Understanding and Evaluating Microbiological Sampling and Testing

May 2019

Science Staff
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
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
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*)
Food Safety and Inspection Service:

Assessing Sampling Plans
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
Food Safety and Inspection Service:

**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

40% of product contaminated by hour 3 of production

“slug”

% contaminated samples

Time of production, hrs

Combo bins

<5 <5 40 30 <10
Food Safety and Inspection Service:

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
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
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
Food Safety and Inspection Service:

**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
Food Safety and Inspection Service:

Pathogen Growth During Enrichment

Log pathogen level (e.g., cfu, MPN/gram)

- lag
- logarithmic
- stationary
- death

Incubation time, hrs

PCR

immunoassay

Possible Loss of Sensitivity Prior to confirmatory retesting
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**

![Diagram](image_url)

- **Lag phase**
- **Log phase**
- **Stationary phase**
- **Death phase**
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)**

- **65 grams + 585 ml buffer = 0.1 grams/mL**

10 mL (0.1 gram x 3)

1 mL 1:10 (0.01 gram x 3)

10 mL 1:100 (0.001 gram x 3)
Food Safety and Inspection Service:

Quantitative Testing - MPN (most probable number)

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)
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)
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
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
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**

![Image of petri dishes and test tubes]

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Colony Count</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>0</td>
<td>$4 \times 329 \div 1/10^{-4} = 1.32 \times 10^7$ CFU/ml</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>329</td>
<td></td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>TNTC</td>
<td></td>
</tr>
</tbody>
</table>
Method Validations
Food Safety and Inspection Service:

**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
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
- 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)
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.)
Food Safety and Inspection Service:

**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
“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
Food Safety and Inspection Service:

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

ISO 17025 Accredited
## FSIS Microbiological Sampling Programs

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Number Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic</td>
<td>136,944</td>
</tr>
<tr>
<td>Import</td>
<td>10,207</td>
</tr>
</tbody>
</table>

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
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(+)
  - $stx(+) \text{ OR } stx(-)$ 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)**

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Sample Prep and Primary Enrichment 42°C±1 for 15-24 hours</th>
<th>Enrichment</th>
</tr>
</thead>
</table>
| Day 2 | Perform PCR  
All samples that do not test PCR negative are carried forward for further analysis | Screening |
| Day 3 | Immunomagnetic Bead Capture & Rainbow Agar Plating | |
| Day 3 | Latex Agglutination & Sheep Blood Agar Plating | |
| 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(+)  
 biochemistry(+)- 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)

<table>
<thead>
<tr>
<th>Day</th>
<th>Process Description</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td><strong>Sample Prep and Primary Enrichment</strong>&lt;br&gt;Somach 25g sample + 225 ml UVM&lt;br&gt;Incubate 30°C for 20-26 hrs</td>
<td>Enrichment</td>
</tr>
<tr>
<td>Day 2</td>
<td><strong>Plating, Secondary Enrichment &amp; Rapid Screen</strong>&lt;br&gt;MOX &amp; MOPS-BLEB&lt;br&gt;Incubate 35°C for 18-24 hrs</td>
<td>confirm (-) possible(+)</td>
</tr>
<tr>
<td>Day 3</td>
<td><strong>Streak plates for next day</strong>&lt;br&gt;Horse blood and MOX plates</td>
<td>Screening</td>
</tr>
<tr>
<td>Day 4</td>
<td><strong>GeneProbe and restreak</strong>&lt;br&gt;Incubate 35°C variable time</td>
<td>presumptive (+)</td>
</tr>
<tr>
<td>Day 5</td>
<td><strong>Biochemical analysis, restreak &amp; GenProbe</strong></td>
<td>presumptive (+) Confirmation</td>
</tr>
<tr>
<td>Day 6</td>
<td><strong>Further characterization, morphological, and atypical isolate analysis</strong></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td><strong>GenProbe</strong></td>
<td>confirm (-/+))</td>
</tr>
</tbody>
</table>
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)*

<table>
<thead>
<tr>
<th>Day</th>
<th>Step</th>
<th>Results</th>
</tr>
</thead>
</table>
| 1    | Sample Prep and Primary Enrichment  
Stomach sample + BPW  
Incubate 35°C for 20-24 hrs | **Enrichment**   |
| 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** |
| 3    | Streak RV and TT on BGS and DMLIA plates  
Incubate 35°C for 18-24 hrs |                |
| 4    | Pick suspect colony from Plate medium to TSI and LIA slants.  
Incubate 35°C for 22-26 hrs | presumptive (+) **Confirmation** |
| 5    | Streak on SBA for biochemical testing  
Incubate 18-24 hrs at 35°C | confirm (-) **Confirmation** |
| 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*
## Campylobacter (MLG 41.04) - Qualitative

<table>
<thead>
<tr>
<th>Day 1-2</th>
<th>Sample Prep and Primary Enrichment or Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample + BF-BEB or plate (Campy-Cefex)</td>
</tr>
<tr>
<td></td>
<td>Incubate 42°C for 48 hrs</td>
</tr>
<tr>
<td></td>
<td>Enrich or plate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 3</th>
<th>PCR Screen &amp; Plating/isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Campy-Cefex</td>
</tr>
<tr>
<td></td>
<td>Incubate 42°C for 48 hrs</td>
</tr>
<tr>
<td></td>
<td>confirm (-)</td>
</tr>
<tr>
<td></td>
<td>Plating/isolation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 5</th>
<th>Microscope examination for morphology/motility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latex agglutination</td>
</tr>
<tr>
<td></td>
<td>confirm (-/+)</td>
</tr>
<tr>
<td></td>
<td>Confirmation</td>
</tr>
</tbody>
</table>

**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
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
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 establishment
- 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 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
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

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**

- **“Cut” Sites**
- **Bacterial Genome**
- **Genome “Fragments”**
- **PFGE Patterns**
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
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.
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)
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)
Food Safety and Inspection Service:

**Whole Genome Sequencing - SNP**

Single Nucleotide Polymorphism (SNP)

ATGTTT

C

CTC isolate A

ATGTTT

G

CTC isolate B

This is a single SNP difference!
Allele Code is more specific than public Sequence Type; one public Sequence Type can be inclusive of many Allele Codes.
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**

- pubST
- Allele Code
- hqSNP
### Whole Genome Sequencing – Reporting from MCB

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

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*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](https://www.fsis.usda.gov/WPS/portal?wps Purpose=PDFDisplay&wpsPrintable=false&docID=9089)
***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](https://www.fsis.usda.gov/WPS/portal?wps Purpose=PDFDisplay&wpsPrintable=false&docID=9089)
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/RLm positive, EIAOs are to make a request through the Outbreaks_WGS@fsis.usda.gov Outlook mailbox for WGS analyses.
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?