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

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

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

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*)

Assessing Sampling Plans

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

Sampling Plans

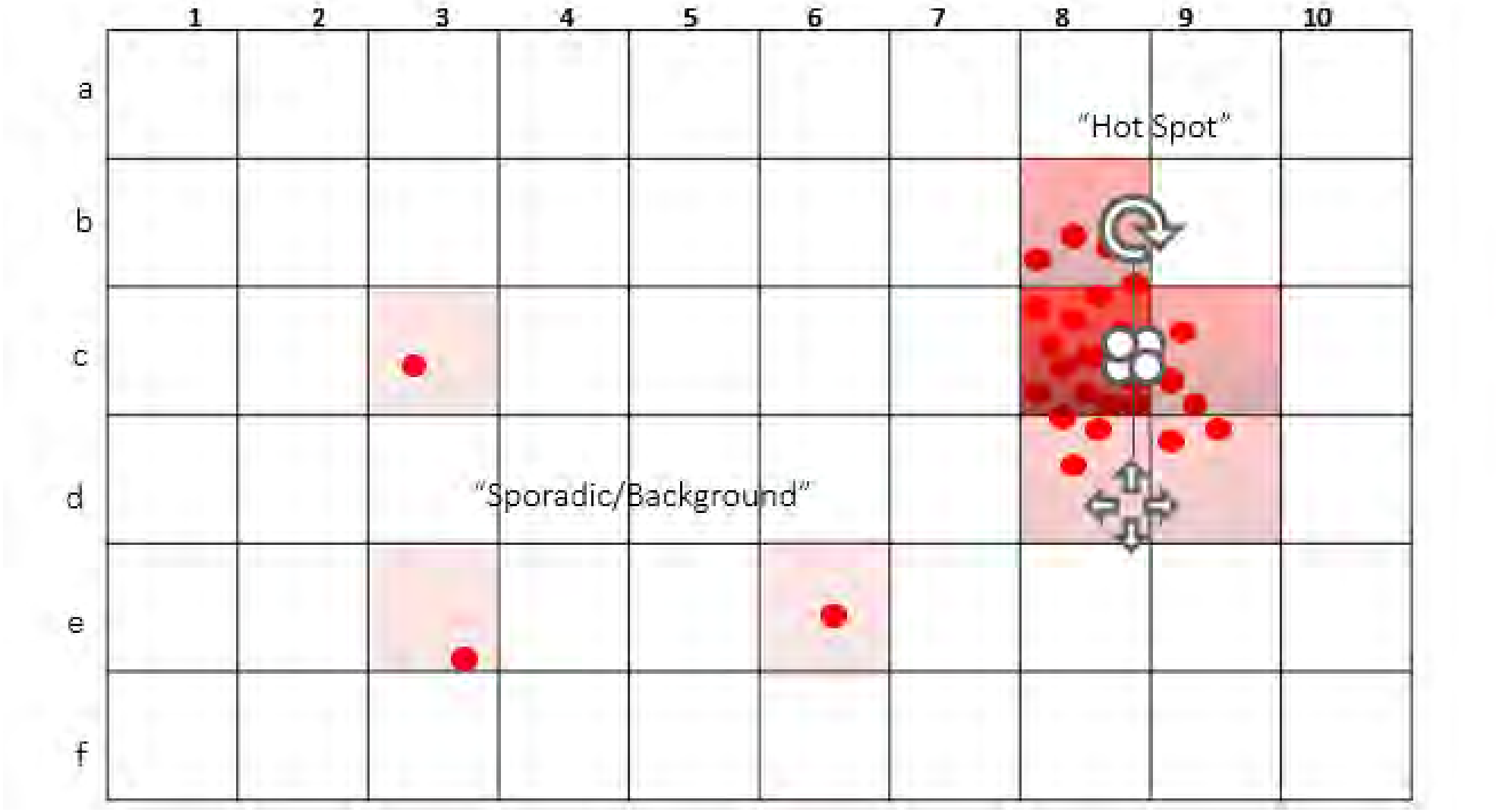
Statistical sampling plans assume:

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

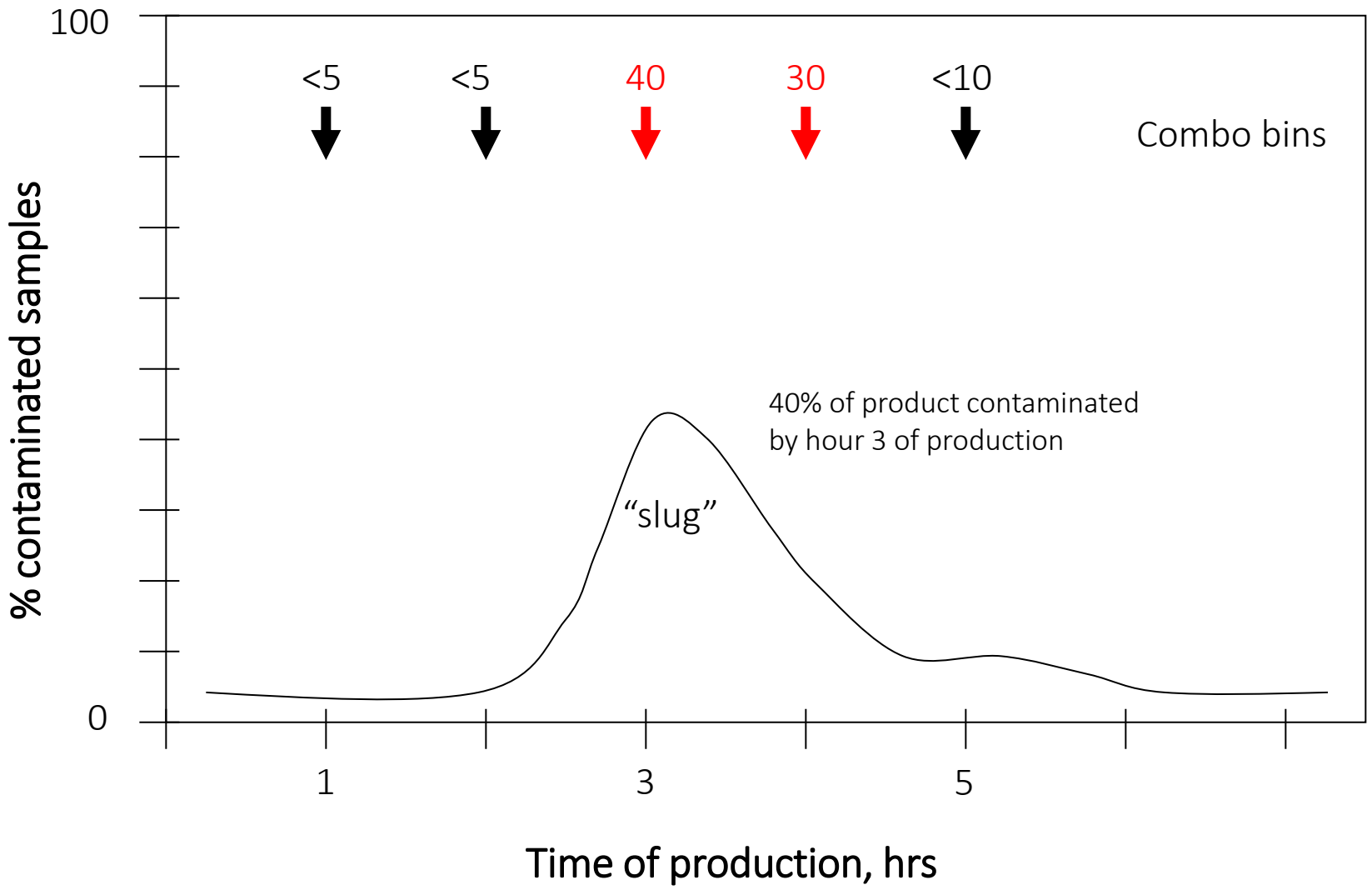
Why are Pathogens Hard to Detect?

- 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

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



E. coli O157:H7 Contamination in Ground Beef



Assessing Testing Methods

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

Steps in Detection Methods

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

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?

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?

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

Enrichment

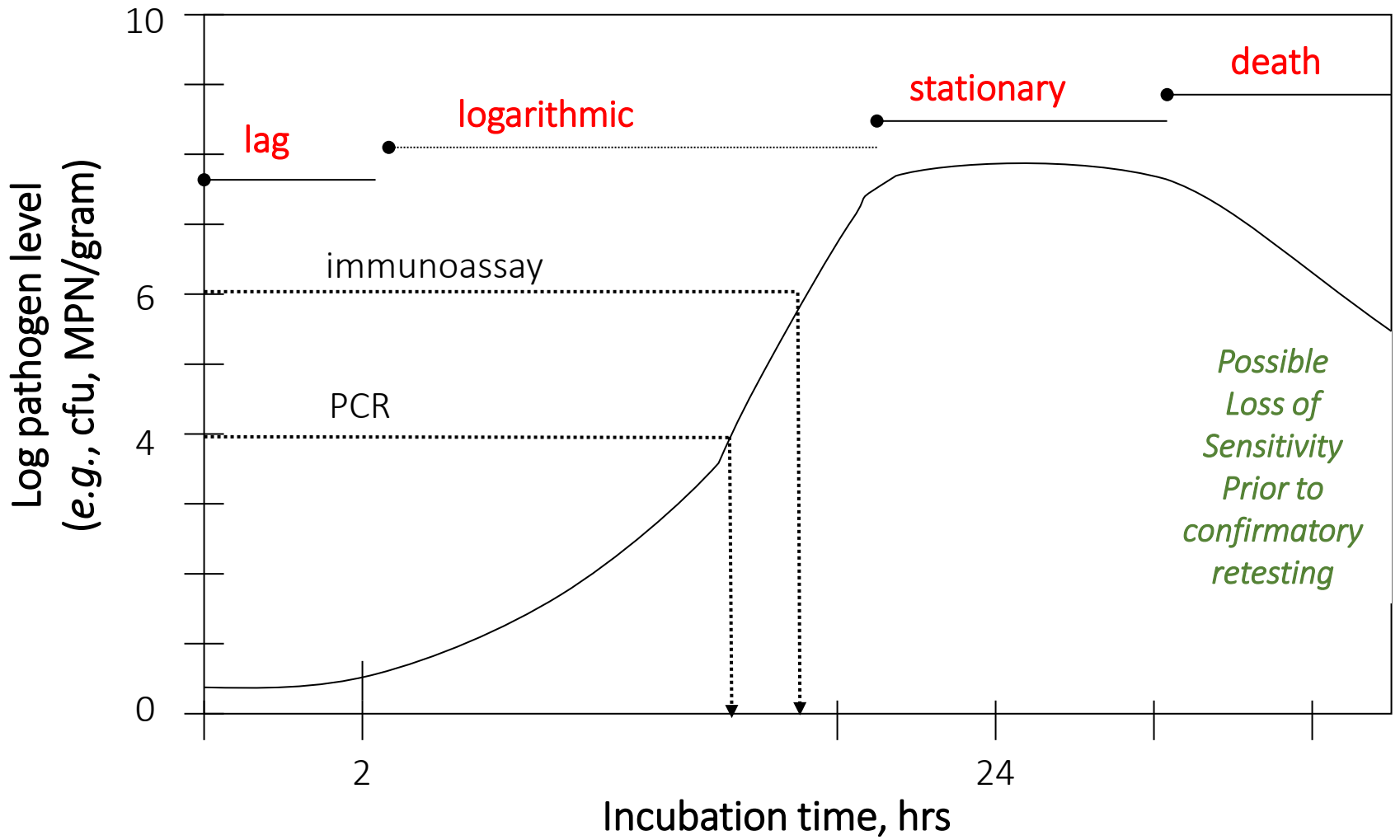
- Test portion is incubated 8-48 hours in a culture broth
  - Why?
    - Contamination levels are too low for detection without enrichment
    - Must grow to high levels so very small volumes have enough pathogen present for later detection steps
- Different pathogens require different enrichment media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

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

Pathogen Growth During Enrichment



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

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)

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

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

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*)

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*)

Method Validations

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

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

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

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)

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

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)

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.)

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

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

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

FSIS Testing Programs

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)

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

FSIS Methods and Pathogen-specific Issues to Consider

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

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.

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

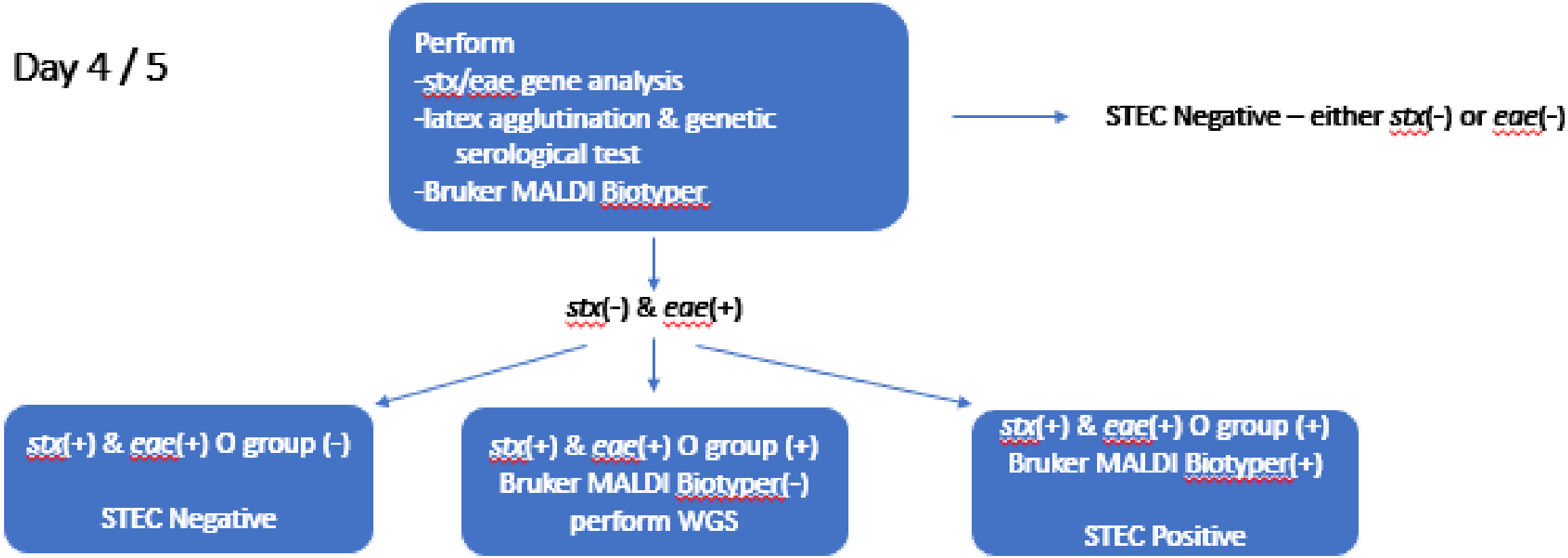
E. coli Top Seven STEC Analysis (MLG 5C.02)

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	Screening negative or potential (+) – stx(+) eae(+) O group(+)
	Immunomagnetic Bead Capture & Rainbow Agar Plating	
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 -stx/eae gene analysis -latex agglutination & genetic serological test - Bruker MALDI Biotyper	

Confirmation – next page

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

Day 4 / 5



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

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

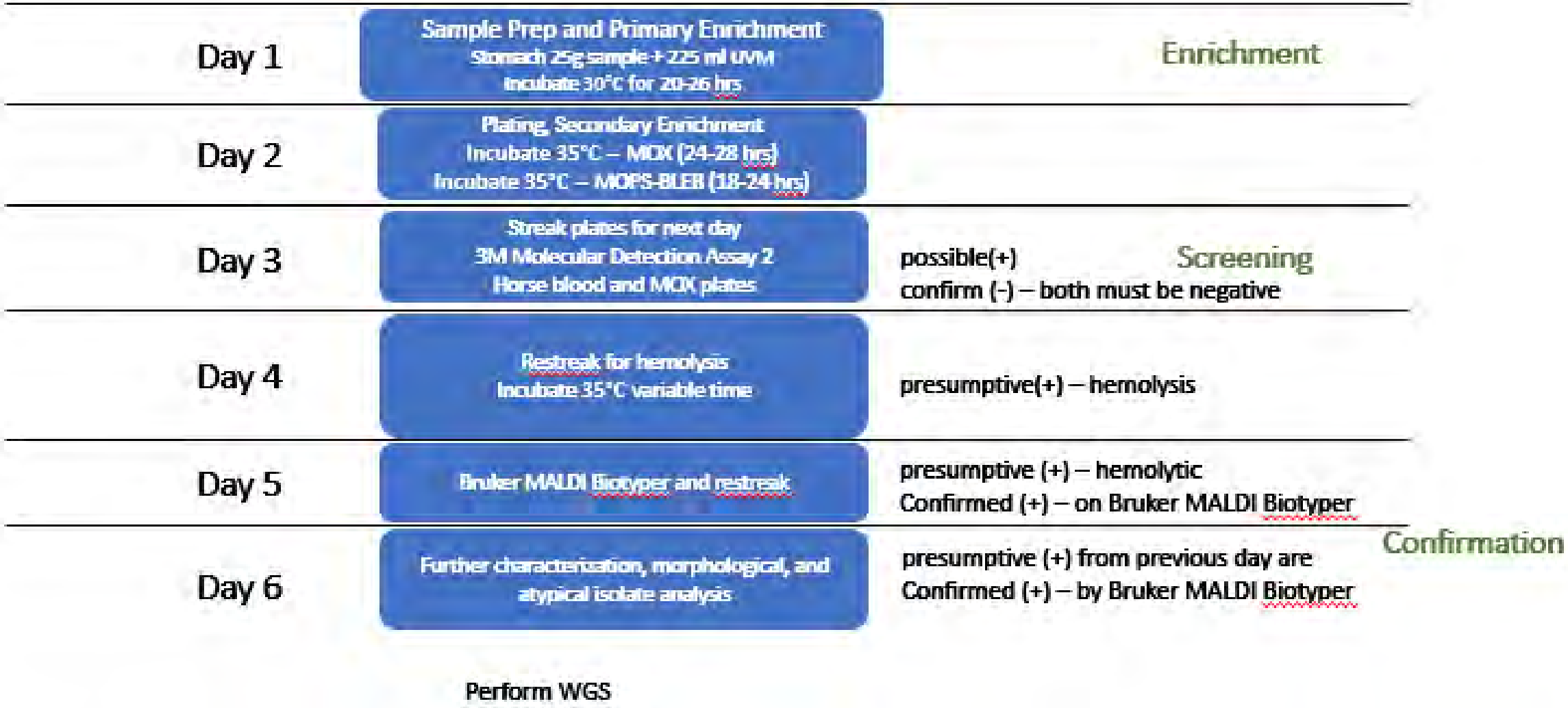
- Culture-based detection and confirmation requires immunomagnetic separation (IMS)

Listeria testing

Includes:

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

Listeria monocytogenes (MLG 8.13)



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

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

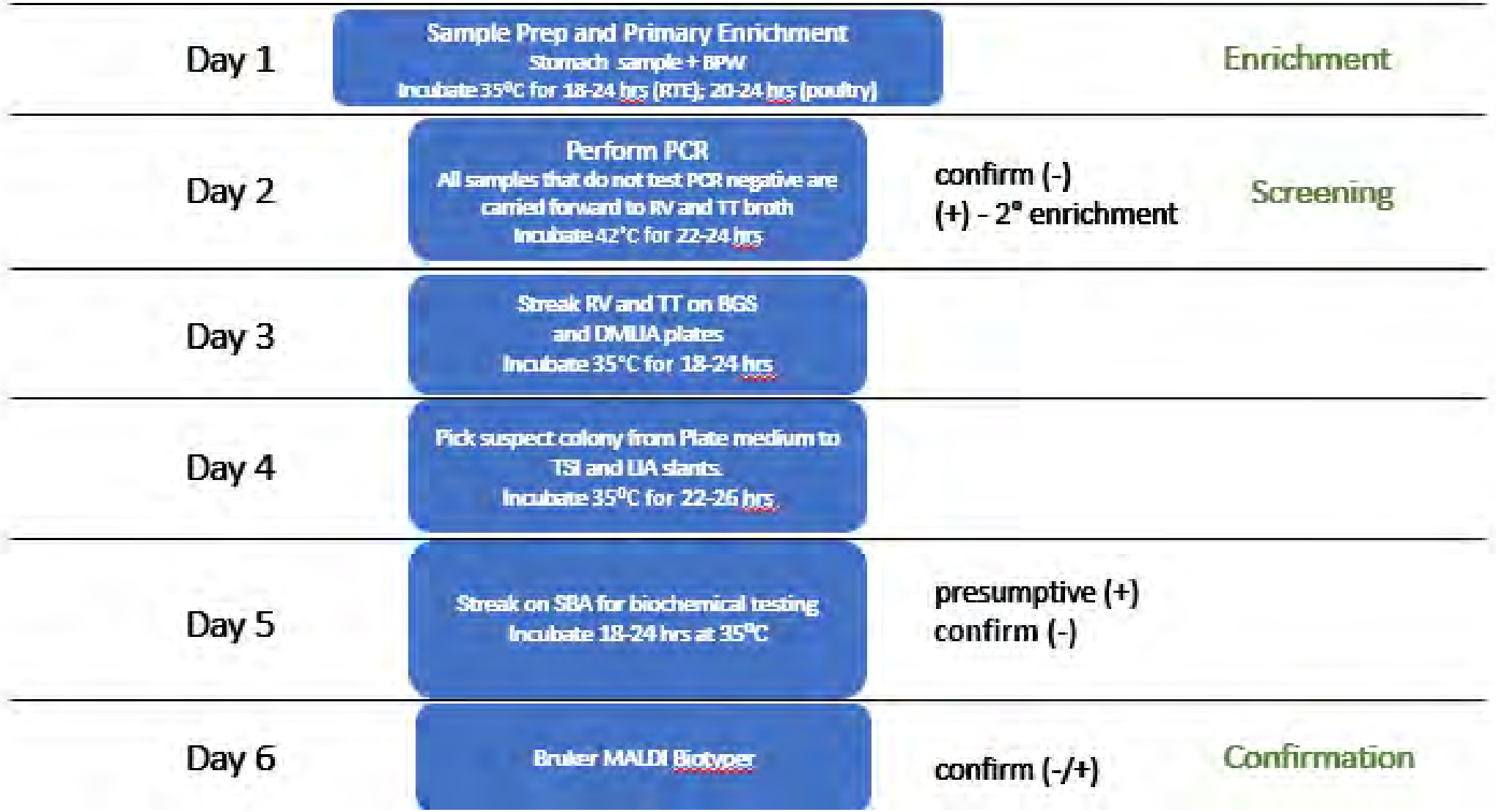
- "*Listeria*-like" indicator bacteria
  - Use the first part of an internationally recognized enrichment-based method to find suspect *Listeria* colonies (*e.g.*, darkened colonies on MOX using the FSIS method)

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

Salmonella (MLG Ch. 4.11)



Campylobacter Testing

Qualitative

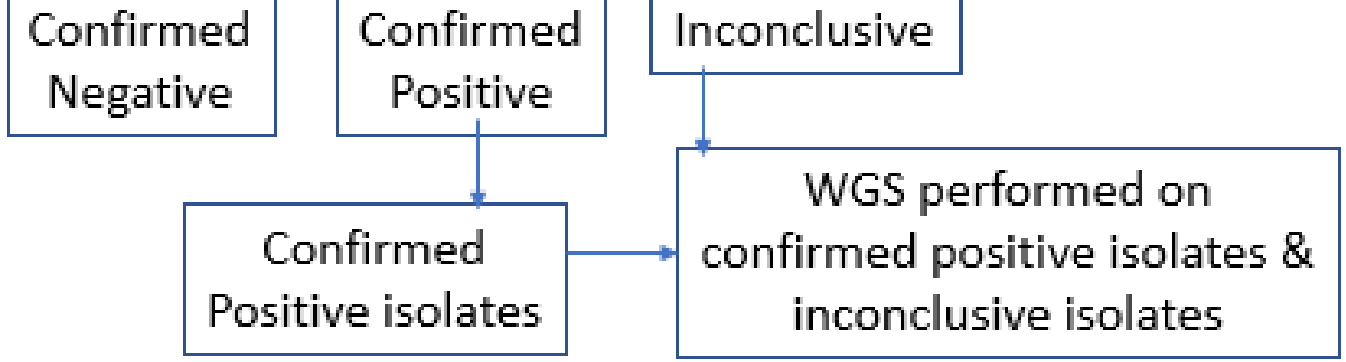
- Enrichment-based (as opposed to direct plating) since Aug 27, 2018 - exception: “other raw chicken parts” (EXP_CPT_OT01 and LO_CPT_OT01)

Targets

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

Campylobacter (MLG 41.06) – Qualitative

Day 1-2	Sample Prep and Primary Enrichment or Plate Sample + BF-BEB or plate (Campy-Cefex) Incubate 42 ± 1°C for 48 ± 2 hrs		Enrich or plate
Day 3	PCR Screen & Plating/Isolation Campy-Cefex Incubate 42 ± 1°C for 48 ± 2 hrs	confirm (-)	Plating/isolation
Day 5	Bruker MALDI Biotyper Analysis		Confirmation
	Latex agglutination	confirm (-/+)	



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

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?

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?

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

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

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?

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)

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)

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/

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)

Whole Genome Sequencing (WGS)

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.

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.

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)

Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)

Single Nucleotide Polymorphism (SNP)

Whole Genome Sequencing – Sequence Typing

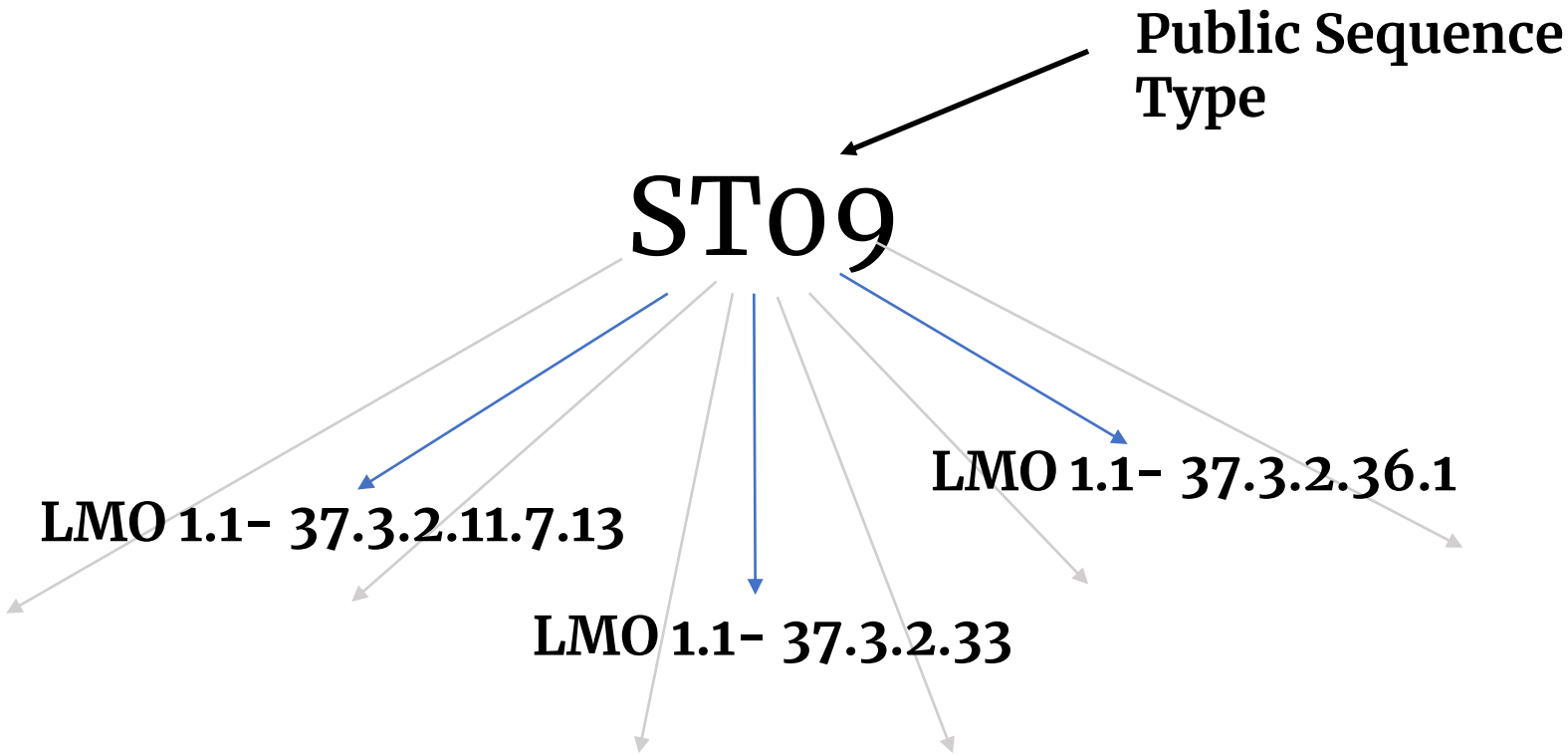
Multi-locus Sequence Typing (MLST)

- 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)

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.

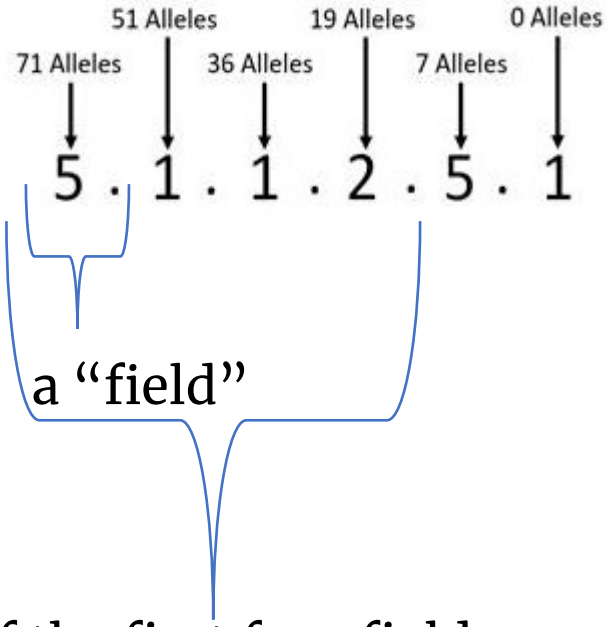
Whole Genome Sequencing – Single Nucleotide Polymorphism (SNP)

Example: LMO1.1 - 5.1.1.2.5.1

LMO – *L. monocytogenes*
Version 1.1

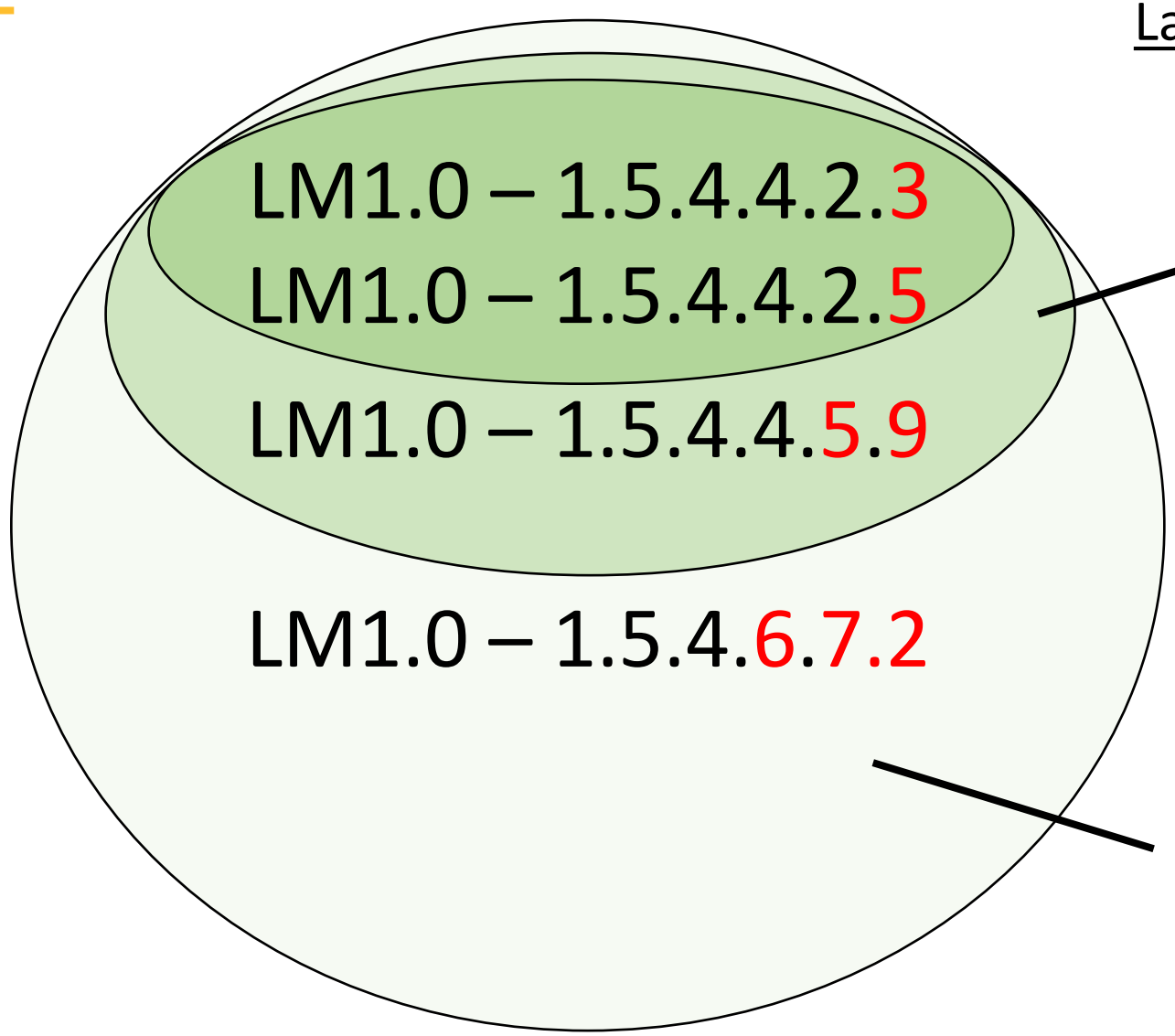
Allele codes are a nomenclature scheme created by CDC

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



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

Background: What does allele code tell you?



Last digit same or different
differ by 0 – 19 alleles
"closely related"

3rd to last digit different
differ by > 19 alleles
"not closely related"

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
M54-P54 (LocID: 9542)	FormID	102595413	102595402	11629154	11610429
	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
	MLST ST	ST204	ST204	publicST204	publicST204
	Project	INTENV_LM_M	INTCONT_LM_M	INTENV	RTE001
	FSIS Identifier	FSIS22029688	FSIS22029687	FSIS11816785	FSIS11816784
	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		

LIMS ID

Form ID

Allele Code

FSIS Identifier

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.*

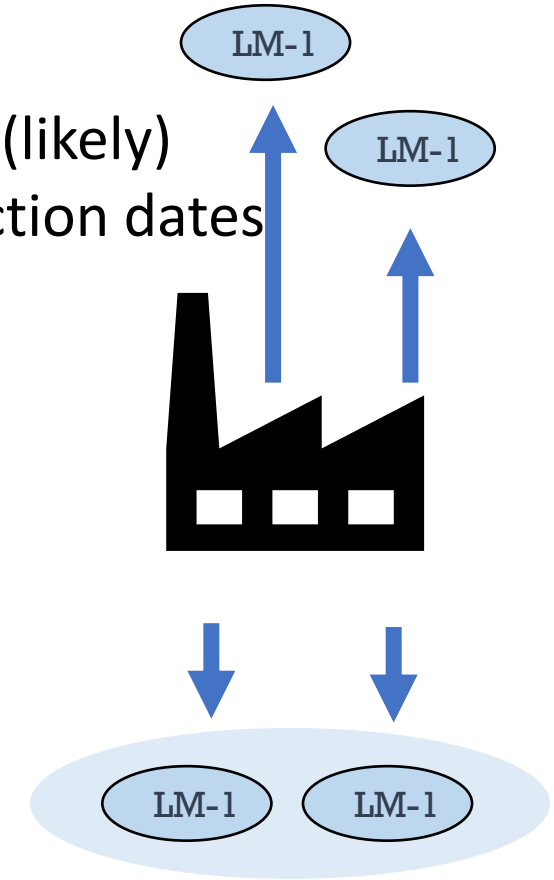
Harborage and Cross-contamination

- **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.

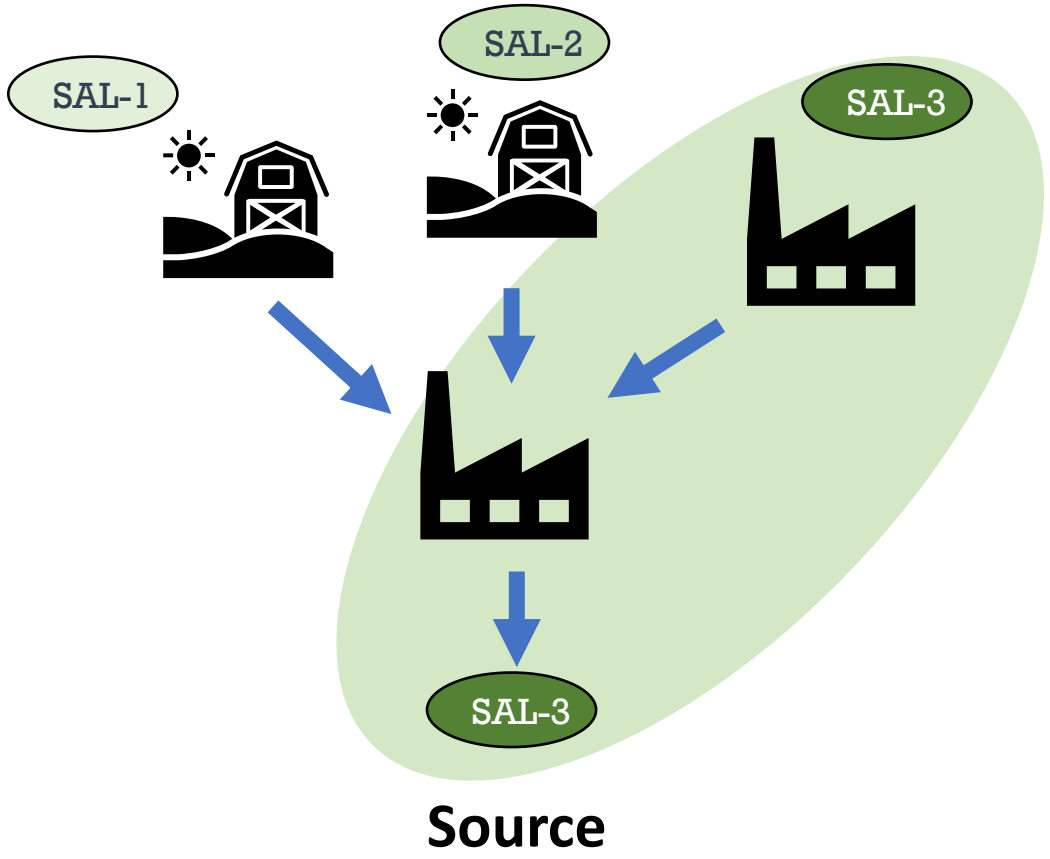
  If *Lm* is isolated from a post-lethality exposed product sample and from a food contact surface sample, the food contact surface is more likely to be the source, unless under-processing of RTE product is suspected.

Harborage and Cross-contamination



Harborage (likely)
different collection dates

Cross-contamination (possible)
same collection date



Source

Recommend corrections to food safety controls

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**.

PulseNet Cluster Search

Sample collection dates (FSIS projects)

Sample collection dates (PulseNet human cases)

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 clincial 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_CARC01	102446119	4/13/2020	FSIS12030359	No	none
P39	HC_CH_CARC01	102446121	4/8/2020	FSIS22027846	No	none
P39	HC_CPT_LBW01	102444257	4/6/2020	FSIS12030175	No	none
P39	F_CH_CARC01	102440871	4/1/2020	FSIS22027733	No	04/20/2020 (PNUSAS143703)
P39	HC_CH_CARC01	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_CARC01	102357006	12/18/2019	FSIS11927558	No	none
P39	HC_CPT_LBW01	102352995	12/12/2019	FSIS11927514	No	none
P39	HC_CH_CARC01	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_CARC01	102270365	8/27/2019	FSIS11924547	No	none
P39	HC_CH_CARC01	102260403	8/6/2019	FSIS3190243	No	none

FSIS sample matches PulseNet cluster (yes/no)

FSIS sample matches recent PulseNet clinical isolate

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.

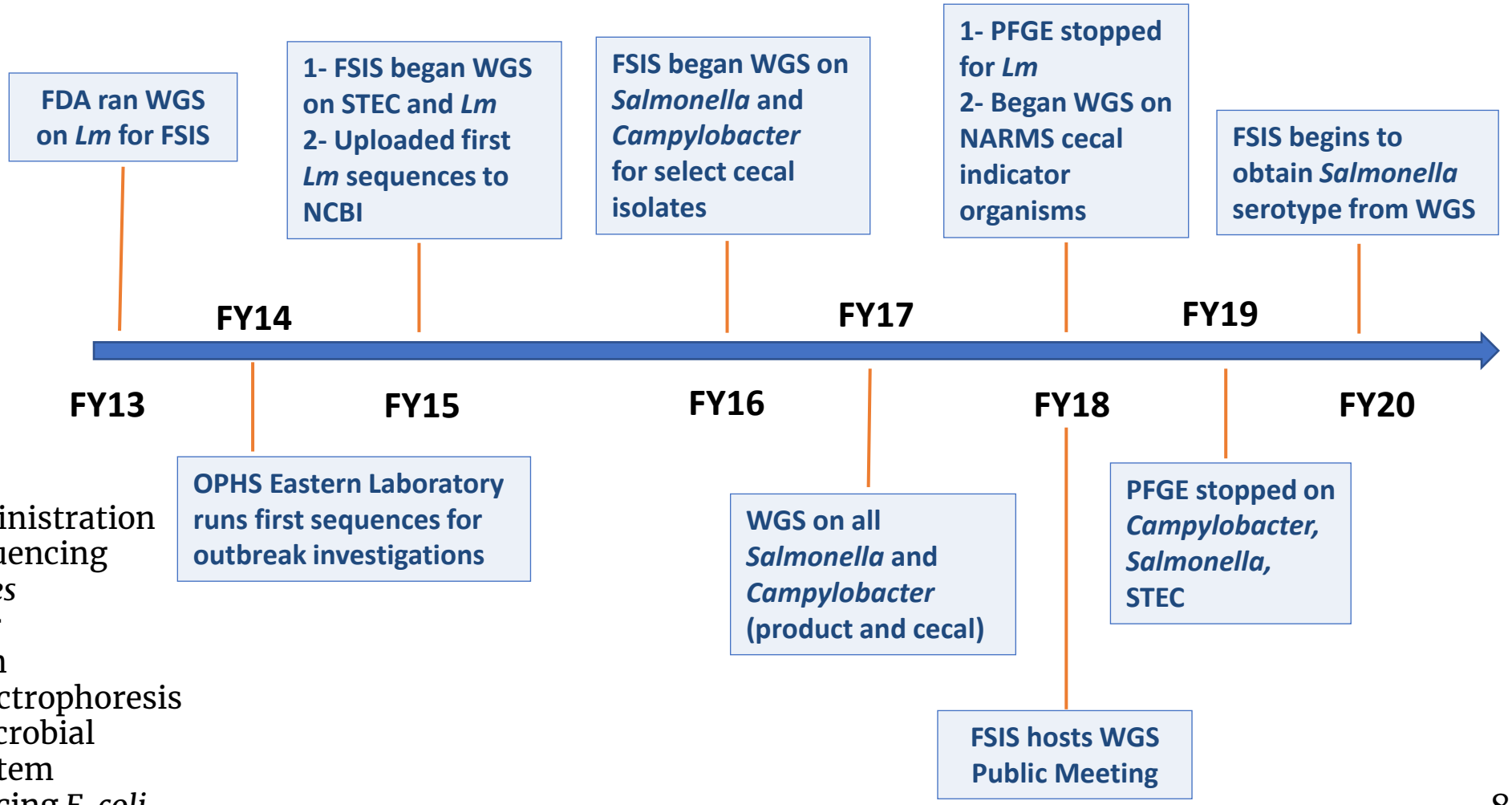
- After an IVT/R*Lm* positive, EIAOs are to make a request through the Outbreaks_WGS@fsis.usda.gov Outlook mailbox for WGS analyses.

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

Whole Genome Sequencing – The Future



Abbreviations
- FDA – Food and Drug Administration
- WGS – Whole genome sequencing
- *Lm* – *Listeria monocytogenes*
- NCBI – National Center for Biotechnology Information
- PFGE – Pulsed field gel electrophoresis
- NARMS – National Antimicrobial Resistance Monitoring System
- STEC – Shiga toxin-producing *E. coli*

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