Food Safety and Inspection Service:

Understanding and Evaluating Microbiological Sampling and Testing

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Office of Public Health Science

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

Sampling Plans

- 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

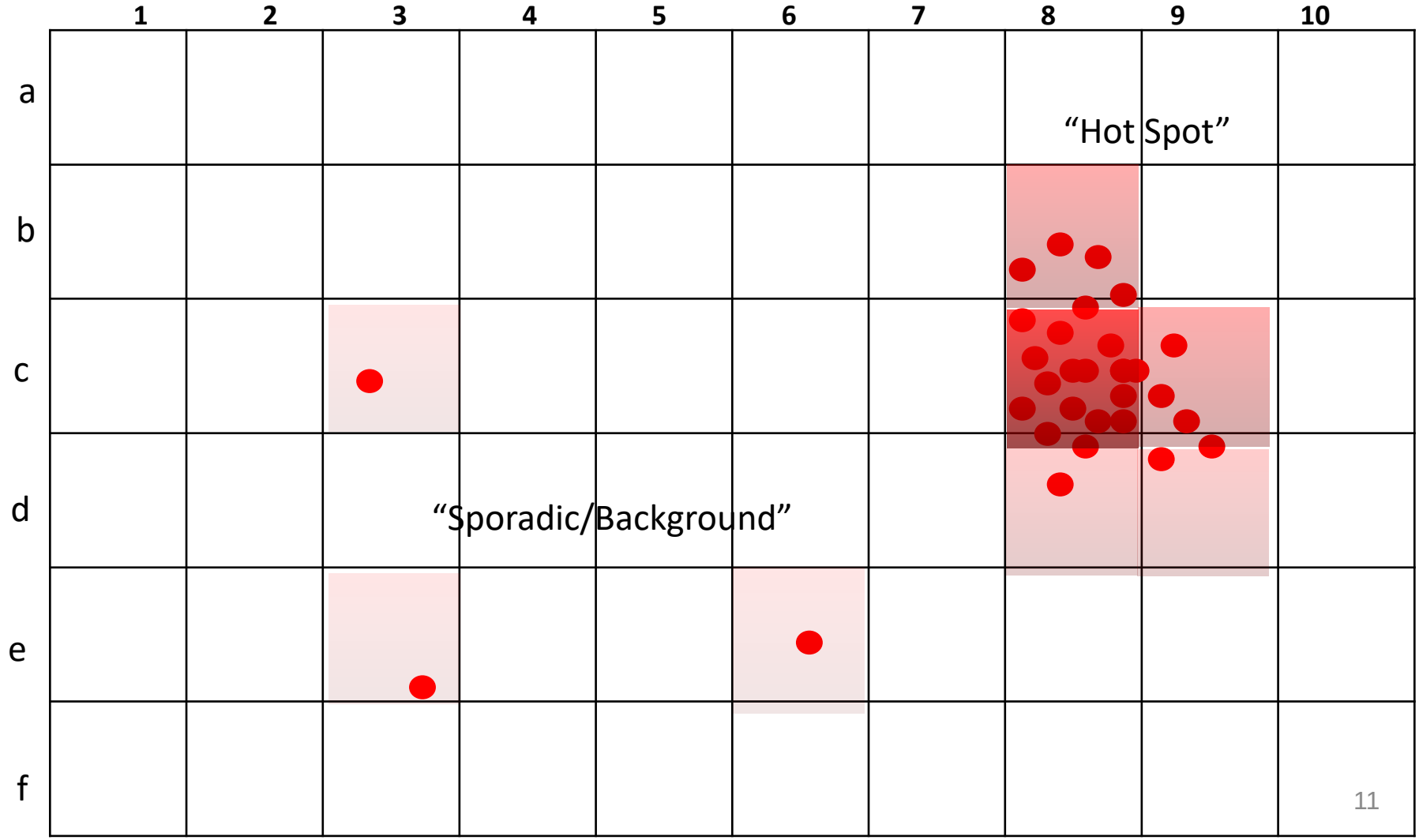
- Statistical sampling plans assume
  - Uniform manufacturing conditions
  - Equal probability of contamination throughout the lot (homogeneous distribution)
  - Independent, random sampling (equal probability of sampling throughout the lot)

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

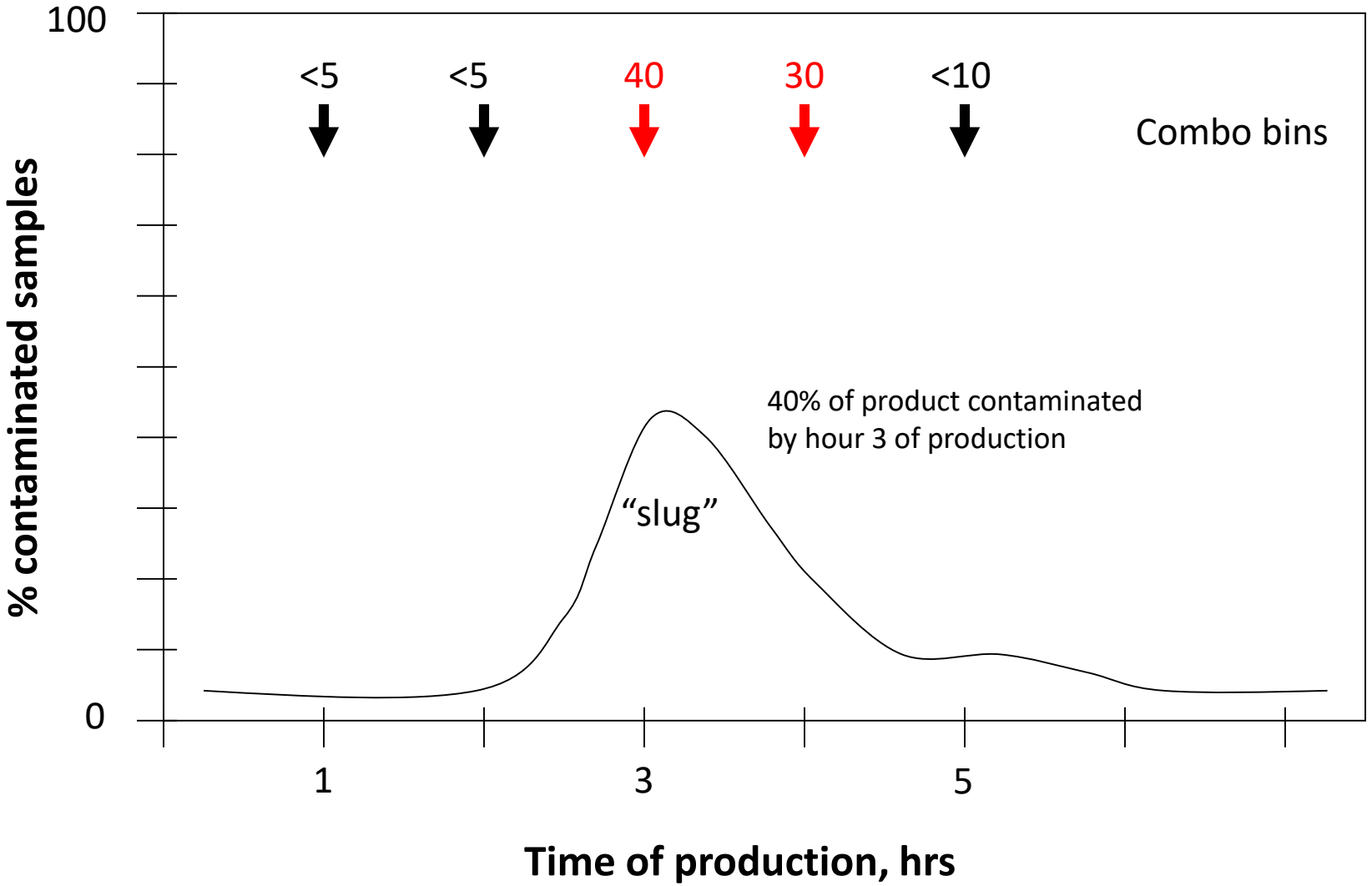
Food Safety and Inspection Service:

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



Food Safety and Inspection Service:

E. coli O157:H7 Contamination in Ground Beef



Assessing Testing Methods

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

Steps in Detection Methods

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

Food Safety and Inspection Service:

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?

Food Safety and Inspection Service:

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

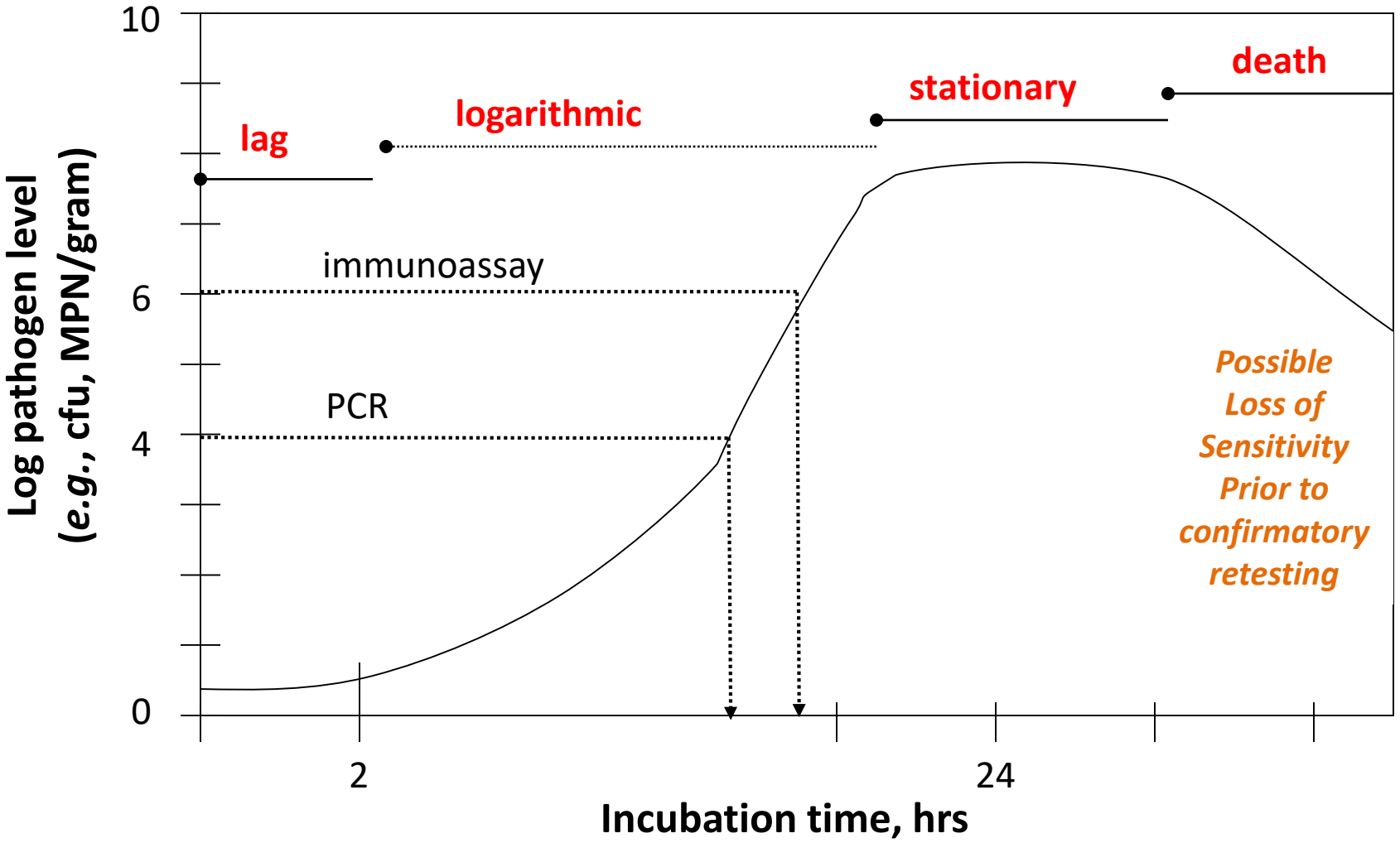
Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

Pathogen Growth During Enrichment



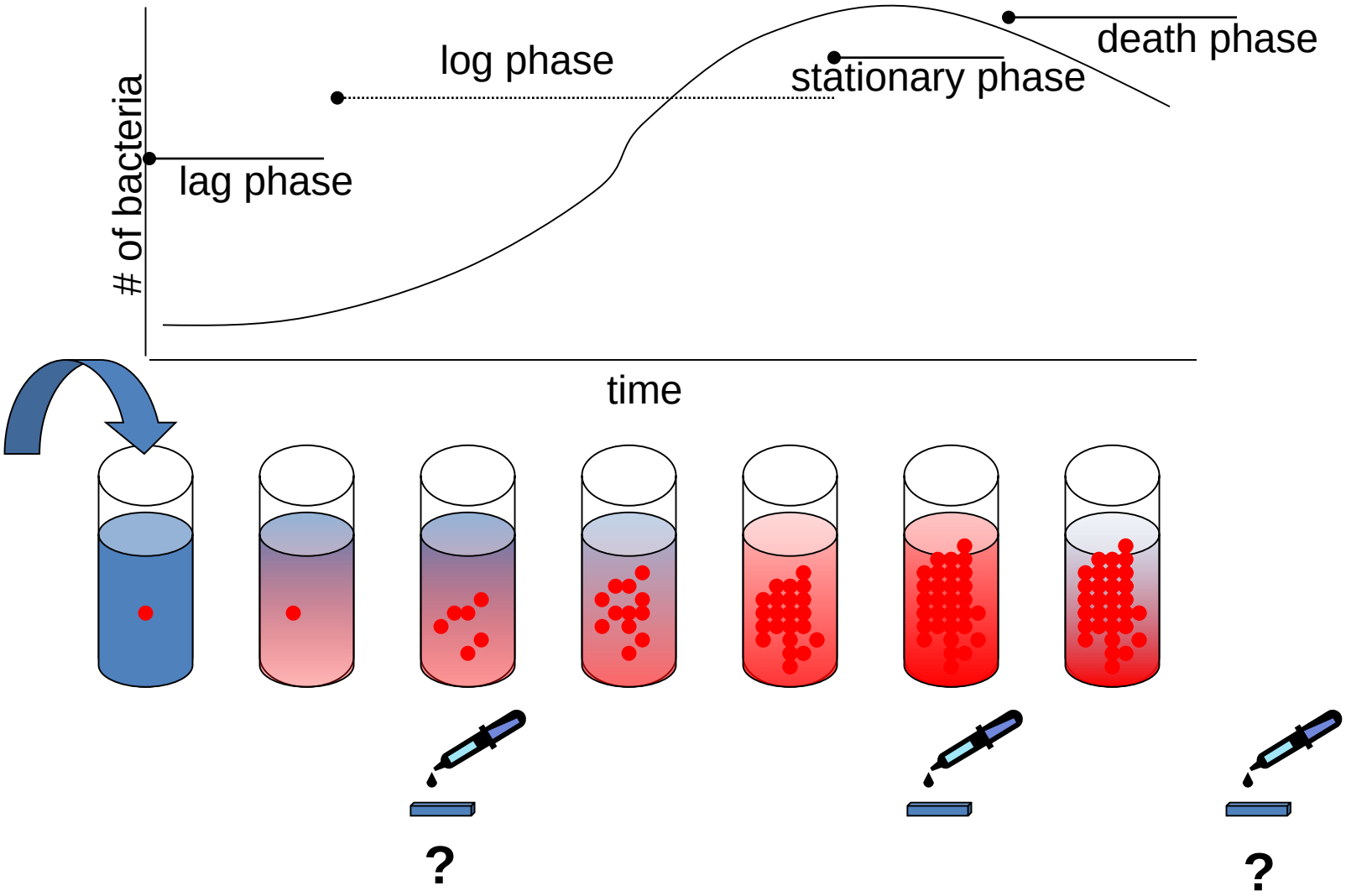
Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

Role of Enrichment



Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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

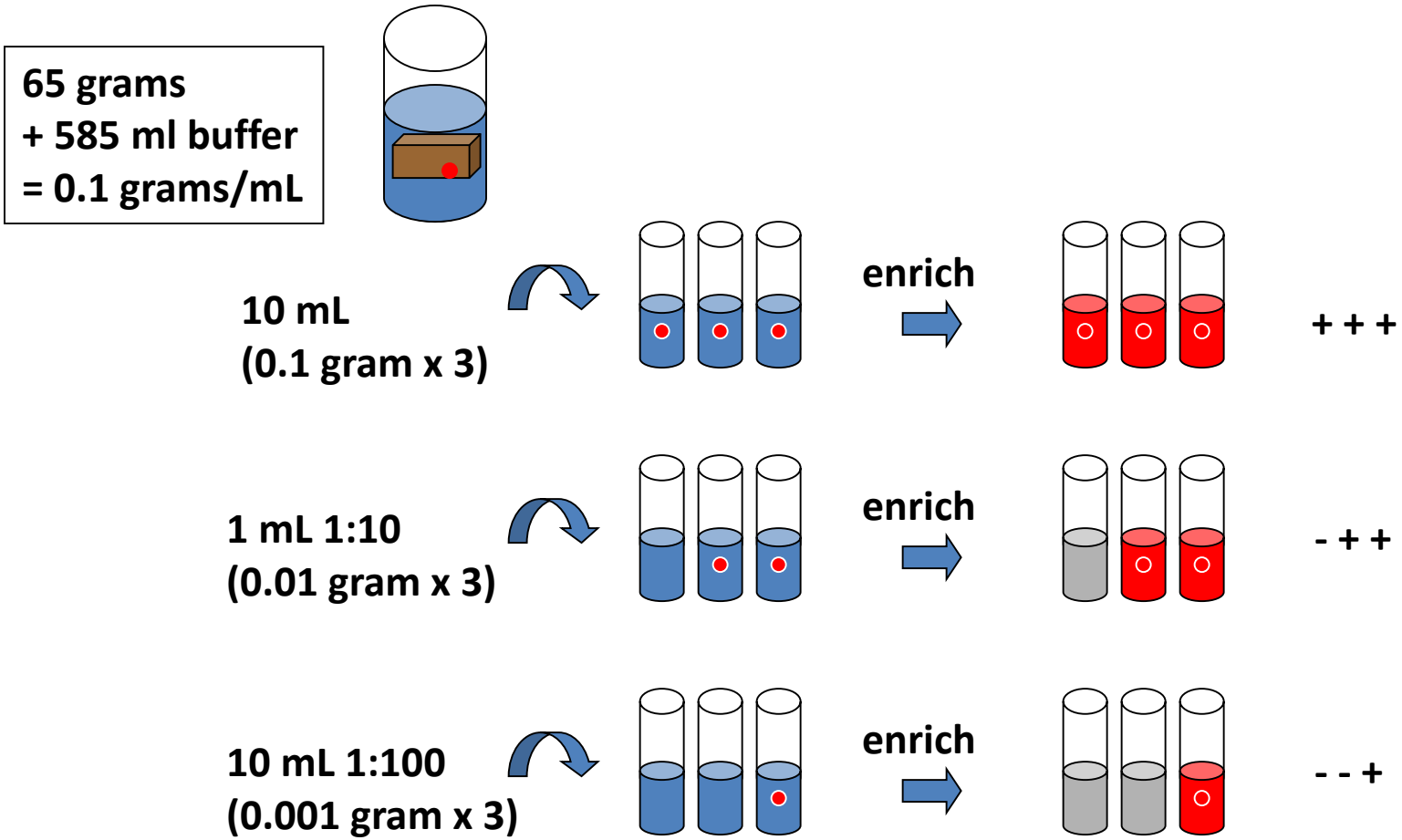
Food Safety and Inspection Service:

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*)

Food Safety and Inspection Service:

Quantitative Testing - MPN (most probable number)



Food Safety and Inspection Service:

Quantitative Testing - MPN (most probable number)

Table 3. MPN Index and 95% Confidence Limits for Various Combinations of Positive Tubes in a 3 Tube Dilution Series Using Inoculum Quantities of 0.1, 0.01 and 0.001 g (ml).

Combination of Positives	MPN Index per g (ml)	95% Confidence Limits Lower	Upper
0-0-0	<3.0	---	9.5
0-0-1	3.0	0.15	9.6
0-1-0	3.0	0.15	11.
0-1-1	6.1	1.2	18.
0-2-0	6.2	1.2	18.
0-3-0	9.4	3.6	38.
1-0-0	3.6	0.17	18.
1-0-1	7.2	1.3	18.
1-0-2	11.	3.6	38.
1-1-0	7.4	1.3	20.
1-1-1	11.	3.6	38.
1-2-0	11.	3.6	42.
1-2-1	15.	4.5	42.
1-3-0	16.	4.5	42.
2-0-0	9.2	1.4	38.
2-0-1	14.	3.6	42.
2-0-2	20.	4.5	42.
2-1-0	15.	3.7	42.
2-1-1	20.	4.5	42.
2-1-2	27.	8.7	94.
2-2-0	21.	4.5	42.
2-2-1	28.	8.7	94.
2-2-2	35.	8.7	94.
2-3-0	29.	8.7	94.
2-3-1	36.	8.7	94.
3-0-0	23.	4.6	94.
3-0-1	38.	8.7	110.
3-0-2	64.	17.	180.
3-1-0	43.	9.0	180.
3-1-1	75.	17.	200.
3-1-2	120.	37.	420.
3-1-3	160.	40.	420.
3-2-0	93.	18.	420.
3-2-1	150.	37.	420.
3-2-2	210.	40.	430.
3-2-3	290.	90.	1000.
3-3-0	240.	42.	1000.
3-3-1	460.	90.	2000.
3-3-2	1100.	180.	4100.
3-3-3	>1100.	420.	---

Example:

"3-2-1" = Y MPN/g

(use MPN table to determine Y)

Total tested:

65 grams FSIS method

Level of Detection =

<0.03 MPN/gram (0-0-0)

Food Safety and Inspection Service:

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*)

Food Safety and Inspection Service:

Quantitative Testing: Direct Plating

Perform a 1:4 dilution using 325 grams of chicken

How much media do I need?

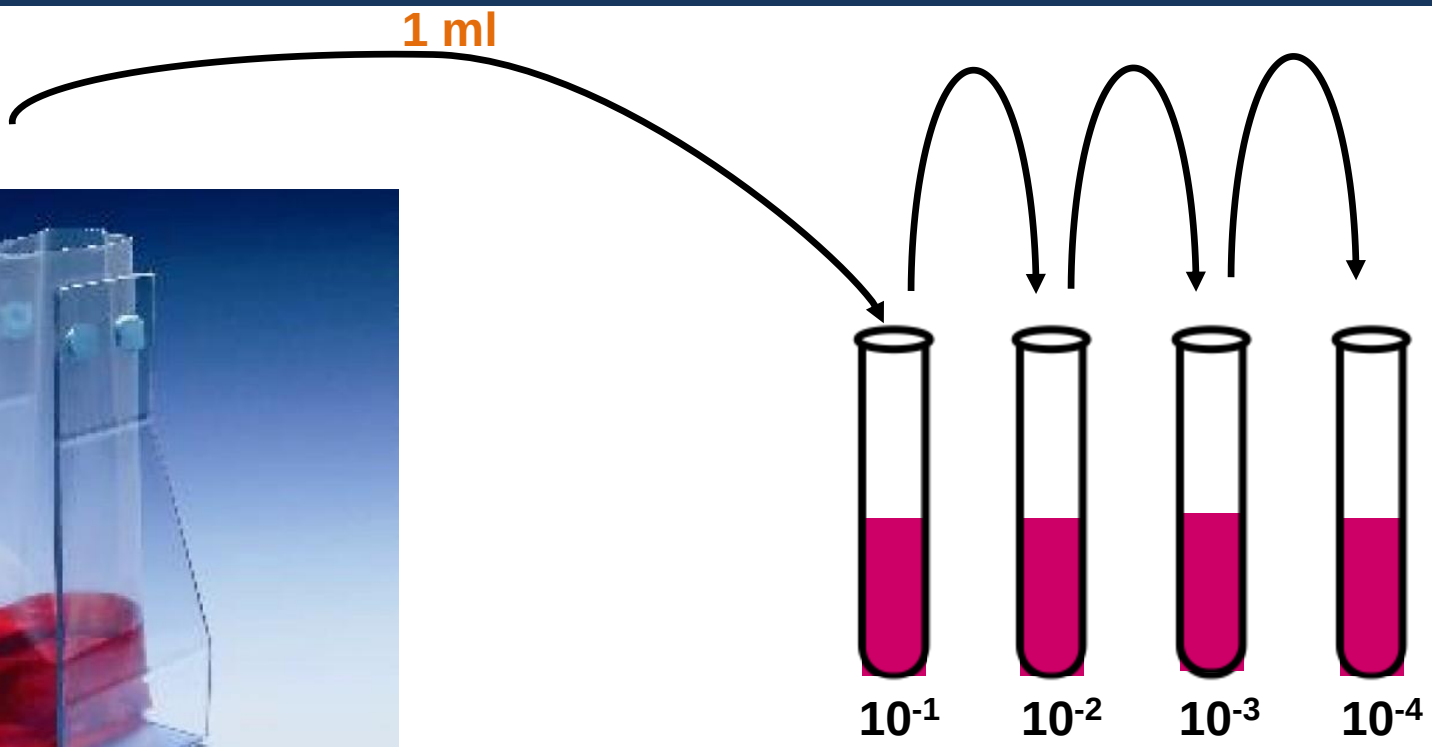
325 g x 4-fold dilution = 1300 g or ml (1 ml = 1g)

1300 ml – 325 g = 975 ml of media

Seal bag and shake

Food Safety and Inspection Service:

Quantitative Testing: Direct Plating



Perform a 1:10 dilutions transferring 1 ml

How much media do I need in each test tube?

1 ml x 10-fold dilution = 10 ml final volume

10 ml – 1 ml = 9 ml of media

Food Safety and Inspection Service:

Quantitative Testing: Direct Plating

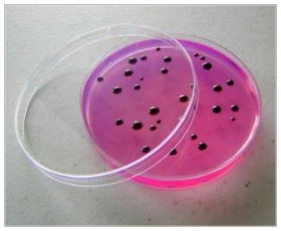
1 ml

1 ml

10^{-1} 10^{-2} 10^{-3} 10^{-4}

0.1 ml

10^{-1}



0.1 ml

10^{-2} 10^{-3} 10^{-4} 10^{-5}

Plate 0.1 ml (or 100 μl) from the test tubes

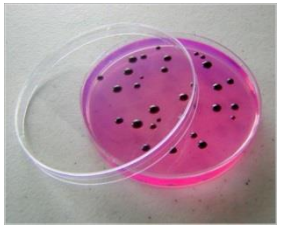
Incubate the plates 37°C for 10 ±2 hr; count the resulting colonies

Food Safety and Inspection Service:

Quantitative Testing: Direct Plating

0.1 ml

	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
colonies	0	6	72	329	TNTC



$4 \times 329 \div 1/10^{-4} = 1.32 \times 10^{7}$ CFU/ml

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

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

Foodborne Pathogen Test Kits Validated by Independent Organizations

- FSIS maintains a list, updated quarterly, of methods that have been validated by independent organizations
  - http://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/New-Technologies
- 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

Food Safety and Inspection Service:

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)?
- If not performed by AOAC, AFNOR, etc., is supplemental validation data available?
  - May require additional scrutiny

Food Safety and Inspection Service:

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)

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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

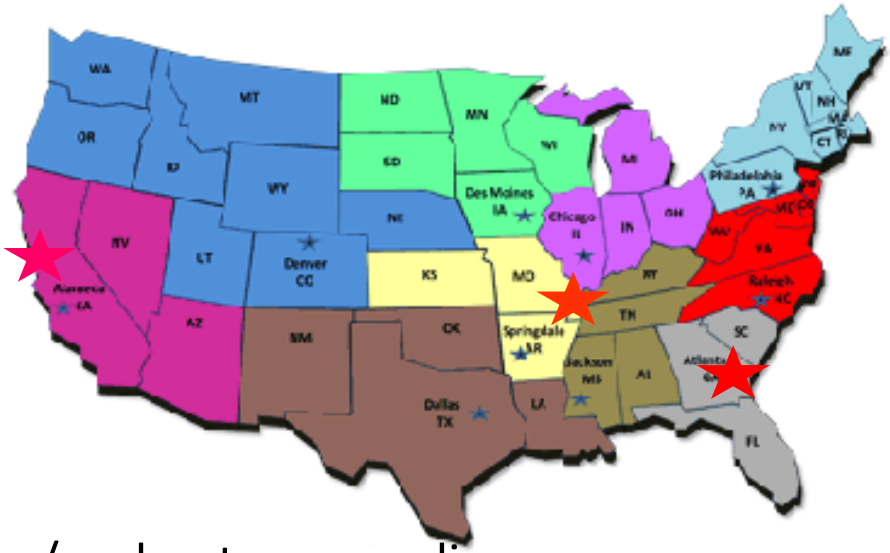
FSIS Testing Programs

Food Safety and Inspection Service:

Microbiological Testing by FSIS Laboratories

- Three Field Service Labs administer regulatory testing programs
  - Washington DC
    Executive Associate for Laboratory Services
  - Athens, Georgia
    - EFSL-routine/other testing
    - LQAS-quality assurance
    - FERN- biosecurity
  - St. Louis, Missouri
    - MWFSL-routine testing
  - Albany, California
    - WFSL-routine testing
    - Canning issues

ISO 17025 Accredited



- Routine monitoring, follow-up, baseline/exploratory sampling programs and investigative sampling

Food Safety and Inspection Service:

FSIS Microbiological Sampling Programs

Sample Type	Number Collected
Domestic	136,944
Import	10,207

Fiscal year 2018 data

Food Safety and Inspection Service:

FSIS Micro 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, PFGE, antimicrobial resistance, whole genome sequencing)

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

Shiga toxin-producing *E. coli* (STEC) Testing

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

Food Safety and Inspection Service:

O157 STEC Program

- Strain must have:
  - O157(+)
  - *stx*(+) OR *stx*(-) and H7(+)
  - biochemical(+)
  - Currently FSIS only analyzes beef manufacturing trimmings (MT60) for non-O157 STECs

Food Safety and Inspection Service:

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(+)
  - Currently FSIS only analyzes beef manufacturing trimmings (MT60) for non-O157 STECs
    - Phased rollout – MT65 – MT64 – MT43

Food Safety and Inspection Service:

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

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

negative or potential (+) – stx(+) & eae(+)

Screening

Day 3

Immunomagnetic Bead Capture & Rainbow Agar Plating

Latex Agglutination & Sheep Blood Agar Plating

negative
presumptive (+) – agglutination(+) and rapid screen (+) or inconclusive

Day 4 / 5

Perform
-stx/eae gene analysis
-latex agglutination & genetic serological test
-biochemical confirmation

Confirmation

Non-O157 Confirmed Positive
stx(+) & eae(+)
biochemical(+)
O-group(+)

O157 Confirmed Positive
O157(+); biochemical(+)
stx(+) OR stx(-) & H7(+)

Food Safety and Inspection Service:

Larger *E. coli* O157:H7 and Non-O157 STEC 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

Food Safety and Inspection Service:

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)

Food Safety and Inspection Service:

Listeria Testing

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

Food Safety and Inspection Service:

Listeria monocytogenes (MLG 8.11)

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 & Rapid Screen MOX & MOPS-BLEB Incubate 35°C for 18-24hrs	confirm (-) possible(+)	
Day 3	Streak plates for next day Horse blood and MOX plates		Screening
Day 4	GeneProbe and restreak Incubate 35°C variable time	presumptive(+)	
Day 5	Biochemical analysis, restreak & GenProbe	presumptive (+)	Confirmation
Day 6	Further characterization, morphological, and atypical isolate analysis		
Day 7	GenProbe	confirm (-/+)	

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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)

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

Salmonella (MLG Ch. 4.10)

Day 1	**Sample Prep and Primary Enrichment** **Stomach sample + BPW** **Incubate 35°C for 20-24 hrs**		**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 (-)**	**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	**Perform biochemical testing and serology using colony from SBA plate.**	**confirm (-/+)**	**Confirmation**

Food Safety and Inspection Service:

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*

Food Safety and Inspection Service:

Campylobacter (MLG 41.04) - Qualitative

Day 1-2	**Sample Prep and Primary Enrichment or Plate** **Sample + BF-BEB or plate (Campy-Cefex)** **Incubate 42°C for 48 hrs**		**Enrich or plate**
Day 3	**PCR Screen & Plating/isolation** **Campy-Cefex** **Incubate 42°C for 48 hrs**	**confirm (-)**	**Plating/isolation**
Day 5	**Microscope examination for morphology/motility**		**Confirmation**
	Latex agglutination	**confirm (-/+)**	

RESULTS ARE NOT USED FOR REGULATORY PURPOSES

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

Microbiological Testing by FSIS-Regulated Establishments

- Industry testing aims to:
  - Fulfill regulatory requirement (9 CFR 310.25, 381.94, 430.4, 590.580)
  - Support decisions made in hazard analysis (9 CFR 417.2 (a))
  - Provide on-going verification of HACCP plan (9 CFR 417.4 (a)(2))
  - Evaluate effectiveness of sanitary SOPs (9 CFR 416.14)
  - Fulfill purchase agreements
  - Respond to process deviations

Food Safety and Inspection Service:

Communication Between Establishment and Laboratory is Vital

- The communication challenge
  - The establishment may not understand the testing conducted on their behalf
  - The laboratory does not necessarily know what the establishment needs
  - The laboratory may not be aware of special validated procedures for larger test portions
- The establishment is ultimately responsible

Food Safety and Inspection Service:

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?

Food Safety and Inspection Service:

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?

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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

Food Safety and Inspection Service:

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?

Helpful Guidance

Food Safety and Inspection Service:

Existing Agency Guidance – Compliance Guides

- RTE
  - 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 (Jun 2017) – **Being updated**
  - 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 (Jun 2017) – **Being updated**

Food Safety and Inspection Service:

Existing Agency Guidance – Compliance Guides

- STEC
  - Compliance Guideline for Minimizing the Risk of Shiga Toxin-Producing *Escherichia coli* (STEC) and Salmonella in Beef (including Veal) Slaughter Operations (2017)
  - Compliance Guideline for Establishments Sampling Beef Trimmings for Shiga Toxin-Producing *Escherichia coli* (STEC) Organisms or Virulence Markers (August 2014)

Food Safety and Inspection Service:

Existing Agency Guidance – Compliance Guides

- HACCP
  - Meat and Poultry Hazards and Controls Guide (Mar 2018)
  - FSIS Compliance Guideline HACCP Systems Validation (April 2015)

Food Safety and Inspection Service:

Existing Agency Guidance – Compliance Guides

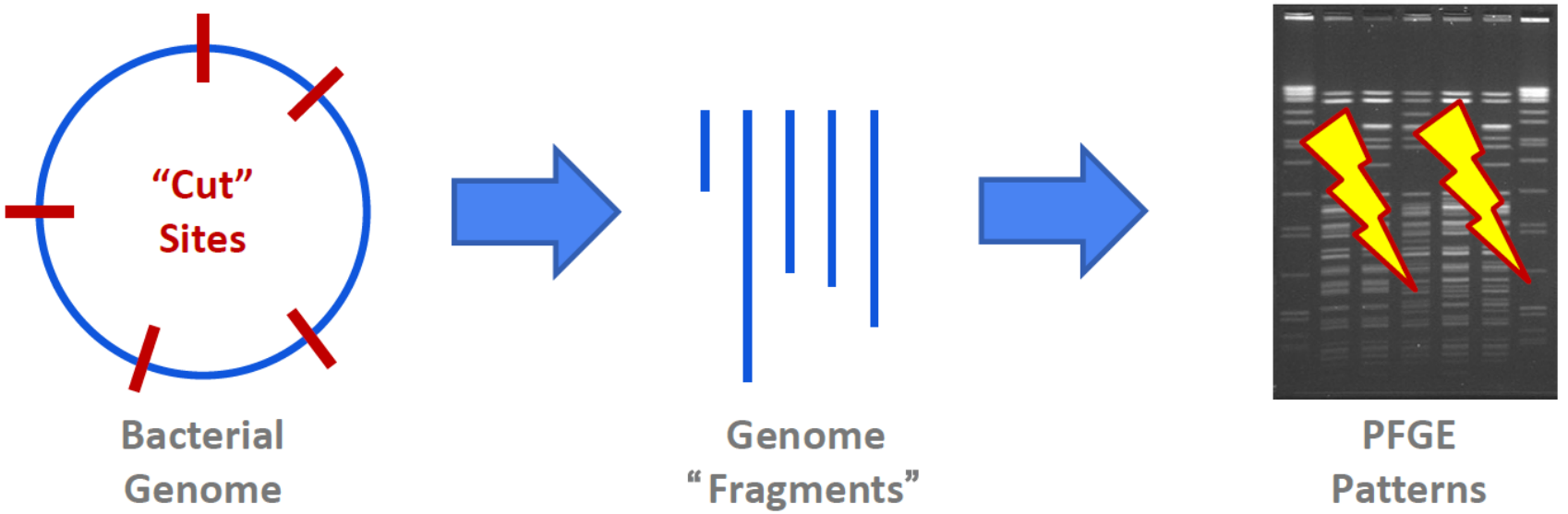
- Microbiological Test Methods and Laboratories
  - Establishment Guidance for the Selection of a Commercial or Private Microbiological Testing Laboratory (June 2013)
  - FSIS Guidance for Evaluating Test Kit Performance (October 2010)
  - Foodborne Pathogen Test Kits Validated by Independent Organizations

Whole Genome Sequencing

Food Safety and Inspection Service:

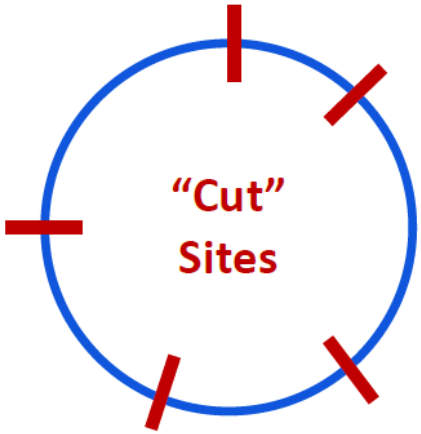
Whole Genome Sequencing – Background before WGS

PFGE



Food Safety and Inspection Service:

Whole Genome Sequencing – PFGE-WGS Comparison



PFGE only gives information at a "cut" site via the banding pattern

WGS has the ability to give us information at nearly every position in the genome

Source: CDC

Food Safety and Inspection Service:

Whole Genome Sequencing - Benefits

- WGS has a number of uses that benefit FSIS and its mission to protect public health.

- These uses include:
  - identifying harborage and cross-contamination of pathogens in FSIS-regulated facilities,
  - tracing human illness outbreak data to regulated food products, and
  - identifying unique genes related to virulence and pathogenicity, survival and adaptation, and resistance to biocides (sanitizers, metal, etc.) and antimicrobials.

Food Safety and Inspection Service:

Whole Genome Sequencing – The Transition

- FSIS began performing WGS in parallel with PFGE for *Lm* starting in FY13 and for all pathogens starting in early FY16.

- Centers for Disease Control and Prevention (CDC) PulseNet partners are transitioned away from using PFGE as the primary molecular characterization tool toward using WGS.

- In coordination with CDC PulseNet, FSIS suspended PFGE for *Lm* and as of January 15, 2018, now generates *Lm* characterization through WGS only.

Food Safety and Inspection Service:

Whole Genome Sequencing – How is WGS analyzed?

- FSIS uses different tools to analyze WGS information including:
  - Multi-locus Sequence Typing (MLST) - resulting in
    - Public Sequence Type
    - Allele Code
  - High-quality Single Nucleotide Polymorphisms (hqSNP)

Food Safety and Inspection Service:

Whole Genome Sequencing – Sequence Typing

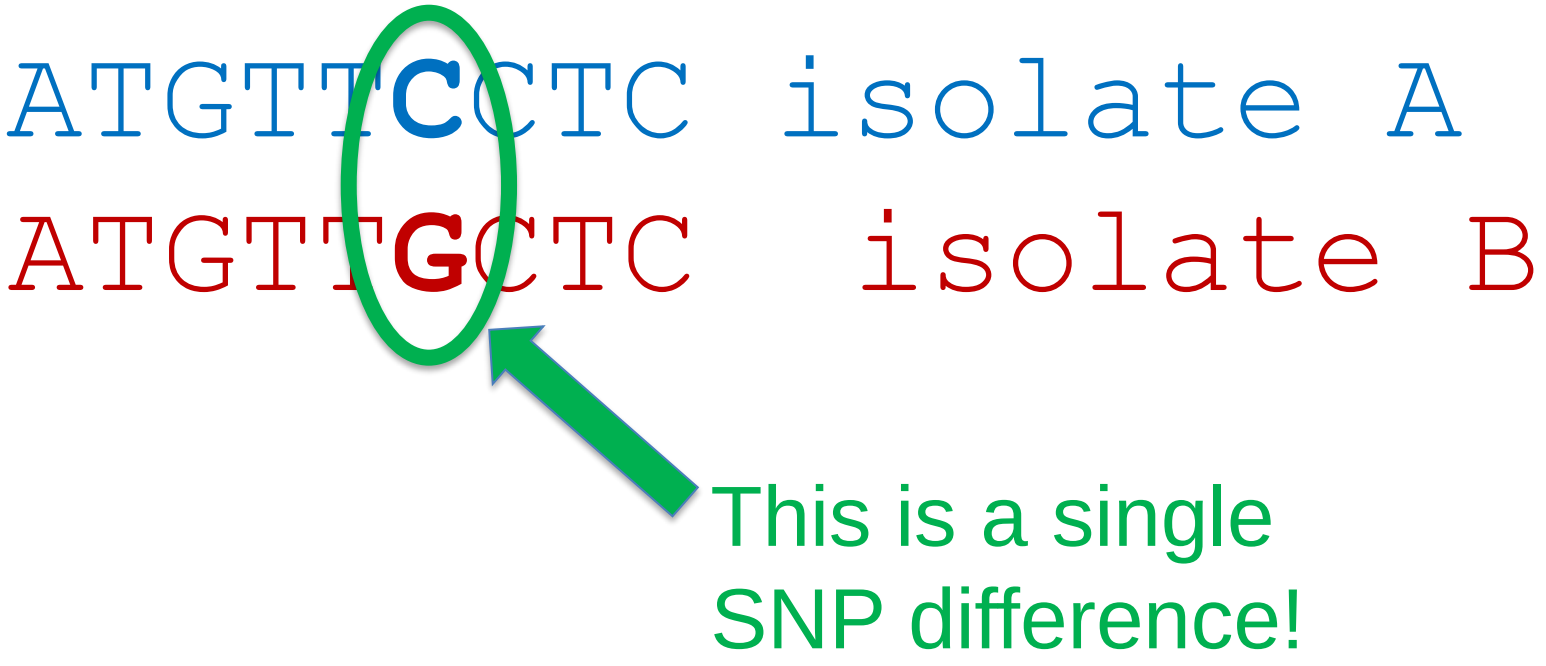
Multi-locus Sequence Typing (MLST)
MLST can generate a **pattern name or designation** (similar to a PFGE pattern name) based on differences in a pre-defined set of genes.

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 over 1,800 genes
  - named by using CDC PulseNet numerical code (e.g., LMO 1.0-5.1.1.2.5.1)

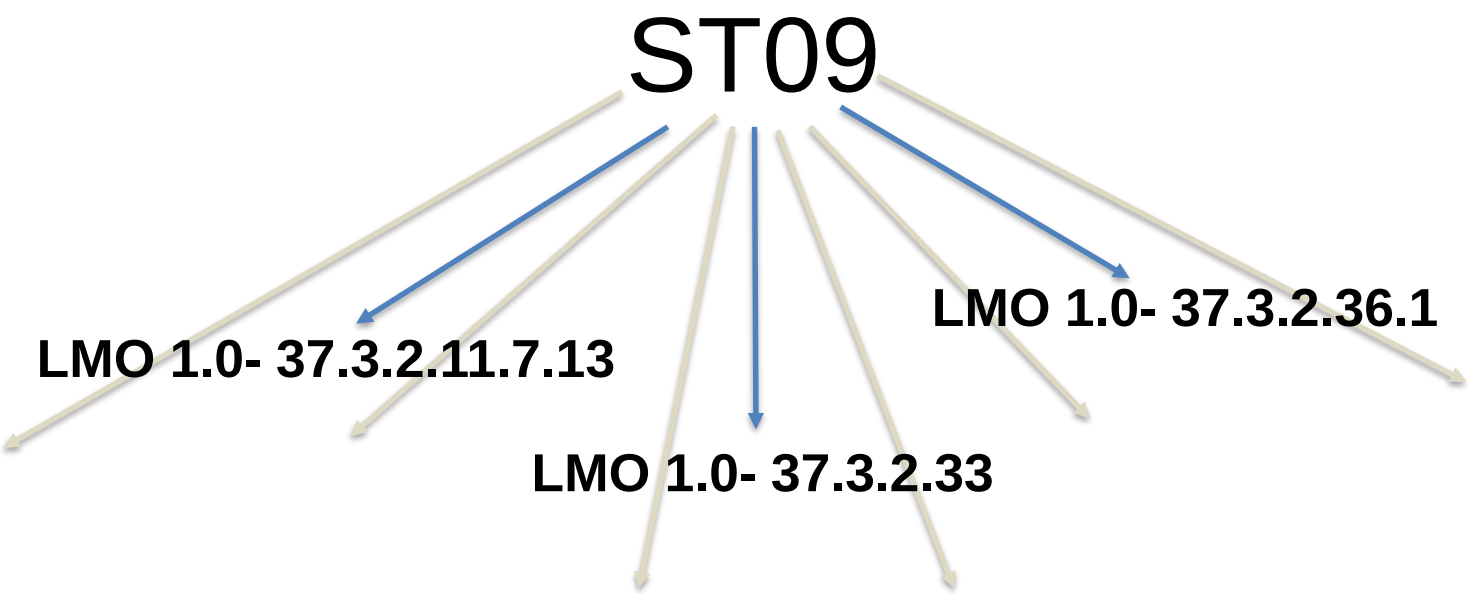
Whole Genome Sequencing - SNP

Single Nucleotide Polymorphism (SNP)



Food Safety and Inspection Service:

Whole Genome Sequencing – Sequence Typing



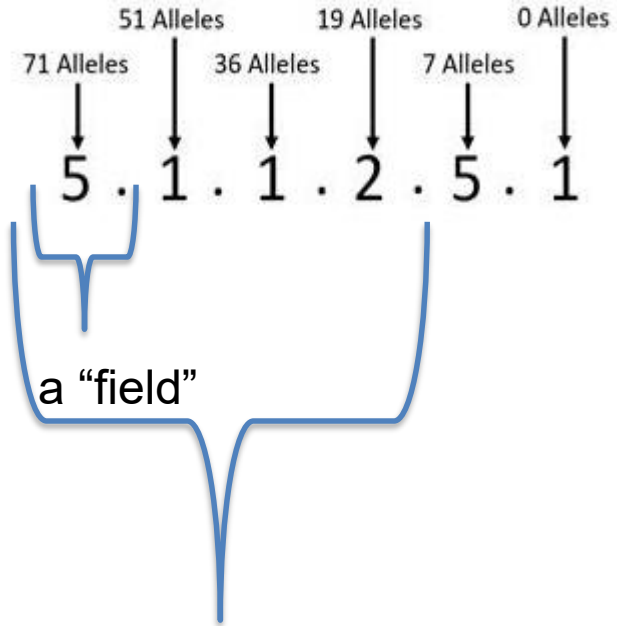
Allele Code is more specific than public Sequence Type; one public Sequence Type can be inclusive of many Allele Codes.

Food Safety and Inspection Service:

Whole Genome Sequencing – Reading Allele Codes

Example: LMO1.0 - 5.1.1.2.5.1

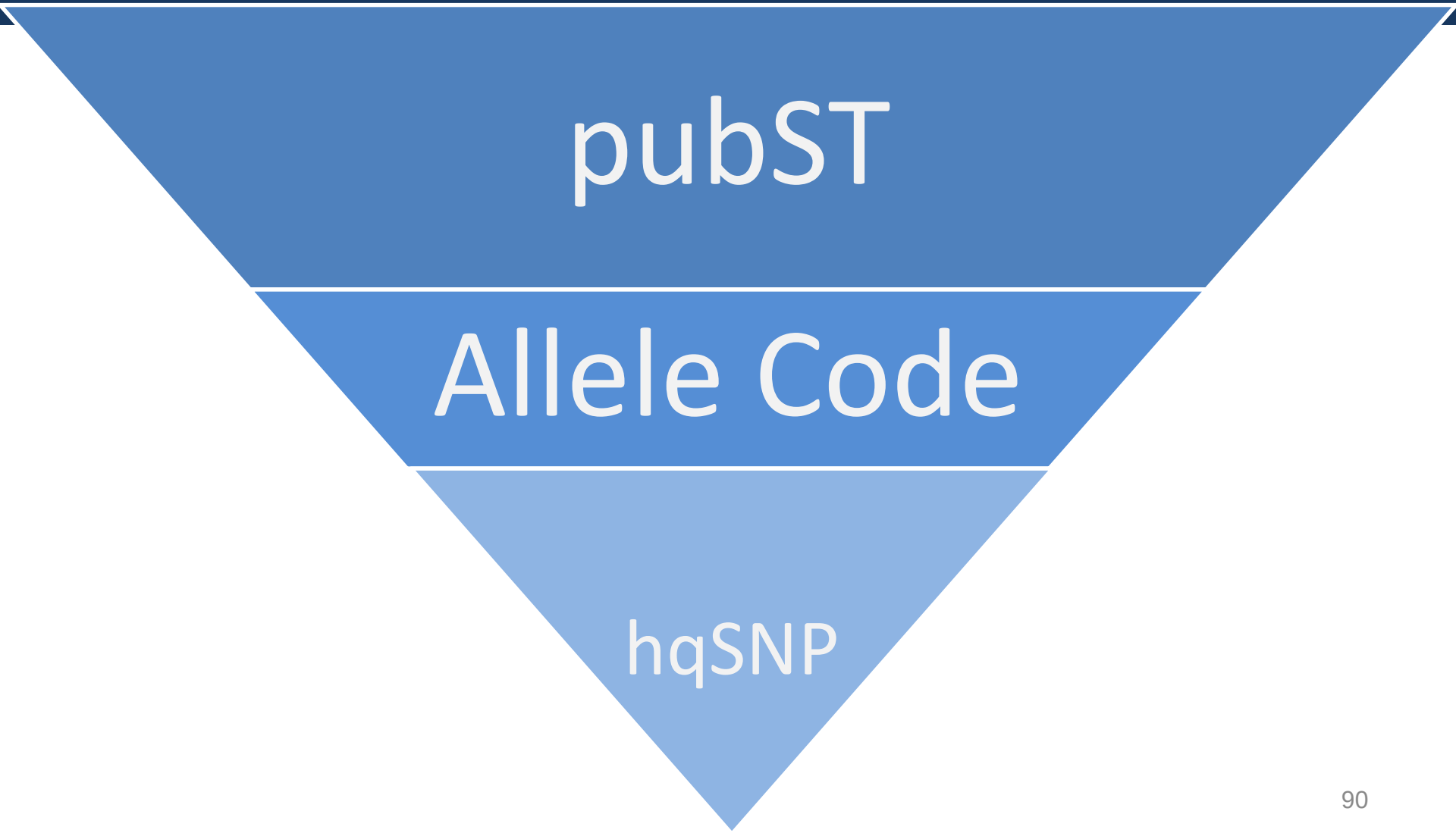
LMO – *L. monocytogenes*
Version 1.0



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

Food Safety and Inspection Service:

Whole Genome Sequencing



Food Safety and Inspection Service:

Whole Genome Sequencing – Reporting from MCB

Email on Lm-positive from Microbial Characterization Branch (MCB; Eastern Lab, Athens GA)

Establishment	Field	853334755	853344746	853344744
M12345 (LocID: 11981)	FormID	102015479	102051750	102051751
	Collect Date	2018-07-30	2018-10-09	2018-10-09
	Project	RTEPROD_RISK	INTENV_LM_W	INTENV_LM_W
	FSIS Identifier	FSIS31800872	FSIS31801180	FSIS31801179
	NCBI Accession Number	SAMN09830008	SAMN10269641	SAMN10269640
	MLST ST	publicST288	publicST288	publicST288
	Allele Code	LMO1.0 - 73.1.1.2.14	LMO1.0 - 73.1.1.2.14	LMO1.0 - 73.1.1.2.14
	NCBI SNP Cluster (Retrieve Date)	PDS000032940.4 2018-10-26	PDS000032940.4 2018-10-26	PDS000032940.4 2018-10-26
	Min Food Env (SNP)*	1	1	2
	Indicative of Potential Harborage**		Yes	Yes
	Indicative of cross-contamination***		Yes	Yes
	Min Clinical (SNP)*	None	None	None
	Potentially related to a clinical isolate		No	No

LIMS ID

Form ID

FSIS Identifier

Allele Code

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*

Food Safety and Inspection Service:

Whole Genome Sequencing – EIAOs needing more info

- When performing a PHRE in establishments with more than one positive RTE sample, EIAOs are to:
  - Request WGS report through Outbreaks_WGS@fsis.usda.gov
  - Use WGS to assess if there is a history of harborage or cross-contamination in the establishment.

Note: OPARM is working on updating the "Public Health Risk Evaluation for Establishment" report to include the MLST designations for any historical samples included in the report.

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

Food Safety and Inspection Service:

Whole Genome Sequencing – The Future

- FSIS continues to work with FDA, CDC PulseNet, local & state health departments.

- WGS will be the primary subtyping tool for *Campylobacter*, Shiga toxin-producing *Escherichia coli* (STEC), and *Salmonella*.

Stay tuned ….

Food Safety and Inspection Service:

Existing Agency Guidance - askFSIS

- askFSIS Q&A sometimes contains additional information on testing methods and WGS

- If you cannot answer your question there, please submit to askFSIS

Questions?

Contact me at udit.minocha@fsis.usda.gov

OR:

- Enter question into askFSIS
- Provide documentation for review
- Request “Sampling Queue”

Food Safety and Inspection Service:

Can't find what you are looking for?