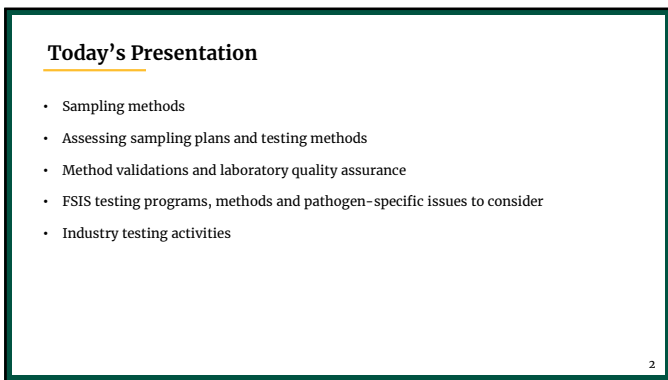
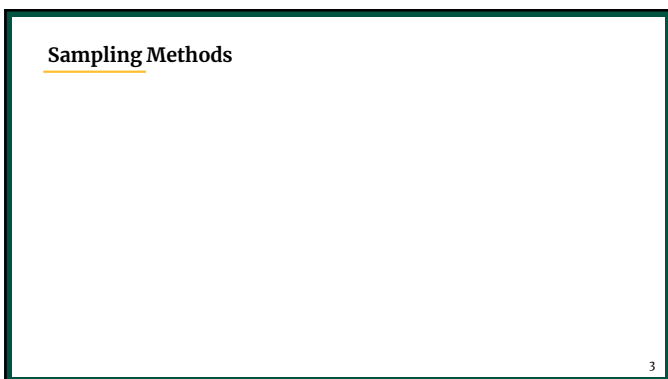


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Sampling Methods

- 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:
 - 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 – stay tuned

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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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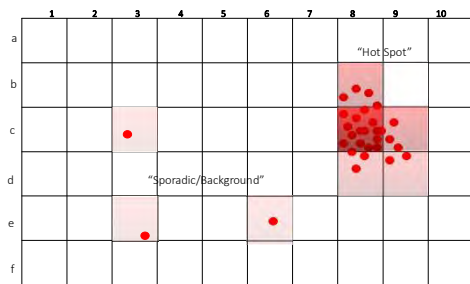
Why are Pathogens Hard to Detect?

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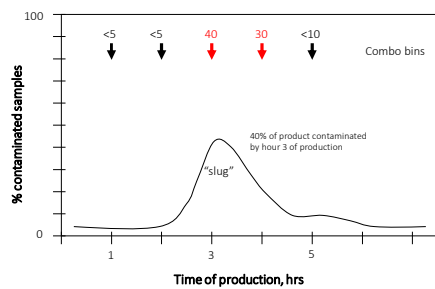
E. coli O157:H7 Contamination in a N60 Sampled Lot



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E. coli O157:H7 Contamination in Ground Beef



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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

14

14

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?

16

16

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?

17

17

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

18

18

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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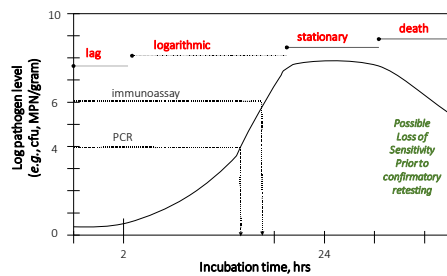
Considerations for Proper Enrichment

- 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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20

Pathogen Growth During Enrichment



21

21

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

22

22

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)

23

23

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

24

24

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

25

25

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*)

26

26

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*)

27

27

Method Validations

28

28

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

29

29

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

30

30

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
- 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

31

31

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)

32

32

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

33

33

Testing Method Specifications

- **Sensitivity:** probability that truly positive samples are detected as positive by analytical test
 - 100 – false negative rate
- **Specificity:** probability that truly negative samples detected as negative by analytical test
 - 100 – 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)

34

34

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.)

35

35

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

36

36

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

37

37

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

38

38

FSIS Testing Programs

39

39

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)

40

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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

41

41

FSIS Methods and Pathogen-specific Issues to Consider

42

42

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

43

43

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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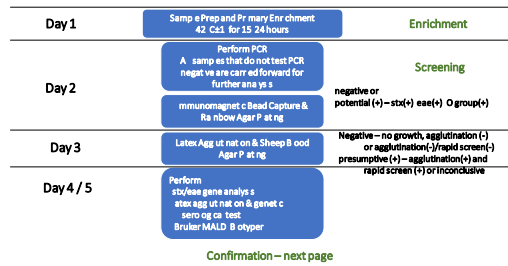
Non-O157 STEC Program

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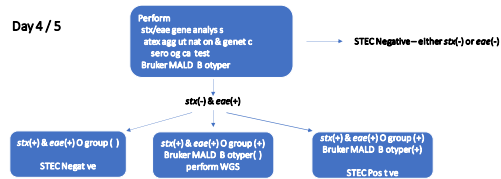
E. coli Top Seven STEC Analysis (MLG 5C.02)



46

46

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



47

47

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

48

48

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)

49

49

Listeria testing

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

50

50

Listeria monocytogenes (MLG 8.13)

Day 1	Sample Prep and Primary Enrichment 5 cm ³ 25g sample + 225 cm ³ BHIW incubate 30°C for 20-26 hrs	Enrichment
Day 2	Plating Secondary Enrichment incubate 35°C - MOP 24-28 hrs incubate 35°C - MOP 18-24 hrs	
Day 3	Screening 3M MoAssoc Detection Assay 2 Horse B. food and MCHP plates	possible(+) confirm (-) – both must be negative
Day 4	Re-streak on hemolysis incubate 35°C overnight	presumptive(+) – hemolysis
Day 5	Brulser MALDI Biotyper and re-streak	presumptive(+) – hemolytic Confirmed (+) – on Brulser MALDI Biotyper
Day 6	For characteristics on morphology and biophysical analysis	presumptive(+) from previous day are Confirmed (+) – by Brulser MALDI Biotyper

Perform WGS

51

51

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

52

52

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)

53

53

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

54

54

Salmonella (MLG Ch. 4.11)

Day 1	Sample Prep and Primary Enrichment S media, incubate 6 hrs incubate 35°C for 18-24 hrs RTE, 20-24 hrs poultry	Enrichment
Day 2	Perform PCR A samples for detection PCR negative and confirmed on IV and TT broth incubate 35°C for 22-24 hrs	confirm (-) (+) - 2 nd enrichment
Day 3	S media IV and TT on BGS and DMLA plates incubate 35°C for 18-24 hrs	Screening
Day 4	Plaque assay only, non-plated media TS and LAs sets incubate 35°C for 22-26 hrs	
Day 5	S media on SBA or biochemical testing incubate 35°C for 22-26 hrs	presumptive (+) confirm (-)
Day 6	Brucella MALDI Biotyper	confirm (-/+)

55

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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)

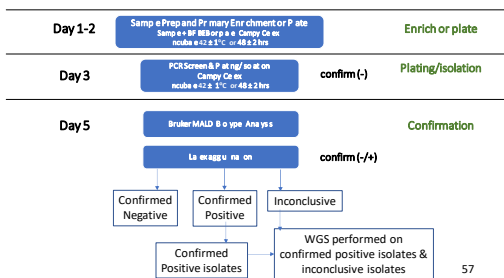
Targets

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

56

56

Campylobacter (MLG 4.1.06) - Qualitative



57

57

57

Issues for *Campylobacter* Testing

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

58

58

Industry Testing Programs

59

59

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?

60

60

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?

61

61

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 25g 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

62

62

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

63

63

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?

64

64

Helpful Guidance

65

65

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**

66

66

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)

67

67

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/>

68

68

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)

69

69

Whole Genome Sequencing (WGS)

70

70

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.

71

71

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, and
 - Identification of unique genes related to virulence, pathogenicity, survival, adaptation, and resistance to biocides (sanitizers, metal, etc.) and antimicrobials.

72

72

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)

73

73

Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)

Single Nucleotide Polymorphism (SNP)

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

74

74

Whole Genome Sequencing – Sequence Typing

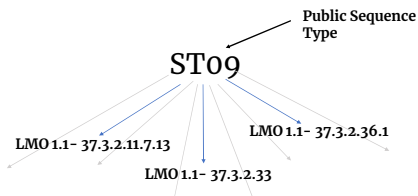
Multi-locus Sequence Typing (MLST)

- 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)

75

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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.

76

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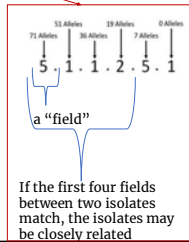
Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)

Example: LMO1.1 - 5.1.1.2.5.1

LMO – L.
monocytogenes
Version 1.1

Allele codes are a
nomenclature scheme
created by CDC

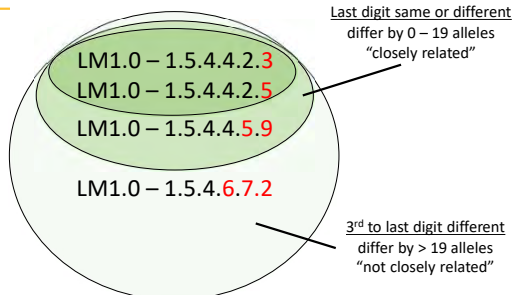
Like PFGE patterns,
allele codes simplify
how we communicate
about pathogen
strains



77

77

Background: What does allele code tell you?



78

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

79

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.

80

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

Establishment	Field	88068019	88068016	201074262	201647723
MS4-P54 (LocID: 9542)	FormID	102396413	102396402	11629154	11619429
	Collect Date	2020-11-09	2020-11-09	2012-03-20	2011-10-11
	Allele Code	LM01.1 - 8.1.2.6.A.1	LM01.1 - 8.1.2.6.A.1	LM01.1 - 8.1.2.6.2	LM01.1 - 8.1.2.6.2
	MLST ST	ST204	ST204	pubMLST204	pubMLST204
	Project	INTENV_LM_M	INTCONT_LM_M	INTENV	RTS001
	FSIS Identifier	FSIS22020688	FSIS22020687	FSIS11816790	FSIS11816794
	NCBP Accession Number	SABN16806333	SABN16806180	SABN16806026	SABN16806029
	NCBP SNP Cluster (Reference Date)	PC0000024493.9 2020-11-23	PC0000024493.9 2020-11-23	PC0000024493.9 2020-11-23	PC0000024493.9 2020-11-23
	Min Pooled Env. (SNP)	0	0	0	0
	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 Select 05.28](#)

***Cross contamination is suggested when closely related Lm isolates are found in product, food contact, and environmental (potential contact)

samples collected during the same sampling event. [FSIS Select 05.28](#)

****Clinical isolates collected and updated within two years of the raw isolate based on isolating NCBP practices

81

81

Harborage and Cross-contamination

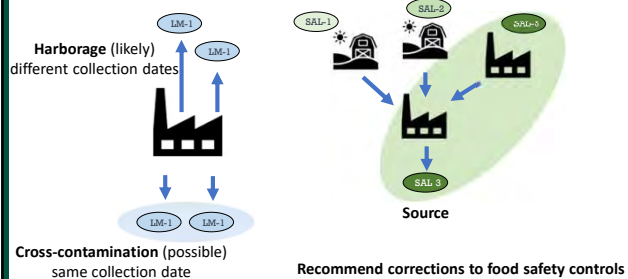
- **Harborage** or persistent contamination of the post-lethality environment, is suggested if WGS analysis indicates closely related *Lm* isolates are found in product, food contact, or non-food contact environmental samples that were collected over multiple days, weeks, months, or years.
- **Cross-contamination**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

82

82

Harborage and Cross-contamination



83

83

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.

84

84

PulseNet Cluster Search

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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.

85

PulseNet Cluster Search

Primary Establishment Number: P09
Establishment Name: P09 - Midwest Inc.
Result Date Range for the Establishment's isolates (52 weeks from report date): 06/13/2020 - 06/18/2020
Date Range for PulseNet (60 days from report date): 04/19/2020 - 08/08/2020

Establishment Number	Project Code	Lab Form ID	Collection Date	P09 Number	Clinical match in P09 Cluster	Must repeat clinical isolation assay
P09	HC_CPT_18W01	10240782	6/2/2020	P0912010128	Sequence Pending	Sequence Pending
P09	HC_CPT_18W01	10247088	5/27/2020	P0912011276	Sequence Pending	Sequence Pending
P09	P_CW_CARC01	102440119	4/13/2020	P0912010109	No	none
P09	HC_CW_CARC01	102440121	4/6/2020	P0912010784	No	none
P09	HC_CPT_18W01	102444257	4/6/2020	P0912010175	No	none
P09	P_CW_CARC01	102440119	4/13/2020	P0912010773	No	06/20/2020 (P0912010141/0103)
P09	HC_CW_CARC01	102407784	2/13/2020	P0912010162	No	none
P09	HC_CPT_18W01	102394292	1/28/2020	P0912010708	No	none
P09	P_CPT_18W01	102397460	1/20/2020	P0912010010	No	01/05/2020 (P0912010144/0101)
P09	HC_CW_CARC01	102377808	11/18/2019	P0911977518	No	none
P09	HC_CPT_18W01	102357995	12/12/2019	P0911977514	No	none
P09	HC_CW_CARC01	102347864	11/26/2019	P0911977511	No	none
P09	HC_CPT_18W01	102320384	11/11/2019	P0911978809	No	none
P09	HC_CPT_18W01	102318007	11/12/2019	P0911978767	No	none
P09	HC_CPT_18W01	102329383	11/11/2019	P0911978145	No	none
P09	HC_CPT_18W01	102303439	10/14/2019	P0911978275	No	06/11/2020 (P0912010140/0106)
P09	HC_CW_CARC01	102277805	8/27/2019	P0911974047	No	none
P09	HC_CW_CARC01	102260401	8/6/2019	P0911974047	No	none

Sample collection dates (FSIS projects)

Sample collection dates (PulseNet human cases)

FSIS sample matches PulseNet cluster (yes/no)

FSIS sample matches recent PulseNet clinical isolate

86

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
 - 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 Outlook mailbox for WGS analyses.

87

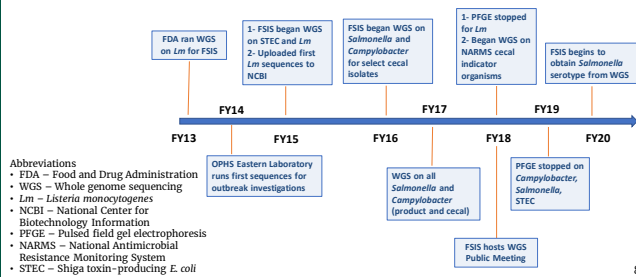
Whole Genome Sequencing – The Future

- 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

88

88

Whole Genome Sequencing – The Future



89

89



90