Non-O157 STEC Policy

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Outline

• Background on non-O157 STEC
• History of FSIS STEC related activities
• Non-O157 STEC Risk Profile/Adulterant Status
• FSIS Verification Testing Program
• FSIS Testing Methodology
• Preliminary Test Results
Non-O157 STEC

• Accumulating evidence of non-O157 STEC as cause of sporadic- and outbreak-associated illness worldwide

• National and regional surveys in the U.S., Australia, and E.U. indicate non-O157 STEC infection associated with a spectrum of outcomes, including severe outcomes (bloody diarrhea, HUS, kidney failure, death)

• Severity of outcome related to pathogen as well as host and environment

• Some outbreaks have been comparable to *E. coli* O157:H7 outbreaks (e.g., 2009 Oklahoma STEC O111 restaurant outbreak, 2011 German STEC O104:H4 outbreak)
FSIS: STEC Related Activities

1994: Large foodborne outbreak of *E. coli* O157:H7
1994: FSIS declares *E. coli* O157:H7 to be an adulterant in raw ground beef, and initiates RGB testing program
1999: *E. coli* O157:H7 declared adulterant in all non-intact products
2004: FSIS begins to test beef trim for *E. coli* O157:H7
2007: FSIS, FDA, and CDC host public meeting on Public Health Significance of non-O157 STEC
2008: non-O157 STEC method development (USDA-ARS collaboration)
2009: FSIS receives citizen’s petition to declare all enterohemorrhagic serotypes of STEC, including non-O157 serotypes, to be adulterants
FSIS: STEC Related Activities

2010: First version of FSIS method for non-O157 STEC published (MLG5B.00)

2011: USDA issues Federal Register Notice (76 FR 58157), draft risk profile, and guidance for evaluating the pathogen test kit methods

2011: Second version of FSIS method for non-O157 STEC published (MLG5B.01)

2012: USDA issues Federal Register Notice (77 FR 31975) announces that FSIS will implement verification testing for the six additional STEC on June 4, 2012

2012: FSIS began testing for six additional STEC in beef manufacturing trimmings on June 4, 2012 (Notice 40-12)
Non-O157 STEC Risk Profile

• Criteria examined for non-O157 STEC as they were for ECH7
  – Can they cause severe illness?
  – Present in cattle and beef products?
  – Can low dose cause illness?
  – Not destroyed by ordinary* cooking?
  – May be spread person-to-person?

• Risk profile supported that six non-O157 STECs (O145, O103, O45, O26, O111, and O121) are adulterants within the meaning of the FMIA

*”ordinary cooking” refers to an overall population distribution where not everyone cooks the product thoroughly.
Adulterant Status

• Raw non-intact beef products that are contaminated with these six STEC O145, O103, O45, O26, O111, and O121 are considered adulterated within the meaning of the FMIA.

• FSIS also considers adulterated intact cuts that are contaminated with these serogroups if they are to be further produced into raw, non-intact products.
In order to verify establishments controls for these pathogens, FSIS will conduct verification testing for six non-O157 STEC.

FSIS began testing for six additional STEC in beef manufacturing trimmings on June 4, 2012.
- Only beef manufacturing trimmings generated at slaughter establishments and from cattle slaughtered on or after June 4th, 2012 are being tested.
- Eligibility is being determined through PHIS questions.
- Samples will also be tested for *E. coli* O157:H7.
FSIS Verification Testing Implementation

• For the first 90 days of FSIS verification testing (FSIS Notice 40-12):
  – If an establishment has a confirmed positive sample they will be required to take corrective actions including ensuring no adulterated product enters commerce.
  – Establishments will not be expected to reassess their HACP plan, nor will for-cause FSAs will be scheduled in response to a positive test result during the 90-day period.
FSIS Verification Testing Implementation

• Eventually testing will be expanded to other raw ground beef components and ground beef.

• Expansion of testing will be announced in the Federal Register.
FSIS Testing Methodology

- Eligible samples be tested for *E. coli* O157:H7 and the top 6 sero-groups of non-O157 STEC (O26, O45, O103, O111, O121, and O145)
- FSIS method MLG 5B.01


7/22/2012
MLG 5B: FSIS Method for Detecting Top Six Serogroups of STEC

- Developed with assistance from FDA, CDC, and ARS
- Major steps:
  - Two-tier real time PCR screen \((stx/eae\text{ followed by } wzx\text{ genes O145, O103, O45, O26, O111, and O121})\)
  - Immuno-magnetic bead concentration
  - Acid treatment of IMS-captured cells
  - Cultural isolation on modified Rainbow agar
  - Colony identification using agglutinating serogroup-specific antisera
  - Biochemical identification
- \textit{E. coli} O157:H7 method modified to allow co-analysis with non-O157 STEC (MLG5.06, May 18, 2012)
MLG 5B: FSIS Method for Detecting Top Six Serogroups of STEC

- Stx and eae positive broths
  - If top 6 serogroup positive: continue with analysis
  - If top 6 serogroup negative:
    1) Refer to ARS Meat Animal Research Center (Clay Center, NE) for further analysis
    2) Provide results to establishments upon request
Disclaimer

- Mention of a specific brand or trade name does not constitute endorsement or selectivity by USDA/FSIS over similar products that might be suitable.
STEC Analytical Procedure

Day 1

Sample Receipt

Sample Prep

325 g of sample

7/22/2012
The NCBI DNA database was probed for variants of the Stx and eaeA genes

- Three variants of stx1 gene
- Seven variants of the stx2 gene
- Twenty-one variants of the eaeA gene
**STEC Analytical Procedure**

Day 2

- **PCR Analysis**
  - If positive, go to Immunomagnetic Bead Retrieval.
  - Potential Positive

- Immunomagnetic Bead Retrieval
  - Modified Rainbow Agar
Modified Rainbow Agar
Agglutination Tests

O157 positive
Card agglutination

O26 positive
Slide agglutination
**STEC Analytical Procedure**

**Confirmation**

**Day 3**
- Rainbow Agar
  - Grey = Typical
  - Purple = Non Typical
- Latex Agglutination
- Presumptive Positive
  - Or
- Conf. Negative

**Day 4**
- Confirmed Positive
- Biochemical Analysis
- Confirmatory PCR (stx, eae, serogroup)
- Analysis Complete
FSIS E. coli O157:H7 and Non-O157 STEC Method Comparison

**Day 0**
- **O157 STEC FSIS Method**
  - Samples Collected from Plant And sent to Lab

**Non-O157 STEC - FSIS Method**

**Day 1**
- Sample Prepared in Enrichment broth
  - Samples incubated at 42°C For 15 – 22 hours

**Day 2**
- **Real Time PCR Screen for stx and eae genes**
  - **O157 specific Immunomagnetic bead capture**
  - **Confirm Negative**

- **O-group specific Immunomagnetic bead capture**
  - Real Time PCR Screen for target O groups
  - **Confirm Negative**

- **Beads plated onto Chromogenic agar**
FSIS E. coli O157:H7 and Non-O157 STEC Method

Day 3

- Agglutination test on Typical colonies (O157)
- SBA
- PRESumptive
- Confirm Negative

Day 4

- O157 and H7 Agglutination Test or wzy, flc PCR
- SBA
- PRESumptive
- Confirm Negative
- Confirmed Negative
- Biochemical Confirmation
- Toxin ELISA or stx PCR
- Confirm Positive

Day 5

- Analysis extends past day 5 if restreaking is required to isolate colonies.

O157 STEC

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

<table>
<thead>
<tr>
<th>Stage</th>
<th>non-O157</th>
<th><em>E. coli</em> O157:H7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential</td>
<td>Sample that causes a positive reaction with both screen tests:</td>
<td>Sample that causes a positive reaction with the screen test</td>
</tr>
<tr>
<td></td>
<td>- stage 1 - for the <em>stx</em> and the <em>eae</em> genes and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- stage 2 (concurrent with stage 1) for one or more of the target</td>
<td></td>
</tr>
<tr>
<td></td>
<td>serogroup genes</td>
<td></td>
</tr>
<tr>
<td>Presumptive</td>
<td>Sample that has typical colonies, observed on Rainbow Agar, and reacts</td>
<td>Sample that has typical colonies, observed on Rainbow Agar, and reacts specifically with one or more of the target serogroup antiserum</td>
</tr>
<tr>
<td></td>
<td>specifically with one or more of the target serogroup antiserum</td>
<td></td>
</tr>
<tr>
<td>Confirmed</td>
<td>An isolate has <em>stx</em>, <em>eae</em>, and one or more of the target serogroup</td>
<td>Biochemically-identified <em>E. coli</em> isolate that is serologically or genetically determined to be 'O157' that meets at least one of the following criteria:</td>
</tr>
<tr>
<td></td>
<td>genes and has been biochemically confirmed to be <em>E. coli.</em></td>
<td>1) positive for Shiga toxin production,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) positive for Shiga toxin gene,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) genetically determined to be &quot;H7&quot;</td>
</tr>
</tbody>
</table>
Stage 1 Positives

• FSIS will send sample enrichment broths that are positive for the stx and eae genes but negative for all of the six non-O157 STEC and E. coli O157:H7 to USDA, ARS for further analysis and will evaluate this data internally to determine whether changes to the policy are needed.
FSIS Testing Methodology

Performance

- PCR screening tests and laboratory reagents are performing as expected.
- Screening test results are available the day after the sample arrives in the lab.
- The number of samples analyzed to date is slowly increasing to close to 150 in the first month.
- Some presumptive-positive samples have required an additional day of analysis.
Preliminary Testing Results

- Sample results can be found on the FSIS website: http://www.fsis.usda.gov/Science/RGBC_STEC_Results/index.asp

- Results are reported on a weekly basis

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Table 2: Non-0157 STEC (by serogroup) and *E. coli* 0157:H7

<table>
<thead>
<tr>
<th>Target STEC</th>
<th>Trim Verification Percent Positive (Number)</th>
<th>Follow-up to RGB Positive at Supplier Percent Positive (Number)</th>
<th>Follow-up to RGB Positive Percent Positive (Number)</th>
<th>Verification/Intensified Percent Positive (Number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157:H7</td>
<td>0.53% (4/759)</td>
<td>0.00% (0/79)</td>
<td>1.72% (1/58)</td>
<td>1.05% (5/478)</td>
</tr>
<tr>
<td>Total non-O157 STEC</td>
<td>2.73% (3/110)</td>
<td>0.00% (0/0)</td>
<td>20.00% (1/5)</td>
<td>0.00% (0/6)</td>
</tr>
<tr>
<td>O26</td>
<td>0.00% (0/110)</td>
<td>0.00% (0/0)</td>
<td>0.00% (0/5)</td>
<td>0.00% (0/6)</td>
</tr>
<tr>
<td>O45</td>
<td>0.91% (1/110)</td>
<td>0.00% (0/0)</td>
<td>0.00% (0/5)</td>
<td>0.00% (0/6)</td>
</tr>
<tr>
<td>O103</td>
<td>0.91% (1/110)</td>
<td>0.00% (0/0)</td>
<td>20.00% (1/5)</td>
<td>0.00% (0/6)</td>
</tr>
<tr>
<td>O111</td>
<td>0.00% (0/110)</td>
<td>0.00% (0/0)</td>
<td>0.00% (0/5)</td>
<td>0.00% (0/6)</td>
</tr>
<tr>
<td>O121</td>
<td>0.00% (0/110)</td>
<td>0.00% (0/0)</td>
<td>0.00% (0/5)</td>
<td>0.00% (0/6)</td>
</tr>
<tr>
<td>O145</td>
<td>0.91% (1/110)</td>
<td>0.00% (0/0)</td>
<td>0.00% (0/5)</td>
<td>0.00% (0/6)</td>
</tr>
</tbody>
</table>

1 Results are posted according to the sample analysis completion date.
Preliminary Testing Results

- As of July 13th there have been a total of 121 samples analyzed for non-O157 STECS from the federal plants and imports
  - 110 samples from the trim verification program (MT60)
  - 5 from the follow-up to RGBC program (MT53)
  - 6 from the imports program (MT51)

- There have been a total of 4 positives through July 8th
  - 3 positives from the trim verification program (MT60)
    - 1 for serogroup O45
    - 1 for serogroup O103
    - 1 for serogroup O145
  - 1 positive from follow-up to RGBC program (MT53)
    - 1 for serogroup O103

- Updated testing results provided on the FSIS website at:
Establishment Testing Methods

- Establishments are not required to test for non-O157 STECs
- FSIS issued guidance for evaluating pathogen test kit methods
- FSIS is evaluating validation data, and has issued “letters of no objection” for use of methods in FSIS-regulated establishments to facilitate the use of these new methods.
  - Submit data to AskFSIS
  - Submission should include
    - inclusivity and exclusivity studies
    - raw data and report
  - Data will be evaluated using FSIS Guidance for Evaluating the Performance of Pathogen Test Kit Methods.
- Guidance and “no objection letters” not intended to supplant role of organizations that certify alternative methods (i.e., AOAC, AFNOR, NordVal, and MicroVal).
Next Steps

• Issue FSIS Notice with instructions to the field following the first 90 days of FSIS verification testing.
  • Inspection program personnel will verify that establishments reassess in response to FSIS or establishment positive non-O157 STEC results.
  • For-cause FSAs will be scheduled in response to a positive test result.

• Intend to issue Federal Register Notice announcing expansion of testing to other raw ground beef components and ground beef.
AskFSIS Q&As

- **Non-O157 STEC isolated from cattle produced outside the United States**
The six target **non-O157** STEC serogroups (O26, O45, O103, O111, O121, and O145) have been isolated from cattle and beef products produced in several countries. Researchers from USDA Agricultural...
  Date Updated: 06/06/2012

- **Confirming Non-O157 STEC Screen Positive Test Results** New
  Establishments may confirm a positive **non-O157** STEC screening result using the FSIS cultural method (available at http://www.fsis.usda.gov/PDF/Mlg_5B_02.pdf, steps 5B.7 and 5B.8), a different...
  Date Updated: 07/12/2012

- **Non-O157 STEC isolated from cattle produced in the United States**
Yes, the six target **non-O157** STEC serogroups (O26, O45, O103, O111, O121, and O145) have been isolated from cattle slaughtered in the U.S. This has been demonstrated in one study by the USDA...
  Date Updated: 06/06/2012

- **Ability of FSIS laboratories to offer non-O157 STEC testing**
No. FSIS laboratories do not offer **non-O157** STEC testing to external entities.
  Date Updated: 06/06/2012
• **Confirmation of establishment screening tests for non-O157 STEC**
  No, FSIS would not require an establishment to perform confirmatory testing. For establishment testing or testing on behalf of an establishment, FSIS recognizes that other criteria, while not used...
  Date Updated: 06/06/2012

• **Availability of latex agglutination reagents for non-O157 STECs**
  No. Latex agglutination reagents for these target organisms are not commercially available at this time. However, these reagents may be prepared. A forthcoming publication by Medina et al. (citation...)
  Date Updated: 06/06/2012

• **Subtypes of Shiga Toxin and Intimin Genes in non-O157 STEC Testing**
  Yes, for both results, if the establishment conducts only screening and does not perform confirmatory testing. Because these samples contain stx, eae, and one of the seven target O-groups, the...
  Date Updated: 06/06/2012

• **Order of establishment screening tests for non-O157 STEC**
  Yes. The order of the screen is not relevant. For establishment testing or testing on behalf of an establishment, FSIS recognizes that other criteria, while not used specifically by FSIS for...