



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



## Understanding and Evaluating Microbiological Sampling and Testing

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### Today's Presentation

- Sampling methods
- Assessing sampling plans and testing methods
- Method validations and laboratory quality assurance
- FSIS testing programs, methods and pathogen-specific issues to consider
- Industry testing activities

## Sampling Methods

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## Sampling Methods

- Destructive sampling – grab, N60, excision
  - e.g., RTE, ground comminuted products, egg products, carcass excision
- Non-Destructive sampling
  - Typically chosen when destructive sampling not an option
  - Examples:
    - Cloth
    - Carcass rinsates or sponge
    - Parts rinsate
    - Environmental sponge

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## What is N60?

- N60 = number of samples (n) = 60
  - Multiple representative samples provides best option for detecting scattered contamination
  - Provides 95% confidence that no more than 5% of food pieces the size of each “n” in the entire lot are contaminated
- Keys to success
  - Must ensure that sampling is as representative as possible across the lot
  - Large composite “N60” samples typical need a larger test portion
- Cloth rather than N60 - in-field study started on January 4, 2021 through June 2021 – its implementation is imminent

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## Common Sampling Problems

- Small sample or sampling method may not be ideal for detection
  - *e.g.*, small swab device or environmental area sampled
- Sanitizer or residual antimicrobial chemicals might interfere with the test
  - Insufficient drip time prior to carcass sample collection
  - Excessive liquid carryover for parts sample collection
- Temperature abuse for the sample prior to testing
  - Holding under refrigeration for long periods allows competing bacteria to grow
  - Freezing can kill some pathogens (*e.g.*, *Campylobacter*)

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## Assessing Sampling Plans

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## Sampling Methods

- All sampling plans have significant limitations
  - Relative rigor of the sampling program must be evaluated
- Best sampling plans provide the opportunity but no guarantee of detection
  - i.e., scattered contamination is difficult to detect
- Frequent sampling and sampling multiple sites/time points provides a better opportunity for detection
  - Examples:
    - Multiple samples per day vs. once per month
    - N60 per lot vs. one grab sample per lot
- Does the type of sampling meet the intended need?
  - Destructive vs. non-destructive sampling

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## Sampling Plans

Statistical sampling plans assume:

- Uniform manufacturing conditions
- Equal probability of contamination throughout the lot (homogeneous distribution)
- Independent, random sampling (equal probability of sampling throughout the lot)

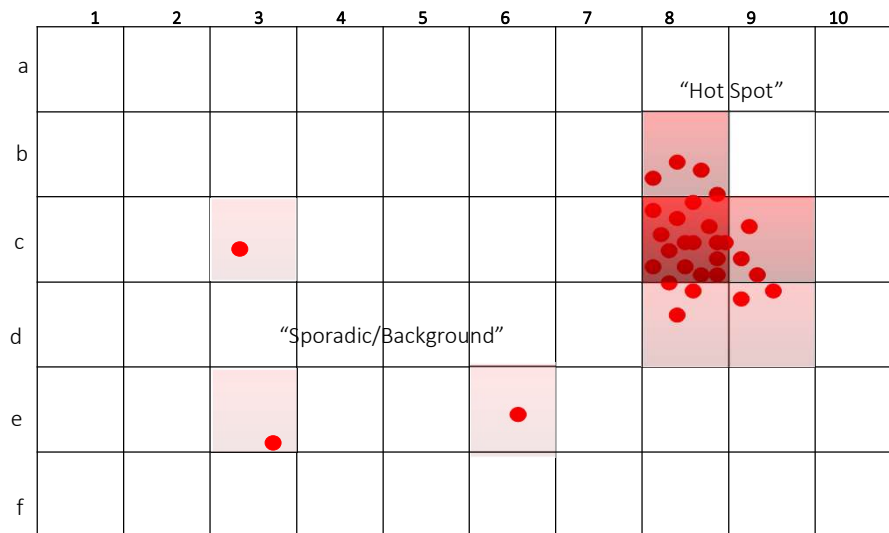
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## Why are Pathogens Hard to Detect?

- They are typically not evenly distributed
- They occur at low levels
- They are often injured when found in the product
- Detection may be inhibited by material in the food product (food matrix)
  - Example: high amounts of fat may inhibit PCR assays; spices, salt, acidulants can affect isolation and detection

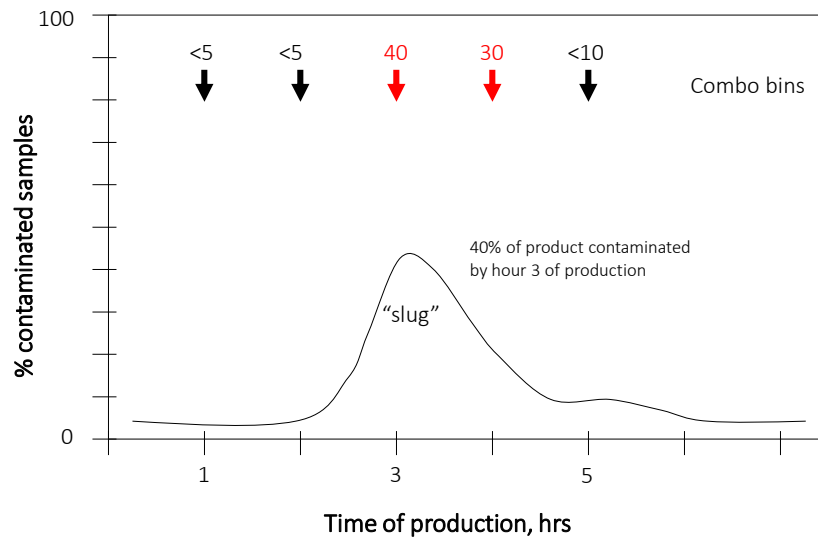
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## *E. coli* O157:H7 Contamination in a N60 Sampled Lot



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## *E. coli* O157:H7 Contamination in Ground Beef



## **Assessing Testing Methods**

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## **Key Players for Ensuring Robust Testing Methods**

- The establishment that needs the testing
- The laboratory they hire
- The manufacturer of the screening test they use
- The organization validating the screening test

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## **Steps in Detection Methods**

- Sample collection
- Sample preparation
- Enrichment for the pathogen
- Screening of the pathogen
- Confirmation of the pathogen

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## **Considerations for Testing Methods**

- Is the method fit for the intended purpose of the analysis?
- Has the method been optimized and experimentally validated for sensitive detection of pathogens?
- Is the laboratory complying to the validated method protocol?

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## Assessing Fitness for Purpose

- Is the test portion appropriate to meet the need?
- Is the method enrichment-based with the intent to detect the lowest possible numbers of stressed pathogen cells?
- Has the food matrix been validated for the method used?
- Are confirmation procedures appropriate for determining true negative samples?

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## The “Test Portion”

- Laboratory sample preparation => “test portion”
  - “analytical unit” or “analytical portion”
  - Definition: the part of the “sample” that is actually tested by the laboratory
- The test portion determines the theoretical (*i.e.*, best possible) sensitivity of the test
  - *e.g.*, 1 cell/test portion
  - 25-gram test portion: detecting 0.04 cells/gram is possible
  - 325-gram test portion: detecting 0.003 cells/gram is possible

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## Enrichment

- Test portion is incubated 8-48 hours in a culture broth
  - Why?
    - Contamination levels are too low for detection without enrichment
    - Must grow to high levels so very small volumes have enough pathogen present for later detection steps
- Different pathogens require different enrichment media (broth)
  - One vs. two-stage enrichment
- Primary enrichment vs. secondary enrichment
  - Resuscitation vs. selective growth

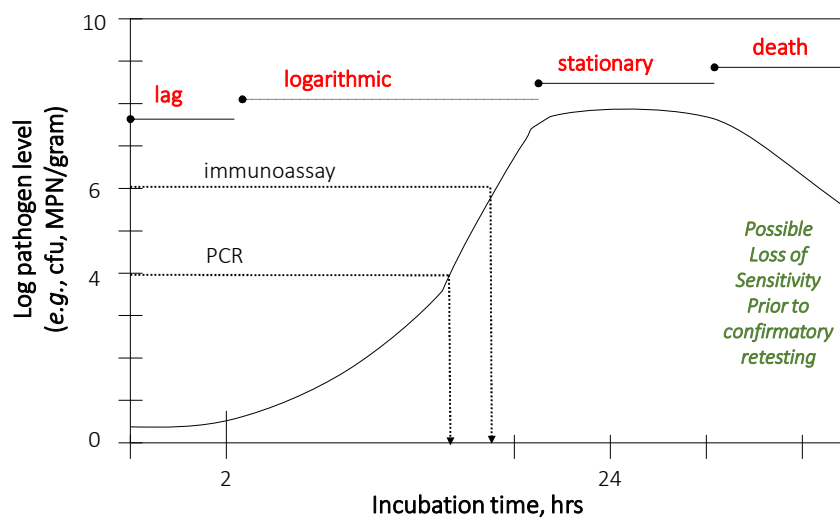
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## Considerations for Proper Enrichment

- Resuscitation (lag phase) can require 2-3 hours before log-phase growth begins
  - Some samples support slower growth
- Has enrichment broth been tempered to warm temperature prior to incubation?
  - Particularly critical for large test portions or shorter incubation periods

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## Pathogen Growth During Enrichment



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## Enrichment Period

- Different screening tests require different levels of enriched pathogen
- Shorter incubation periods (<15 hours) may warrant additional scrutiny of laboratory compliance to the validated protocol
- Has enrichment/screening combination been validated for a larger test portion?
  - Particular concern for large test portions incubated for shorter periods - e.g., 375-gram test portion incubated for 8 hours
- Proposed incubations <8 hours may warrant OPHS review

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## Confirmatory Testing

- Non-culture confirmation (*e.g.*, PCR)
- Culture confirmation (*e.g.*, FSIS confirmation)
  - Plating the enrichment on selective and differential agar media
  - Immunomagnetic separation (IMS) necessary prior to plating for *E. coli* O157:H7 and non-O157 STECs
    - Suspect colonies = “presumptive positive”
- Purification and confirmatory identification tests including:
  - Biochemical (*e.g.*, identifies “*E. coli*”)
  - Serological (*e.g.*, identifies “O157” and “H7”)
  - Genetic (*e.g.*, identifies “*stx*” = Shiga toxin genes)

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## Concerns for Confirmation

- Do not re-sample the lot or sample reserve!
- Non-culture confirmation
  - Same considerations as the screening test
  - Used under validated conditions
  - Transport and storage of enrichment
- Culture confirmation- carefully assess!
  - Typically expect that methods comply with a validated procedure (*e.g.*, MLG, FDA-BAM, ISO)
  - Small changes can affect ability to recover pathogen of interest

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## Quantitative Testing

Two options:

- MPN
- Direct plating

**NOTE:** Quantitative testing typically cannot accommodate larger test portions and provide the opportunity for detection that a qualitative test can provide

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## Most Probable Number (MPN) Enumeration Analysis

- Traditional enrichment-based analyses are performed on three or more dilutions, each typically in triplicate, from a single sample homogenate (*i.e.*, MPN = method format, not a specific method per se)
- Advantages:
  - Better sensitivity (lower LOD) than direct plating
- Disadvantages:
  - Very resource intensive/expensive
- Application:
  - For quantifying low levels of pathogens (*e.g.*, *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*)

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## Direct Plating Enumeration Methods

- Product is homogenized in diluent and small volume is directly dispensed onto agar media (*i.e.*, sometimes there is a 1–2 h “resuscitation” step, but enrichment is never used prior to plating)
- Advantages:
  - Allows easy inexpensive quantitative analysis
- Disadvantages:
  - Accommodates only a very small test portion
  - Higher LOD (*i.e.*, often 100 CFU/g) not suitable for detecting low levels of pathogens
- Application:
  - Expedient for higher level analytes (*e.g.*, indicators, *Campylobacter*, *S. aureus*, *C. perfringens*, *B. cereus*)

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## Method Validations

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## Value of Validation

- Determines performance characteristics of the method in comparison to a gold standard (reference) method (*e.g.*, usually FSIS or FDA method)
- Independent evaluation provides credibility
- Rigor varies (multilab vs. single lab, # tests, etc)
- Still must consider fitness for purpose and how the method is applied
  - *e.g.*, some AOAC-validated methods are not consistent with FSIS goals or Compliance Guidelines

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## Method Validation

- Recognized independent method validation organizations:
  - Government: FSIS (MLG) and FDA (BAM)
  - AOAC International (U.S.A.)
    - AOAC Official Methods of Analysis (OMA) validations
    - AOAC-RI “Performance Tested Method” validations
  - AFNOR (France)
    - *e.g.*, bioMerieux Vitek biochemical confirmation tests
  - Others (ISO, MicroVal, NordVal, AENOR, etc.)
- However, past validations conducted by these organizations may not be relevant to larger test portions or other testing scenarios

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## **Foodborne Pathogen Test Kits Validated by Independent Organizations**

- FSIS maintains a list, updated quarterly, of methods that have been validated by independent organizations
  - [https://www.fsis.usda.gov/sites/default/files/media\\_file/2021-05/Validated-Test-Kit.pdf](https://www.fsis.usda.gov/sites/default/files/media_file/2021-05/Validated-Test-Kit.pdf)
- None of the test kits listed are implicitly approved by USDA FSIS
  - A validated test kit must also be fit for purpose and appropriate for the specific application in a food safety program

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## **Process for Validating Qualitative Pathogen Methods**

- Series of laboratory experiments using inoculated samples under controlled conditions
- Inoculate portions with pathogen strain at very low level where only 20-80% of samples are positive (*i.e.*, fractional recovery)
- Statistically compare percent of positive samples in alternative method to reference method (FSIS MLG)

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## Considerations for Validation Data

- Was method compared to an appropriate reference method (*e.g.*, FSIS MLG; FDA)?
- If not performed by AOAC, AFNOR, etc., is supplemental validation data available?
  - May require additional scrutiny

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## Testing Method Specifications

- Sensitivity: probability that truly positive samples are detected as positive by analytical test
  - $100 - \text{false negative rate}$
- Specificity: probability that truly negative samples detected as negative by analytical test
  - $100 - \text{false positive rate}$
- Level of detection (LOD): lowest level of contamination reliably detected by analytical test
  - LOD expressed as ratio of organisms to quantity tested material (*e.g.*, CFU per gram, MPN per mL, CFU per square-ft) but definitions vary (*e.g.*, LOD95, POD)

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## Factors Impacting Detection and Method Specifications

- Detection as measured by sensitivity, specificity, and LOD can vary based on:
  - Specific strains of pathogen
  - Intrinsic factors for the sample matrix
    - Levels of competing bacteria
    - Fat, salt, pH and additives
- Experimental design for the validation study (*e.g.*, cell stress, etc.)

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## Complying with the Validated Protocol

- Do AOAC/AFNOR/ISO citations match the protocol in use?
  - Modifications are common, and some contribute to greater potential for false negative result
- Compare the lab procedure to the validated protocol (*i.e.*, package insert)
- If culture confirmation is used, verify that it follows validated method as well

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## Methods not Validated by Recognized Organizations

- “Supplemental” or “extension” validations
- *E. coli* O157:H7 and non-O157 STEC testing for 325-375g test portions
  - Modifications required for AOAC validated procedures based on 25g
  - Instructions for sample preparation may not be clear for the lab

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## Laboratory Accreditation and Quality Assurance

- ISO 17025 = protocol for establishing and documenting a microbiology laboratory quality program (*i.e.*, “HACCP” for labs)
- Accrediting bodies = A2LA and others
- Accreditation implies robust quality program but does not necessarily indicate methods meet FSIS expectations
  - Laboratories are able to perform the methods they use as expected, but methods are not “accredited” to be fit for purpose
- Laboratories are not required to be ISO accredited, but should have quality assurance programs that ensure results are reliable and accurate

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## **FSIS Testing Programs**

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## **FSIS Microbiological Sampling Program Objectives**

- Assess effectiveness of industry process controls
- Provide critical feedback to industry
- Monitor compliance with performance standards, zero-tolerance policies
- Allow FSIS to monitor industry-wide trends
- Serve as a strong incentive to reduce the occurrence of pathogens in products
- Capture pathogen characterization information (*i.e.*, serotype, speciation, antimicrobial resistance, whole genome sequencing)

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## FSIS Sampling Programs

- Sampling plans measure compliance with performance standards:
  - *Salmonella* and *Campylobacter* verification programs (raw poultry)
- Zero-tolerance policies for food pathogens
  - *E. coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* (non-O157 STEC) (raw non-intact beef or components of raw ground beef)
  - *Listeria monocytogenes* in RTE and pasteurized egg products and on food contact surfaces
  - *Salmonella* in RTE and pasteurized egg products

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## FSIS Methods and Pathogen-specific Issues to Consider

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## Shiga Toxin-Producing E. coli (STEC) Testing

Includes:

*E. coli* O157:H7 and the six non-O157 Shiga toxin-producing *E. coli* (STEC) (non-O157 STEC) – O26, O45, O103, O111, O121, and O145

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## O157 STEC Program

- Strain must have:
  - O157(+)
  - *stx*(+) OR *stx*(-) and H7(+)
  - biochemical(+) or Bruker MALDI Biotyper
- Currently FSIS only analyzes beef manufacturing trimmings (MT60) for non-O157 STECs
- FSIS plans to expand non-O157 STEC verification testing (85 FR 34397; June 2020):
  - Ground beef (MT43), bench trim (MT65), raw ground beef components other than trim (MT64)
  - Responding to comments; final rule; grace period, etc.

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## Non-O157 STEC Program

- Six non-O157 STEC = O26, O45, O103, O111, O121, O145
  - Strain must have:
    - *stx*(+) and *eae*(+) genes
    - one of the six O-groups
    - biochemical(+) or Bruker MALDI Biotyper
- Currently FSIS only analyzes beef manufacturing trimmings (MT60) for non-O157 STECs
  - Phased rollout – MT65 – MT64 – MT43

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## E. coli Top Seven STEC Analysis (MLG 5C.02)

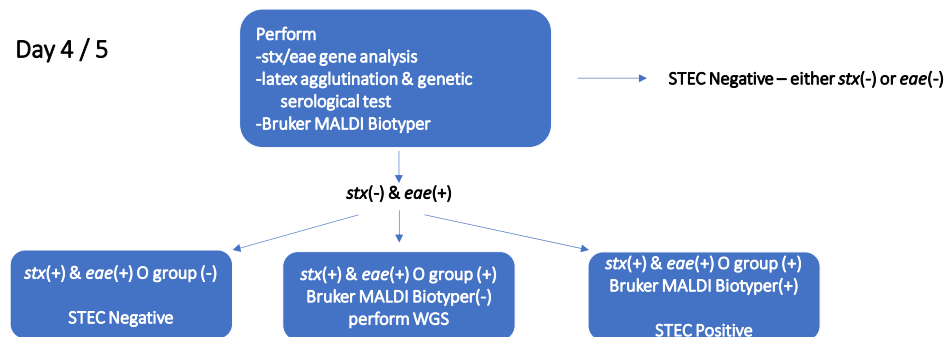
Day 1	Sample Prep and Primary Enrichment 42°C±1 for 15-24 hours	Enrichment
Day 2	Perform PCR All samples that do not test PCR negative are carried forward for further analysis Immunomagnetic Bead Capture & Rainbow Agar Plating	Screening negative or potential (+) – <i>stx</i> (+) <i>eae</i> (+) O group(+)
Day 3	Latex Agglutination & Sheep Blood Agar Plating	Negative – no growth, agglutination (-) or agglutination(-)/rapid screen(-) presumptive (+) – agglutination(+) and rapid screen (+) or inconclusive
Day 4 / 5	Perform - <i>stx</i> / <i>eae</i> gene analysis -latex agglutination & genetic serological test - Bruker MALDI Biotyper	

Confirmation – next page

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## E. coli Top Seven STEC Analysis (MLG 5C.02) – continued

Day 4 / 5



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## Larger E. coli O157:H7 and Non-O157 Test Portions

- Larger test portions (325–375 grams) are most important for N60 and other composite samples containing many samples
- Less important for single “grab” samples of ground beef final product testing when:
  - Trim and components have already been tested using robust sampling and 325–375-gram test portions
  - Multiple samples are collected throughout the production day
- Methods must be adapted, optimized and validated for effective use with 325–375 gram test portions

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## **E. coli O157:H7 and Non-O157 STEC Testing Concerns**

- Supplemental validation and special instructions for testing larger test portions
  - For enrichment periods <15 hours
  - 325-375g test portions typically often require longer minimum enrichment period than 25g
- Culture-based detection and confirmation requires immunomagnetic separation (IMS)

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## **Listeria testing**

Includes:

- L. monocytogenes* testing (FSIS)
- Listeria*-like or *Listeria* spp. testing (industry)

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## Listeria monocytogenes (MLG 8.13)

Day 1	Sample Prep and Primary Enrichment Stomach 25g sample + 225 ml UVM Incubate 30°C for 20-26 hrs	Enrichment
Day 2	Plating, Secondary Enrichment Incubate 35°C -- MOX (24-28 hrs) Incubate 35°C -- MOPS-BLEB (18-24 hrs)	
Day 3	Streak plates for next day 3M Molecular Detection Assay 2 Horse blood and MOX plates	possible(+) confirm (-) – both must be negative Screening
Day 4	Restreak for hemolysis Incubate 35°C variable time	presumptive(+) – hemolysis
Day 5	Bruker MALDI Biotyper and restreak	presumptive (+) – hemolytic Confirmed (+) – on Bruker MALDI Biotyper
Day 6	Further characterization, morphological, and atypical isolate analysis	presumptive (+) from previous day are Confirmed (+) – by Bruker MALDI Biotyper Confirmation

Perform WGS

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## Expectations for Listeria Environmental Testing Equivalence

- Compliance Guidelines – Controlling Lm in Post-lethality Exposed RTE Meat and Poultry Products Jan 2014
- For optimal sensitivity of detection, method for food contact surface testing must:
  - Validated by a recognized body (e.g., AOAC, AFNOR)
  - Be enrichment-based
  - Enrich the entire sponge/swab sample
    - e.g., an aliquot from sponge/swab does not provide opportunity to detect bacteria trapped in the sponge

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## Analytes for Industry Food Contact or Environmental Surface Testing

Establishment laboratories test for one of the following:

- *Listeria monocytogenes*
  - Use internationally recognized **enrichment-based method** that biochemically confirms culture as *L. monocytogenes*
- *Listeria* spp.
  - Use internationally recognized **enrichment-based method** that uses ELISA, PCR or other screening technology to provide more rapid but less specific *Listeria* spp. result
- “*Listeria*-like” indicator bacteria
  - Use the first part of an internationally recognized enrichment-based method to find suspect *Listeria* colonies (*e.g.*, darkened colonies on MOX using the FSIS method)

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## Salmonella Testing

- Raw products
  - Meat and turkey carcass sponge samples
  - Chicken carcass/parts rinsates
  - Raw meat and comminuted poultry
- Processed products
  - RTE (325g portion)
  - Pasteurized egg

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## Salmonella (MLG Ch. 4.11)

Day 1	Sample Prep and Primary Enrichment Stomach sample + BPW Incubate 35°C for 18-24 hrs (RTE); 20-24 hrs (poultry)		Enrichment
Day 2	Perform PCR All samples that do not test PCR negative are carried forward to RV and TT broth Incubate 42°C for 22-24 hrs	confirm (-) (+) - 2° enrichment	Screening
Day 3	Streak RV and TT on BGS and DMLIA plates Incubate 35°C for 18-24 hrs		
Day 4	Pick suspect colony from Plate medium to TSI and LIA slants. Incubate 35°C for 22-26 hrs		
Day 5	Streak on SBA for biochemical testing Incubate 18-24 hrs at 35°C	presumptive (+) confirm (-)	
Day 6	Bruker MALDI Biotyper	confirm (-/+)	Confirmation

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## Campylobacter Testing

### Qualitative

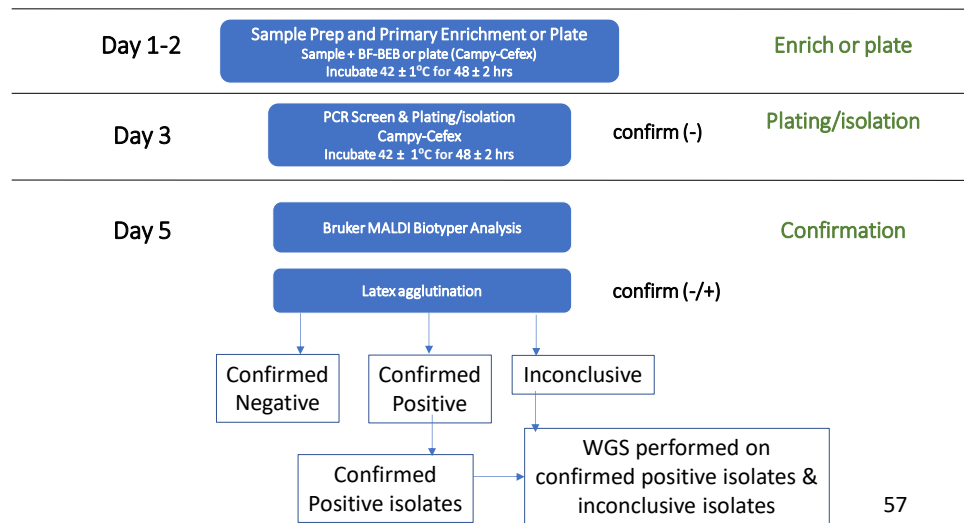
- Enrichment-based (as opposed to direct plating) since Aug 27, 2018 - exception: "other raw chicken parts" (EXP\_CPT\_OT01 and LO\_CPT\_OT01)

### Targets

- C. jejuni*, *C. lari* or *C. coli*

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## Campylobacter (MLG 41.06) – Qualitative



## Issues for Campylobacter Testing

- *Campylobacter* is highly vulnerable to freezing
  - Do not freeze samples
- Can be a challenging test (inconsistent results across labs)

## Industry Testing Programs

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## Establishment Documentation for Testing Methods

- Does the establishment have the necessary documentation?
  - Can the establishment provide the method used for microbial detection?
  - Can the establishment provide evidence that the method used was properly validated by an independent body?
  - Can the establishment explain why the method fits the need?

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## Issues for Industry Labs

- On-site vs. off-site labs
  - Shipment of samples/handling during shipment
- Overarching concerns for on-site labs
  - Is testing effective?
  - Is testing safe in that facility?
    - Enrichment of pathogens in an establishments
- Evaluate the following:
  - Are personnel qualified?
  - Does the lab have proper equipment and materials for testing and disposal of contaminated media?
  - Do they follow the validated testing protocol?

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## Establishment Responsibilities for Laboratory Testing

- The establishment is ultimately responsible for the testing they request from private laboratories
- Has the establishment properly conveyed testing needs?
  - *e.g.*, test portion equivalent to FSIS as opposed to the default 25 g in protocols
- Is the laboratory aware of FSIS expectations?
  - Directives, Notices and guidance
- Establishment should provide documented detailed methodology and validation information for FSIS review

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## **FSIS Verification of Establishment Sampling and Testing Programs**

Effectiveness verified by FSIS

- Reviews/observations of EIAOs during FSA
- Establishment provides supporting documentation
- Technical and policy support provided through askFSIS
- Establishment, not laboratory, is responsible for implementing effective program

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## **FSIS Verification of Establishment Sampling and Testing Programs**

Focus of FSIS' evaluation

- Is the method fit for the intended purpose?
- Does the method support the hazard analysis decisions?
- Is the method comparable to the appropriate FSIS method (or is there justification for an alternative)?
- Is a comparable or appropriate test portion used?
- Is the method validated and used under validated conditions?
- Does the laboratory assure the quality of the results?

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## Helpful Guidance

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## Existing Agency Guidance – Compliance Guides

### Ready-to-Eat

- **FSIS-GD-2021-0014** - Appendix A – “FSIS Salmonella Compliance Guidelines for Small and Very Small Meat and Poultry Establishments that Produce Ready-to-Eat (RTE) Products and Revised Appendix A” (December 2021) – **Being updated**
- **FSIS-GD-2021-0013** - Appendix B – “FSIS Compliance Guideline for Stabilization (Cooling and Hot-Holding) of Fully and Partially Heat-Treated RTE and NRTE Meat and Poultry Products Produced by Small and Very Small Establishments and Revised Appendix B” (December 2021) – **Being updated**
- **Notice 41-22 (7/19/2022)** Instructions on the 2021 Cooking Guideline (Revised Appendix A) and Stabilization Guideline (Revised Appendix B)

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## Existing Agency Guidance – Compliance Guides

### Shiga Toxin-producing *E. coli* (STEC)

- **FSIS-GD-2021-0007** – FSIS Industry Guideline for Minimizing the Risk of Shiga Toxin-Producing *Escherichia coli* (STEC) in Beef (including Veal) Processing Operations (July 2021)
- **FSIS-GD-2021-0008** – FSIS Industry Guideline for Minimizing the Risk of Shiga Toxin-Producing *Escherichia coli* (STEC) in Beef (including Veal) Slaughter Operations (July 2021)

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## Existing Agency Guidance – Compliance Guides

### HACCP

- **FSIS-GD-2018-0005** – “Meat and Poultry Hazards and Controls Guide” (March 2018)
- **FSIS-GD-2015-0011** – “FSIS Compliance Guideline: HACCP Systems Validation” (April 2015)

### WGS

- News & Events (under Full Menu; right side) – Events & Meetings (left side under the picture) – search for WGS in the advanced search
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6653787/>

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## Existing Agency Guidance – Compliance Guides

### Microbiological Test Methods and Laboratories

- **FSIS-GD-2013-009** -Establishment Guidance for the Selection of a Commercial or Private Microbiological Testing Laboratory” (June 2013)  
go to: Policy-FSIS Guidelines – search for “selection of a commercial lab”
- **FSIS-GD-2010-0004** – “FSIS Guidance for Test Kit Manufacturers, Laboratories: Evaluating the Performance of Pathogen Test Kit Methods” (October 2010)
- **FSIS-GD-2019-0008** – “Foodborne Pathogen Test Kits Validated by Independent Organizations” (February 2020)

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## Whole Genome Sequencing (WGS)

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## Whole Genome Sequencing – A Collaborative Approach

- FSIS worked with the Food and Drug Administration, the Centers for Disease Control and Prevention (CDC), with PulseNet partners on:
  - How to perform WGS – methodology (aligned methods)
  - Analyze WGS data
  - Interpret WGS data
- FSIS began performing WGS for *Listeria monocytogenes* (Lm) in FY13 (along side PFGE) and for all pathogens starting in early FY16.
- FSIS suspended PFGE analysis for Lm and started using WGS data Jan 15, 2018.

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## Whole Genome Sequencing – Benefits

- WGS benefits FSIS and its mission to protect public health:
  - Detects harborage and cross-contamination of pathogens in FSIS-regulated facilities
  - Traceback from human illness outbreak data to regulated food products
  - Identification of unique genes related to virulence, pathogenicity, survival, adaptation, and resistance to biocides (sanitizers, metal, etc.) and antimicrobials.

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## Whole Genome Sequencing – Analysis

- FSIS uses different tools to analyze WGS information including:
  - Public Sequence Typing
  - Multi-locus Sequence Typing (MLST)
  - Core genome analysis (~1800 genes for Lm)
  - Phylogenetic analysis
  - High-quality Single Nucleotide Polymorphisms (hqSNP)

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## Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)

Single Nucleotide Polymorphism (SNP)

ATGTT**C**CTC isolate A  
ATGTT**G**CTC isolate B

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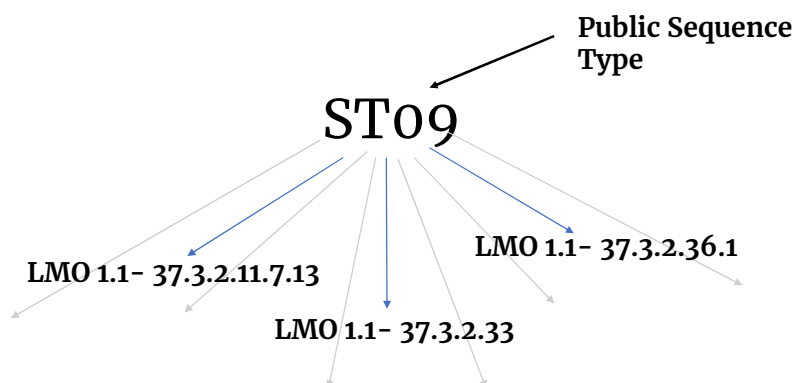
## Whole Genome Sequencing – Sequence Typing

### Multi-locus Sequence Typing (MLST)

- MLST can generate a **pattern name or designation** based on differences in a pre-defined set of genes.
- MLST Results will be Provided by FSIS as Follows:
  - **Public Sequence Type** (“MLST ST”, “ST”, or “pubST”)
    - small number of genes (i.e., 6-12)
    - named using the publicly available database developed by Jolley & Maiden (2010) (e.g., publicST09)
- **Allele Code**
  - compares ~1,800 genes for Lm
  - named by using CDC PulseNet numerical code (e.g., LMO1.1-5.1.1.2.5.1)

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## Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)



Allele Code is more specific than Public Sequence Type; one Public Sequence Type can be inclusive of many Allele Codes.

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## Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)

Example: LMO1.1 - 5.1.1.2.5.1

LMO – *L. monocytogenes*  
Version 1.1

Allele codes are a nomenclature scheme created by CDC

Like PFGE patterns, allele codes simplify how we communicate about pathogen strains

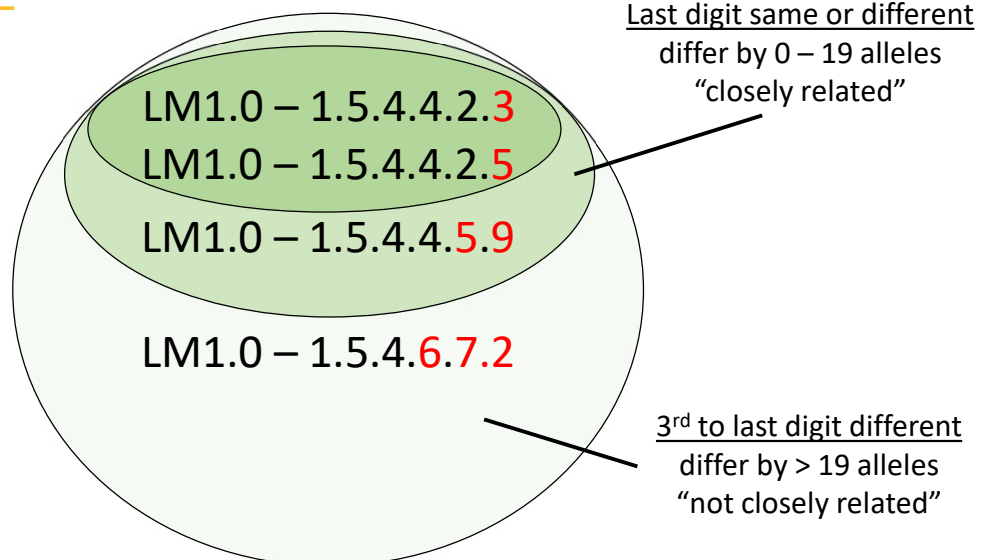
71 Alleles 51 Alleles 36 Alleles 19 Alleles 7 Alleles 0 Alleles  
5 . 1 . 1 . 2 . 5 . 1

a “field”

If the first four fields between two isolates match, the isolates may be closely related

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### Background: What does allele code tell you?



## Establishment-specific Datasets

Allele codes for Lm have been reported since 2019

Fields were created for *Salmonella* and STEC allele codes (*Campylobacter* in development)

Date Stamp format (allele code) (space) date mm/dd/yyyy

LM1.0-23.5.6.0 04/05/2022 → Retrieval of the allele code from PulseNet

Allele codes may change over time, a date-stamp supports use of the data in static reports

## Whole Genome Sequencing – Allele Codes

- Allele codes are a nomenclature scheme created by CDC.
- Like PFGE patterns, allele codes simplify how we communicate about pathogen strains.
- Allele codes can be used for trend analysis and to interpret relatedness.



## Whole Genome Sequencing – Analysis – Microbial Characterization Branch (MCB) – Eastern Lab, Athens, GA

Establishment	Field	590668019	590668018	201074252	201047328	LIMS ID
M54-P54 (LocID: 9542)	FormID	102595413	102595402	11629154	11610429	Form ID
	Collect Date	2020-11-09	2020-11-09	2012-03-20	2011-10-11	
	Allele Code	LMO1.1 - 5.1.2.5.4.1	LMO1.1 - 5.1.2.5.4.1	LMO1.1 - 5.1.2.5.2	LMO1.1 - 5.1.2.5.2	Allele Code
	MLST ST	ST204	ST204	publicST204	publicST204	
	Project	INTENV_LM_M	INTCONT_LM_M	INTENV	RTE001	
	FSIS Identifier	FSIS22029688	FSIS22029687	FSIS11816785	FSIS11816784	FSIS Identifier
	NCBI Accession Number	SAMN16839333	SAMN16839186	SAMN10645629	SAMN10645628	
	NCBI SNP Cluster (Retrieve Date)	PDS000024493.9 2020-11-23	PDS000024493.9 2020-11-23	PDS000024493.9 2020-11-23	PDS000024493.9 2020-11-23	
	Min Food Env (SNP)*	0	0	8	5	
	Indicative of Potential Harborage**	Yes	Yes			
	Indicative of cross-contamination***	Yes	Yes			
	Min Clinical (SNP)*	None	None	None	None	
	Potentially related to a clinical isolate****	No	No			

\*A value of "None" indicates greater than ~50 SNPs for this isolate source

\*\*Harborage, or repeated introduction is suggested if WGS analysis indicates closely related *Lm* isolates are found in product, food contact, or non-food contact environmental samples collected over multiple days, weeks, months, or years. [FSIS Notice 48-18](#)

\*\*\*Cross-contamination is suggested when closely related *Lm* isolates are found in product, food contact, and environmental (nonfood contact) samples collected during the same sampling event. [FSIS Notice 48-18](#)

\*\*\*\* Clinical isolates collected and uploaded within two years of the new isolate based on available NCBI metadata.

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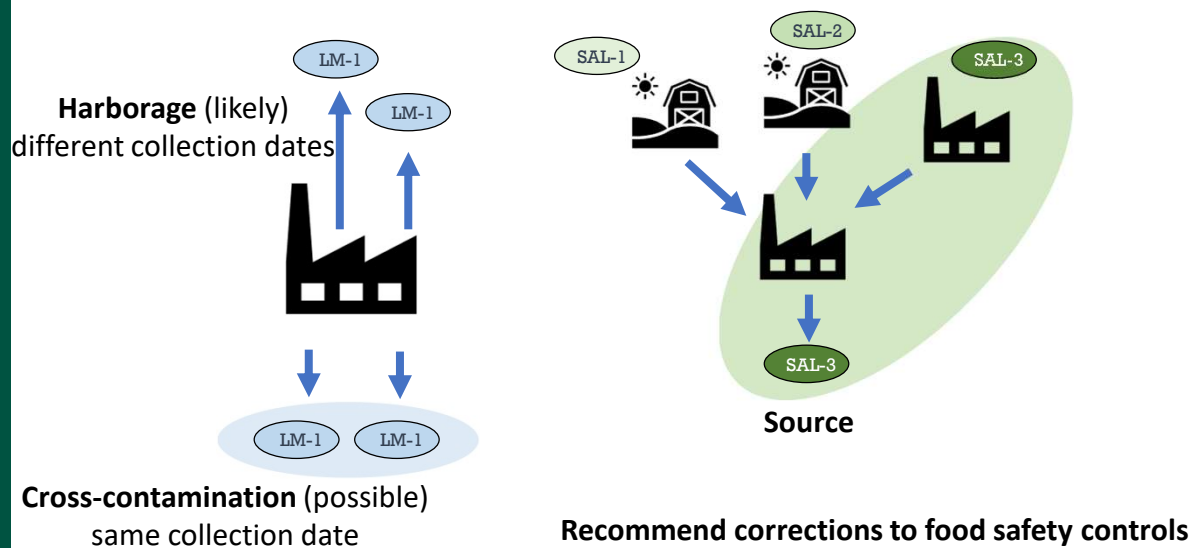
## Harborage and Cross-contamination

- **Harborage** or **persistent** contamination of the post-lethality environment, is suggested if WGS analysis indicates closely related *Lm* isolates are found in product, food contact, or non-food contact environmental samples that were collected over multiple days, weeks, months, or years.
- **Cross-contamination** is suggested when closely related *Lm* isolates are found in product, food contact, and environmental (non-food contact) samples collected during the same sampling event.

If *Lm* is isolated from a post-lethality exposed product sample and from a food contact surface sample, the food contact surface is more likely to be the source, unless under-processing of RTE product is suspected.

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## Harborage and Cross-contamination



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## PulseNet Cluster Search

- EIAOs assigned to perform PHRE at Cat 3 Est request search through AskFSIS (Directive 10,250.2)

### **Search strategy:**

- Obtain all *Salmonella* WGS from all raw poultry sampling projects obtained in past 52-weeks from the establishment.
- Determine if any such sequences are closely related to a **recent** clinical isolate **associated with a PulseNet cluster**.

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## PulseNet Cluster Search

Primary Establishment Number: P39  
 Establishment Name: Pine Manor Inc.  
 Result Date Range for the Establishment's isolates (52 weeks from report date): 06/17/2019 - 06/16/2020  
 Date Range for PulseNet (60 days from report date): 04/17/2020 - 06/16/2020

Establishment Number	Project Code	Lab Form ID	Collection Date	FSIS_Number	Clinical match in PN Cluster	Most recent clinical isolation date
P39	HC_CPT_LBW01	102482782	6/2/2020	FSIS12031324	Sequence Pending	Sequence Pending
P39	HC_CPT_LBW01	102472866	5/27/2020	FSIS12031276	Sequence Pending	Sequence Pending
P39	F_CH_CARCO1	102446119	4/13/2020	FSIS12030359	No	none
P39	HC_CH_CARCO1	102446121	4/8/2020	FSIS22027846	No	none
P39	HC_CPT_LBW01	102444257	4/6/2020	FSIS12030175	No	none
P39	F_CH_CARCO1	102440871	4/1/2020	FSIS22027733	No	04/20/2020 (PNUSAS143703)
P39	HC_CH_CARCO1	102402754	2/11/2020	FSIS32003362	No	none
P39	HC_CPT_LBW01	102394292	1/28/2020	FSIS22027008	No	none
P39	F_CPT_LBW01	102381960	1/20/2020	FSIS22026910	No	05/05/2020 (PNUSAS144635)
P39	HC_CH_CARCO1	102357006	12/18/2019	FSIS11927558	No	none
P39	HC_CPT_LBW01	102352995	12/12/2019	FSIS11927514	No	none
P39	HC_CH_CARCO1	102347464	11/26/2019	FSIS11927022	No	none
P39	HC_CPT_LBW01	102329284	11/21/2019	FSIS11926869	No	none
P39	HC_CPT_LBW01	102326607	11/18/2019	FSIS11926797	No	none
P39	HC_CPT_LBW01	102329283	11/11/2019	FSIS21926145	No	none
P39	HC_CPT_LBW01	102303639	10/14/2019	FSIS21925875	No	05/11/2020 (PNUSAS145056)
P39	HC_CH_CARCO1	102270365	8/27/2019	FSIS11924547	No	none
P39	HC_CH_CARCO1	102260403	8/6/2019	FSIS319021	No	none

Sample collection dates (FSIS projects)

Sample collection dates (PulseNet human cases)

FSIS sample matches recent PulseNet clinical isolate

FSIS sample matches PulseNet cluster (yes/no)

## Asking for More Information

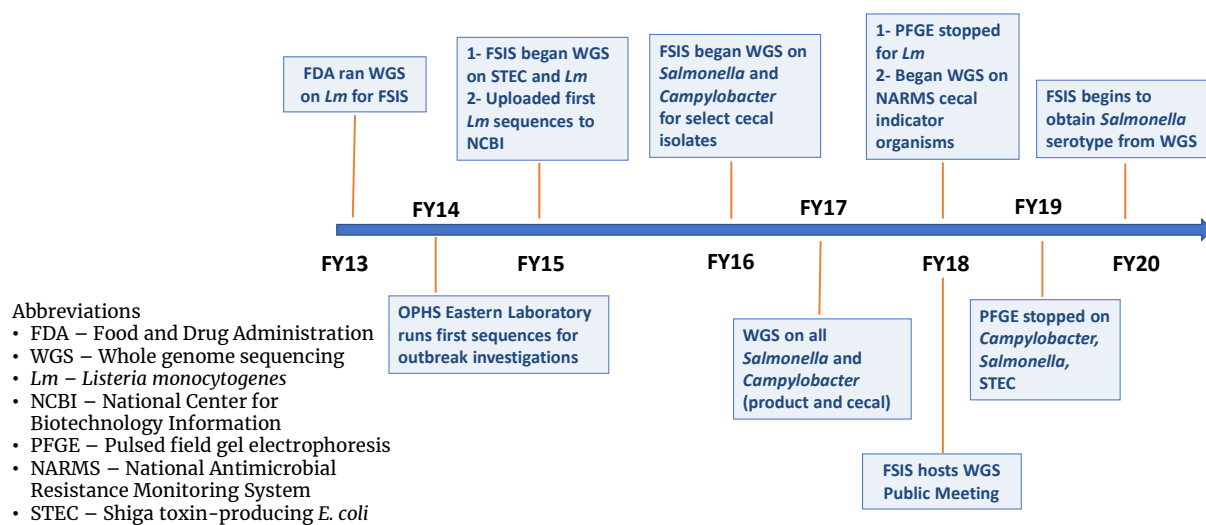
- When performing a PHRE in establishments with more than one positive RTE sample, EIAOs are to:
  - Use the Form ID to Request WGS analysis of previous matches from the OPHS - Microbial Characterization Branch (OPHS-MCB) from [Outbreaks\\_WGS@fsis.usda.gov](mailto:Outbreaks_WGS@fsis.usda.gov)
  - The WGS analysis will indicate if there is a history of harborage or cross-contamination in the establishment.
- After an IVT/RLm positive, EIAOs are to make a request through the [Outbreaks\\_WGS@fsis.usda.gov](mailto:Outbreaks_WGS@fsis.usda.gov) Outlook mailbox for WGS analyses.

## Whole Genome Sequencing – The Future

- FSIS continually works with FDA, CDC PulseNet, local & state health departments to harmonize interpretation and reporting.
- Future plans -- pathogens that will be reported by allele code:
  - STEC and *Campylobacter jejuni* allele codes were released in early 2021
  - *Salmonella* is still being finalized by PulseNet

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## Whole Genome Sequencing – The Future



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