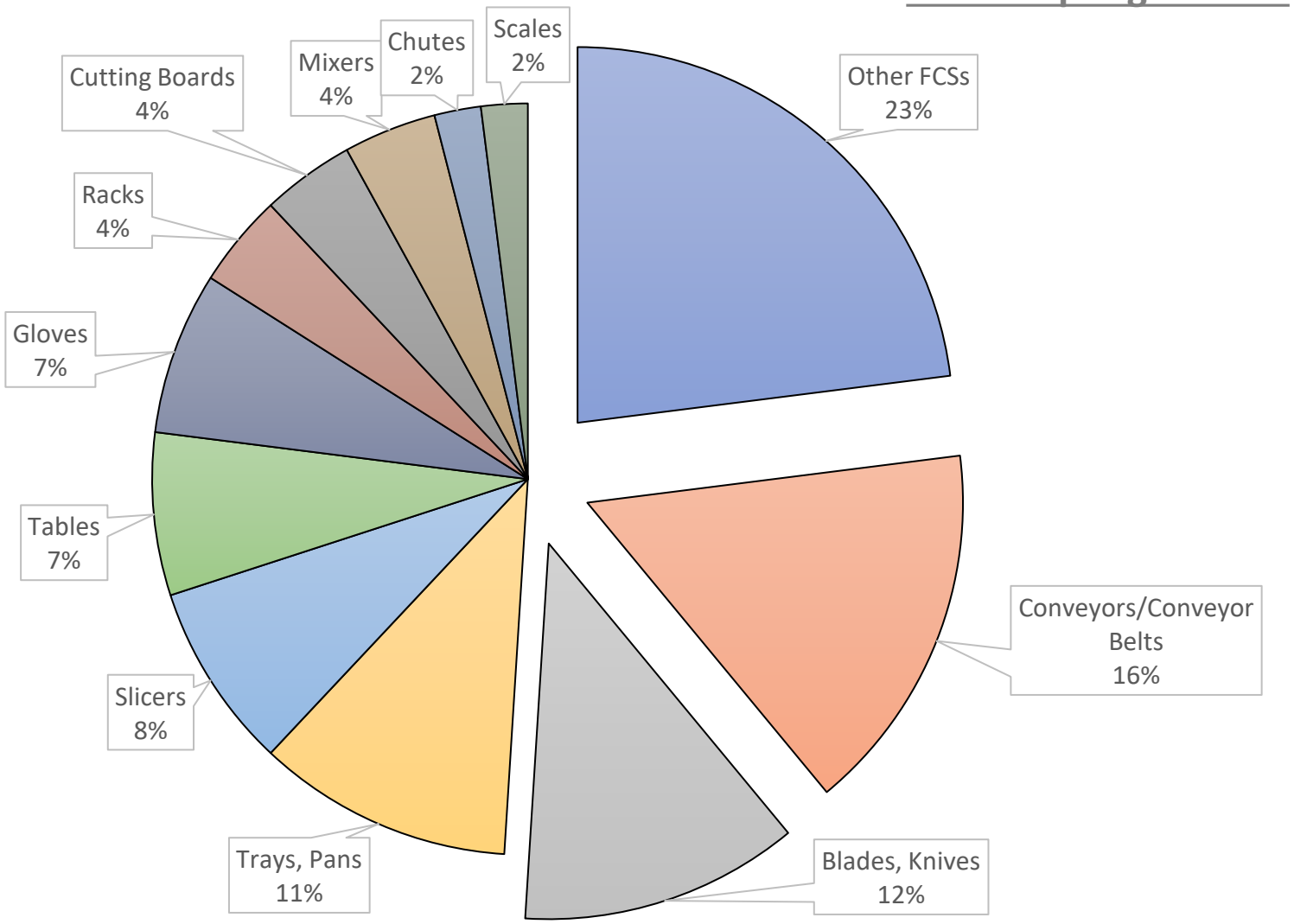
- Products with the most positive results:
  - FC Multicomponent Products
  - Salads, Spreads, Pâtés



FC = fully cooked
Other = individually have not come up positive often or are not sampled often

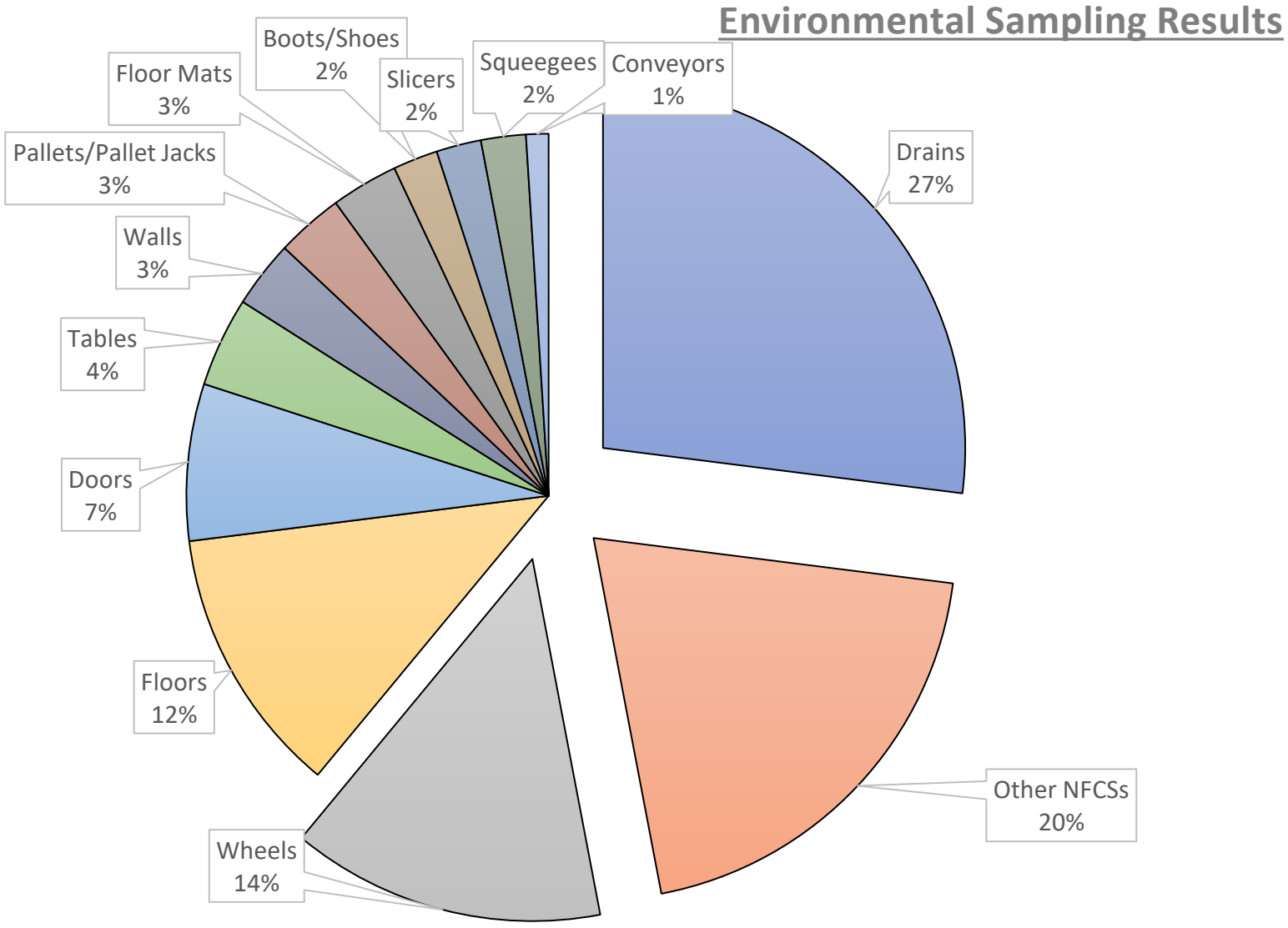
IVT FCS Results (2005-2014)

- FCSs with the most positive results:
  - Conveyors/Conveyor Belts
  - Blades, Knives



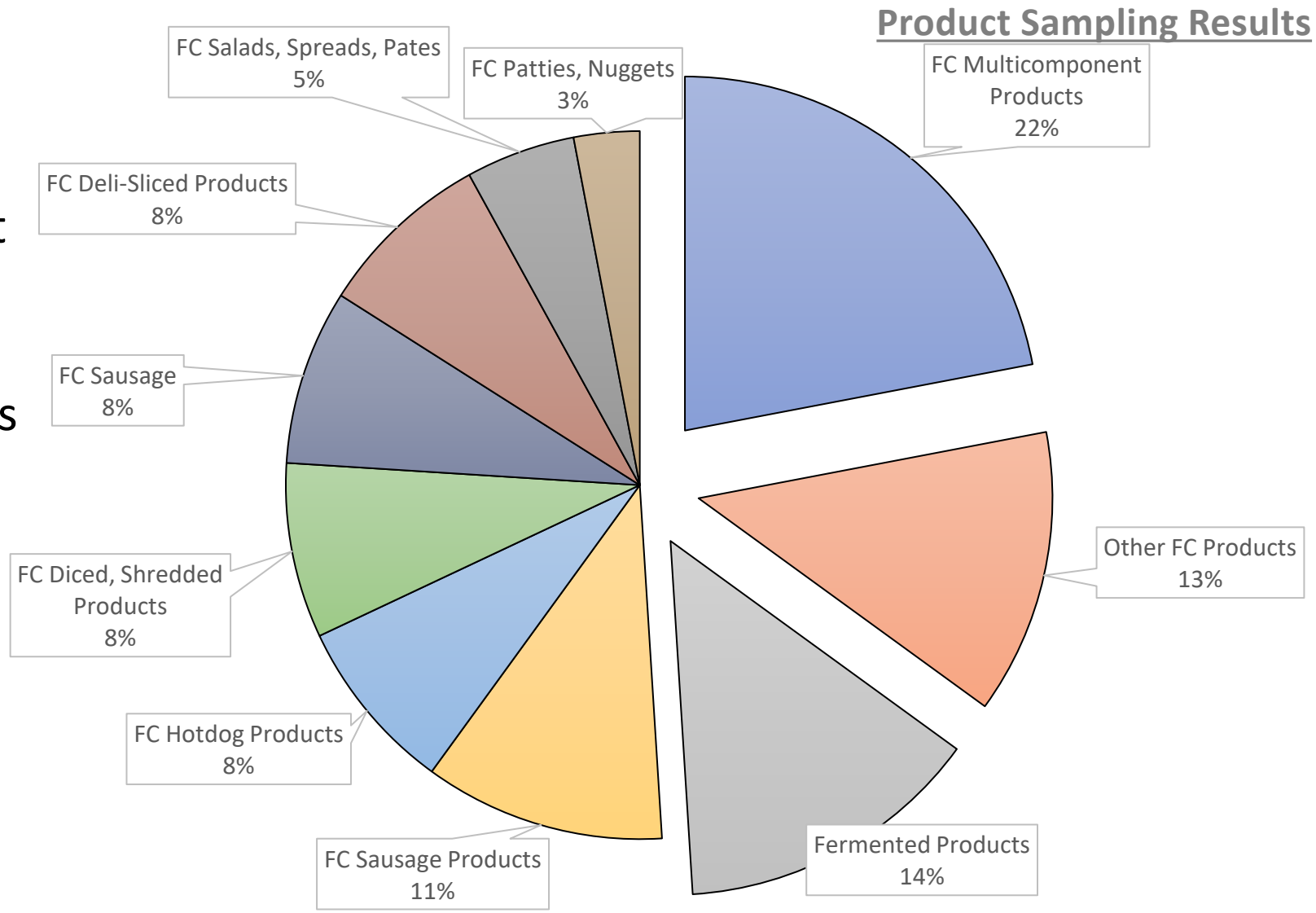
IVT NFCS Results (2005-2014)

- NFCSs with the most positive results:
  - Drains
  - Wheels
  - Floors



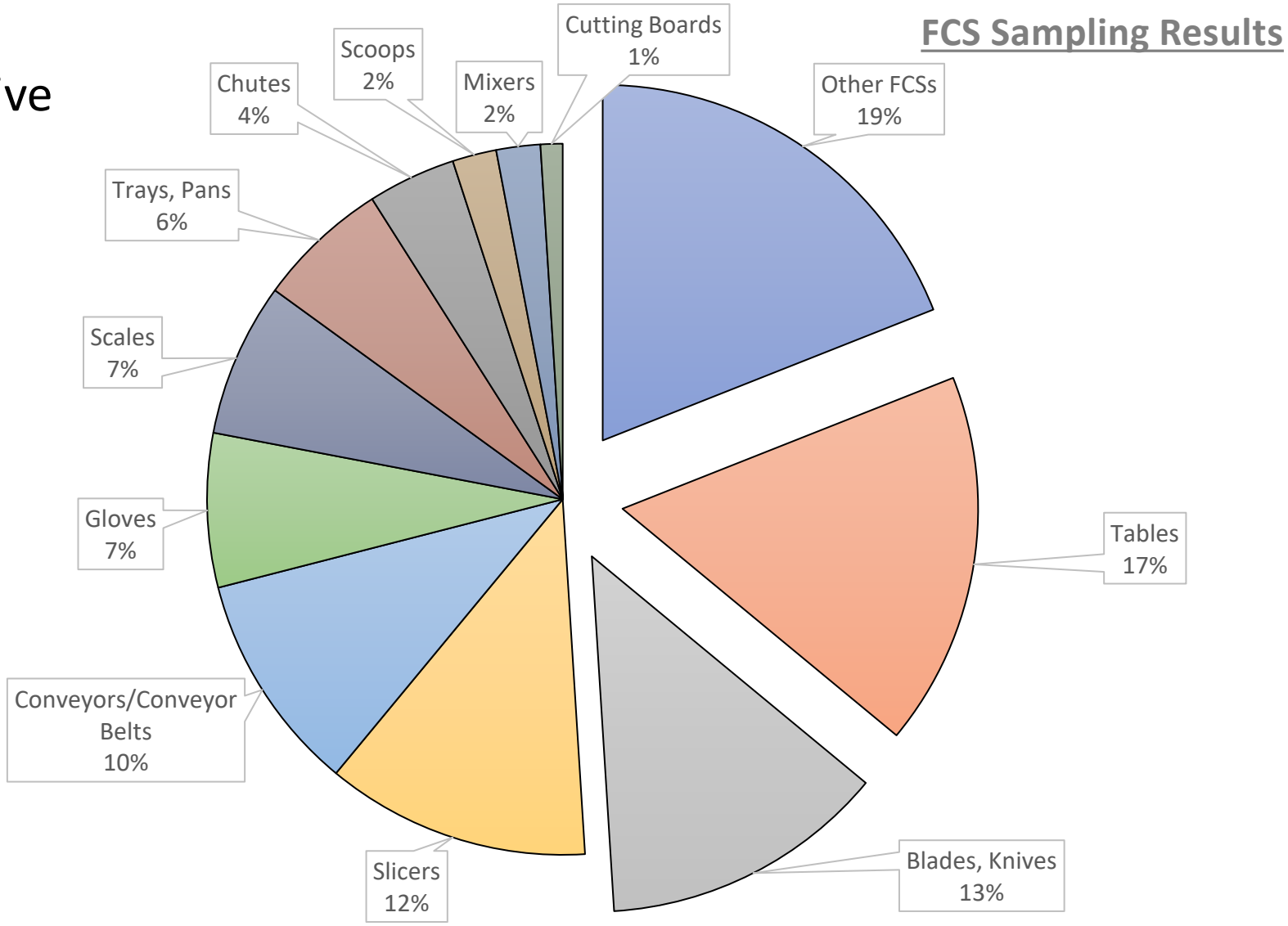
RLm Product Results (2006-2014)

- Products with the most positive results:
  - FC Multicomponent Products
  - Other FC Products
  - Fermented Products



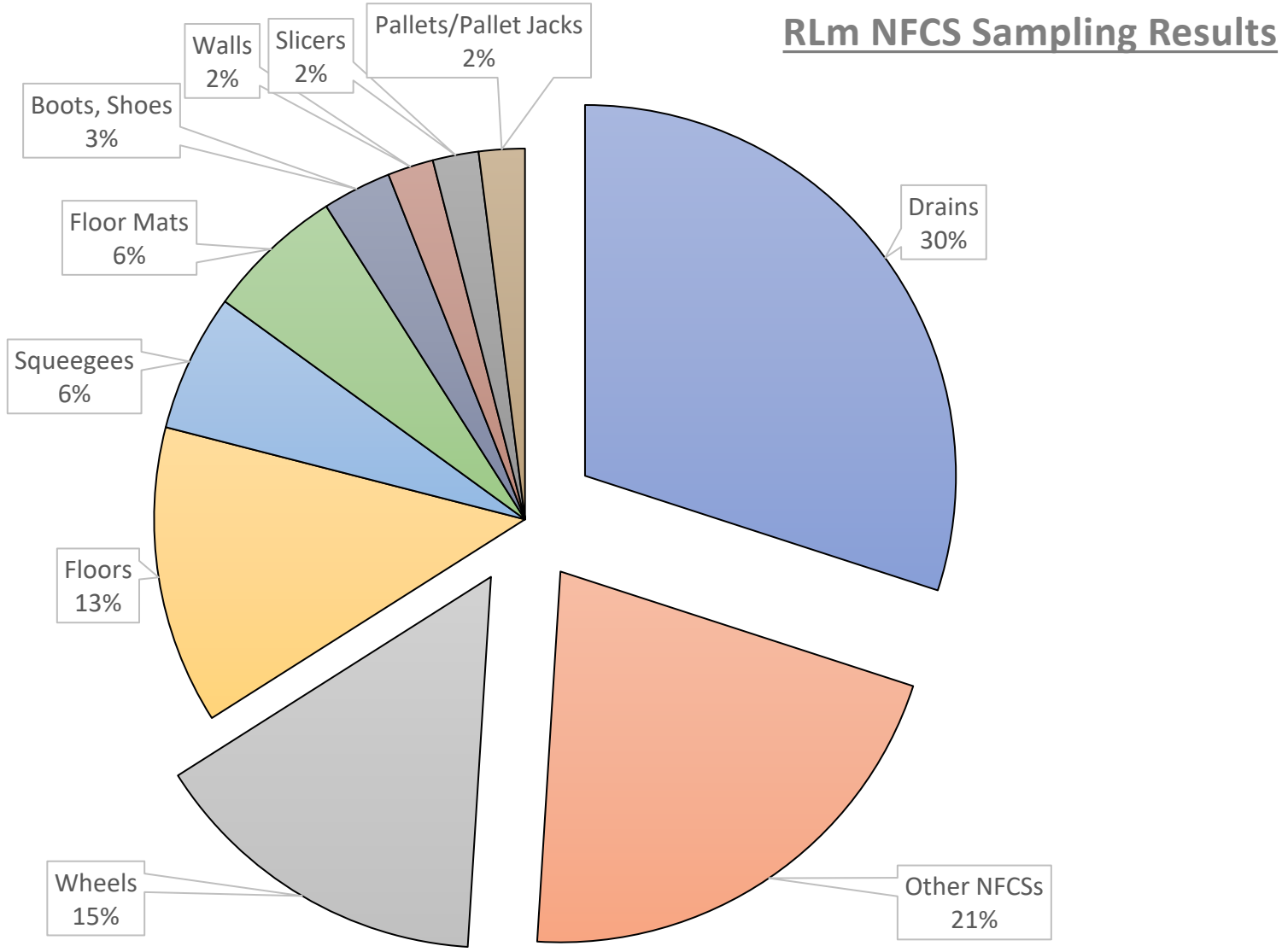
RLm FCS Results (2006-2014)

- FCSs with the most positive results:
  - Tables
  - Blades, Knives



RLm NFCS Results (2006-July 2009)

- NFCS locations with the most positive results:
  - Drains
  - Wheels
  - floors



Common Issues with EIAO Testing

EIAO Sampling, Shipping, or Packaging Issues.

- Not check sampling supplies upon receipt, only to realize they're short of supplies when they arrive at the establishment.

- Improper use of gel packs. Frozen gel packs should be removed from the freezer and placed into shipping containers on the day of shipment.

- Breaking establishment procedures due to not reviewing the establishment's operational SSOPs, GMPs, etc., before sampling.

EIAO Sampling, Shipping, or Packaging Issues -2

- Allowing an establishment to drastically shorten its RTE lot size to the degree it is no longer representative of routine processing.
- Collecting samples only on the day of packaging, when there was processing, handling, etc., the day before.
- Not understanding the difference between the **sampled lot** vs. a possible **implicated lot** (see page 4 of Directive 10,240.3).

EIAO Sampling, Shipping, or Packaging Issues -3

- 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.

EIAO Sampling, Shipping, or Packaging Issues – 4

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?

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, revision 3.

DE Broth and Pre-hydrated Sponges

- DE 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.

Results and Enforcement

Results and Enforcement – 1

- Check LIMS or PHIS for results.
- Inform the establishment of the results and the required corrective actions
- If any product sample tests positive, the entire sampled lot is adulterated.
- If a FCS sample tests positive, all product which passed over the FCS (the sampled lot) is adulterated.
- If FCS, NFCS, or product test positive for Listeria spp.

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

- Notice 50-24
- Results reported in PHIS not reported in Bites
- Corrective Actions per 416.15
- Product okay to ship
- Indication that sanitation program is ineffective at preventing conditions where Lm may be present

Results and Enforcement – 2

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, **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.

FSIS Directive 10,240.6

Use of Whole Genome Sequencing (WGS) Results for FSIS Ready to Eat (RTE) Sampling Programs. (Published on 01/19/2024).

“*The WGS Directive.*”

WGS not performed for non-*Lm Listeria spp.* results

FSIS Directive 10,240.6 - 2

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.

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

FSIS Directive 10,240.6 - 3

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.

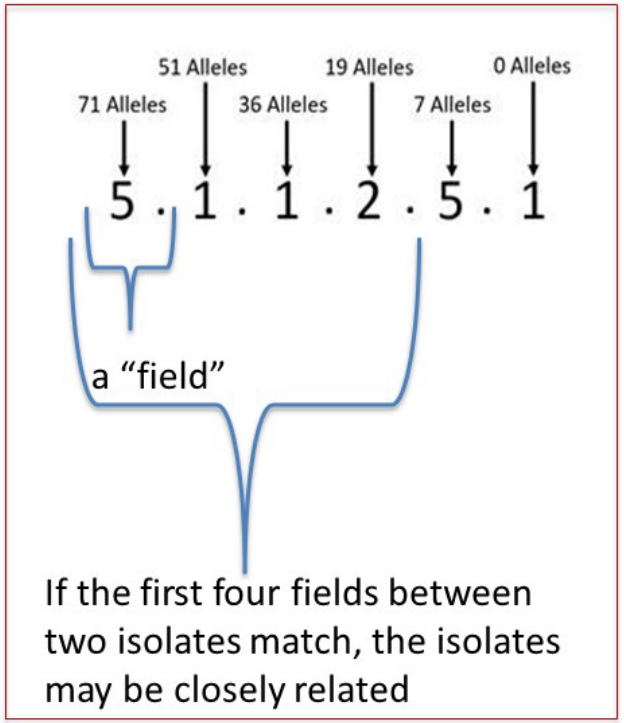
Cross-contamination;

- 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.

FSIS Directive 10,240.6 - 4

- FSIS uses allele codes to show relatedness.
- A complete allele code is written as: the organism abbreviation, allele code version followed by a dash and a series of numbers, called “fields,” distinguished by periods.

- Allele codes sharing the first 4 fields (5.1.1.2) differ by no more than 19 alleles, and those sharing the first 5 digits (5.1.1.2.5) differ by no more than 7 alleles.



- FSIS considers isolates from the same establishment that have the first 4 digits (fields) of an allele code in common to be closely related.

FSIS Directive 10,240.6 - 5

Sample Number	FormID	Collect Date	Allele Code**	MLST ST***	Project	FSIS Number	Indicative of Potential Harborage*	Indicative of cross-contamination*	Potentially related to a clinical isolate****
300425674	103237463	2023-07-26	LMO1.1 - 840.1.2.19.7.4 | 08/09/2023	ST6	INTENV_LM_M	FSIS22313744	Yes	Yes	No
300425675	103237466	2023-07-26	LMO1.1 - 840.1.2.19.7 | 08/09/2023	ST6	INTENV_LM_M	FSIS22313745	Yes	Yes	No
300389219	103193844	2023-05-22	LMO1.1 - 43.2.2.85.48.6 | 08/09/2023	ST5	RTEPROD_RAND	FSIS12322481			
590407357	101704082	2017-06-13	LMO1.1 - 840.1.2.19.7 | 03/04/2020	publicST6	INTENV_LM_M	FSIS1702561			
590407611	101704081	2017-06-13	LMO1.1 - 43.2.2.85.48 | 03/04/2020	publicST5	INTENV_LM_M	FSIS1702579			
590407612	101704079	2017-06-13	LMO1.1 - 43.2.2.85.48 | 03/04/2020	publicST5	INTENV_LM_M	FSIS1702580			
201081400	11632213	2012-05-15	LMO1.1 - 840.1.2.19.7 | 03/04/2020	publicST6	RLMENVC	FSIS1702438			

840.1.2.19 – found twice in two locations on 7/26/2023 = cross contamination
- also found previously on 6/13/2017 and 5/15/2023 = harborage

43.2.2.85 - found twice in two locations in 2017, found again on 05/22/2023

FSIS Directive 10,240.6 – 6

EIAOs and DO personnel are not to wait for WGS results prior to verifying the establishment's immediate corrective actions to FSIS positive Lm sampling results.

Once received, findings of harborage or cross-contamination can be used to further demonstrate an establishment's failure to control Lm in the post-lethality environment and to support enforcement.

WGS reports will indicate whether there is potential harborage, cross-contamination, or both. The report will also indicate 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.

FSIS Directive 10,240.6 - 7

EIAOs and DO personnel are to share the Listeria WGS report with the establishment management and are to document the discussion in a Memorandum of Interview.

If an enforcement action was issued prior receipt of the WGS report, the DO is to share how the findings in the report further support the enforcement action.

Make the establishment management aware of the recommendations in the FSIS' 2014 Lm Compliance Guide.

FSIS Directive 10,240.6 - 8

When sharing a WGS report, this Directive instructs the EIAO and DO to make the establishment aware of relevant FSIS guidance in the 2014 Lm Compliance Guide related to harborage and cross contamination.

- All of the following should be escalated in the event of consecutive positives and potential harborage (page 78):
  - Determine Listeria trends (page 122).
  - Perform a comprehensive investigation (page 122).
  - Conduct intensified sanitation (pages 78 and 117).
  - Conduct Employee training - Equipment cleaning (page 82), product handling (pages 74 and 81).
  - Conduct intensified sampling (pages 117 and 123).
    - Intensified sampling should include collection of product, FCS, and NFCS samples.

FSIS Directive 10,240.6 – 9

If DO personnel recommend an enforcement action associated with the RLm or IVT sampling, the DO personnel should include the findings of the WGS report in the enforcement letter (FSIS Directive 5100.1).

FSIS may determine, based on other findings;

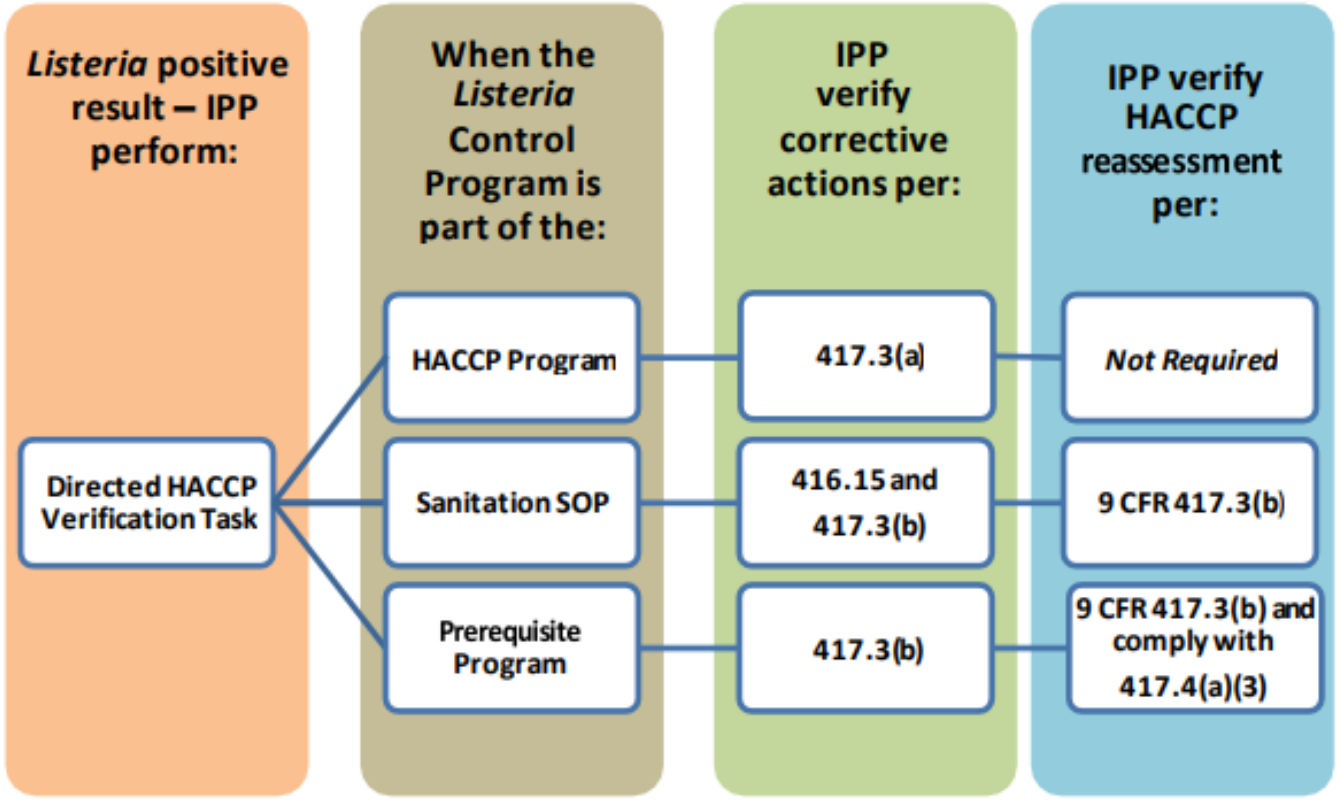
- That the establishment's food safety system is inadequate to control Lm in the post-lethality environment.
- That the Sanitation Standard Operating Procedure is not properly implemented or maintained, or
- The establishment has not maintained sanitary conditions to prevent Lm product adulteration (9 CFR 500).

The enforcement letter should include the WGS findings identifying harborage or cross-contamination, in addition to the specific compliance history.

FSIS Directive 10,240.6 – 10

- EIAOs are to verify the establishment meets corrective action requirements of 417.3(a), 417.3(b), and/ or 416.15. 1.

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



FSIS Directive 10,240.6 – 11

- EIAOs are to consider findings of harborage and cross-contamination as part of any follow-up verification activities, per FSIS Directives 5100.1 and 5100.3.
- EIAOs are to be aware that repetitive positives indicate previous corrective actions were ineffective, and retraining is not sufficient on its own.
- FSIS will conduct a PHRE;
  - May recommend IVT sampling and an FSA per FSIS Directive 5100.4, FSIS Directive 10,300.1 and FSIS Directive 5100.1, to verify corrective actions.

- Enforcement may result from multiple, recurring non-compliances, ineffective corrective actions, multiple adulterant positive results from FSIS testing, or shipping adulterated product.

Questions?