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.

FSIS Verification Testing

- 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.

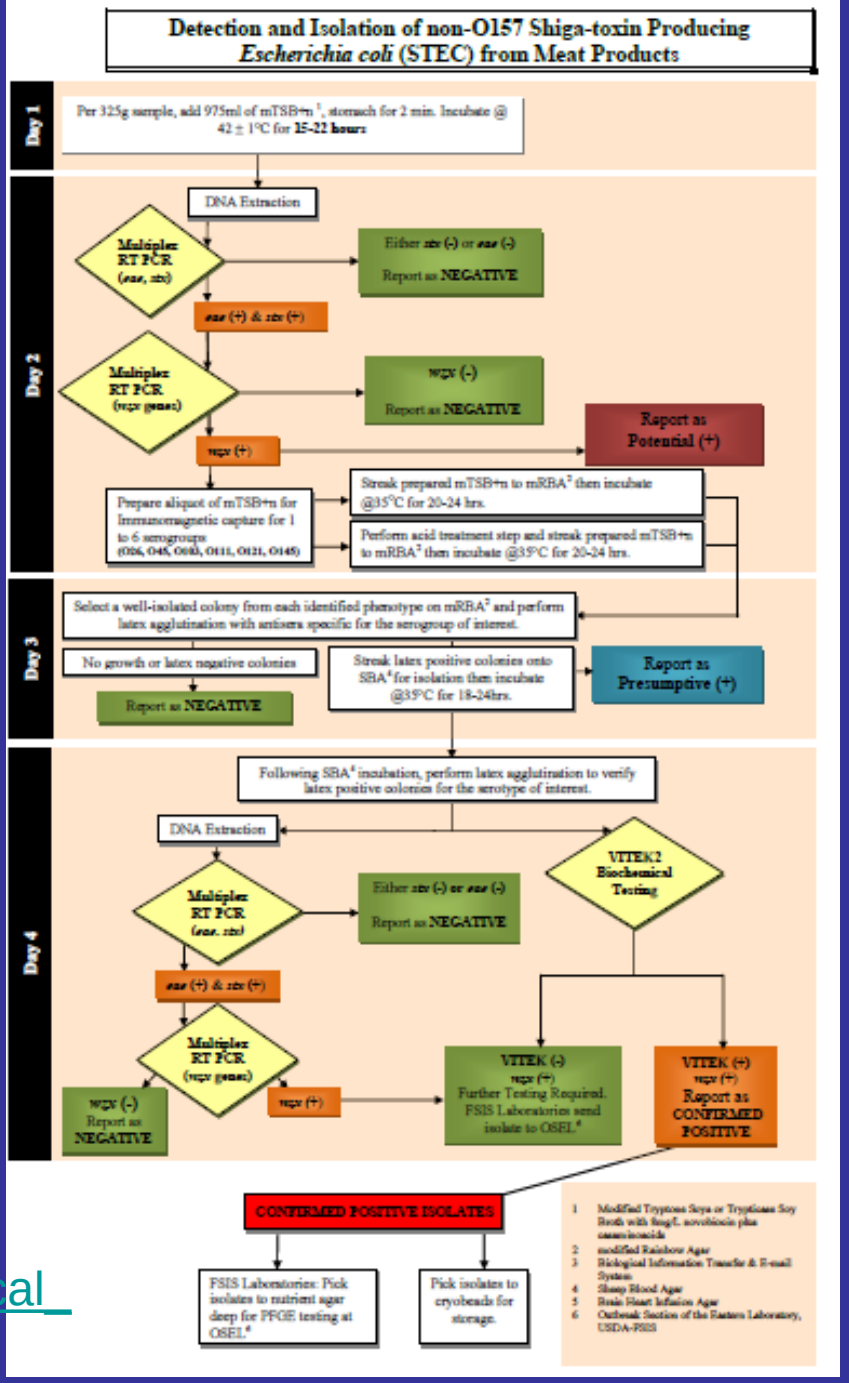
FSIS Verification Testing Implementation

- 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



http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp

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* followed by *wzx* 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
- *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.

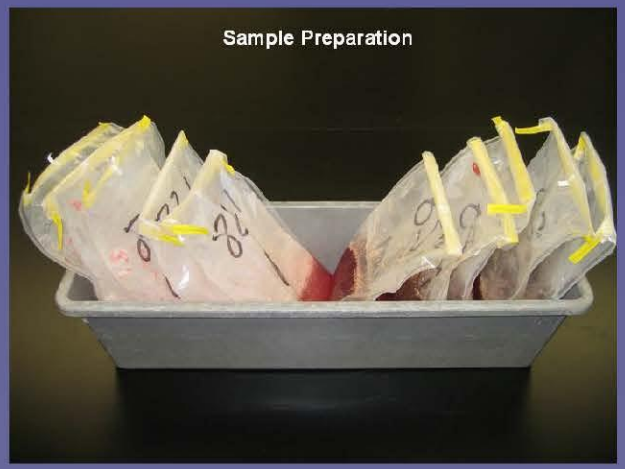
STEC Analytical Procedure

Day 1

Sample Receipt

325 g of sample

Sample Prep



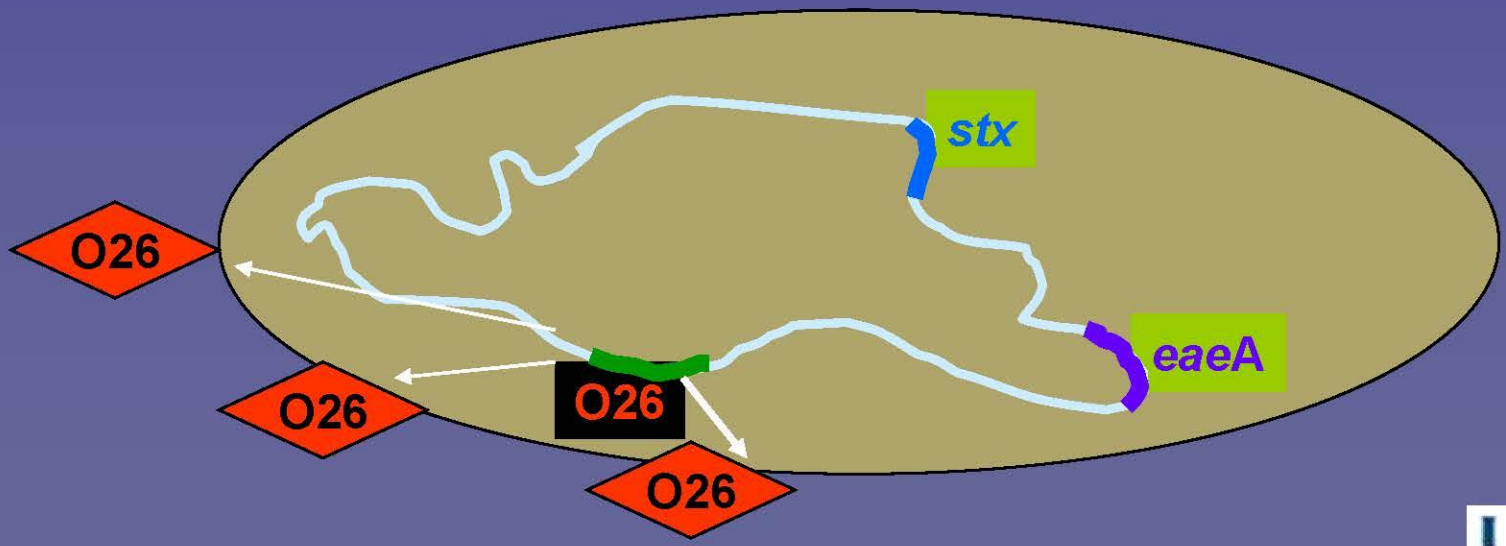
eae and *stx sequences*

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

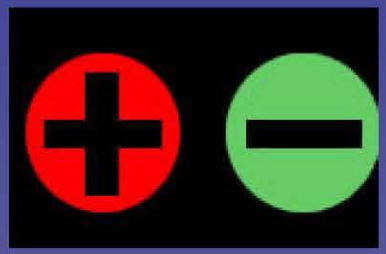
-Twenty-one variants of the eaeA gene



STEC Analytical Procedure

Day 2

PCR Analysis

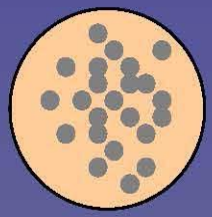


If

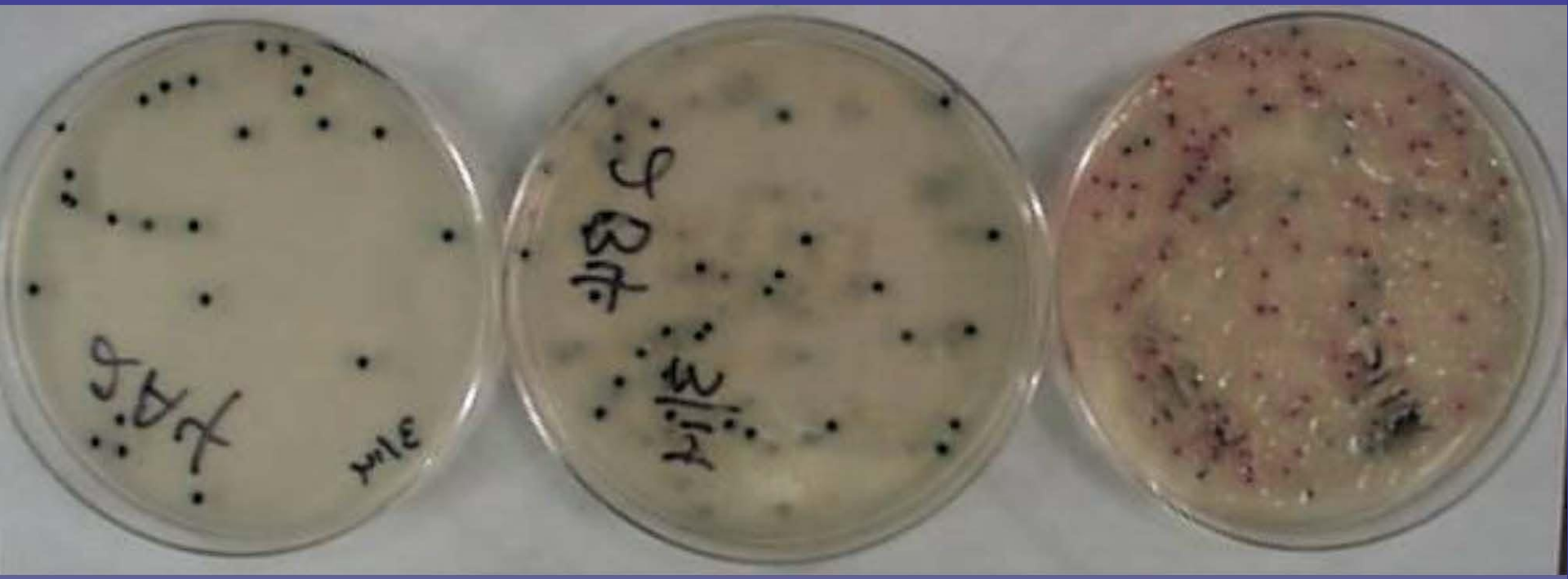
Potential Positive

Immunomagnetic Bead Retrieval

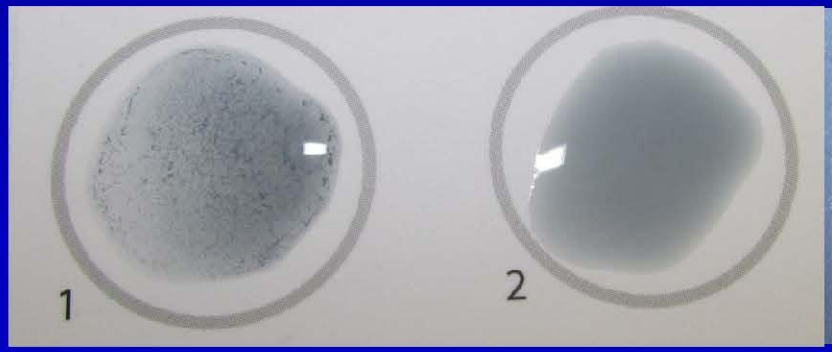
Modified Rainbow Agar



Modified Rainbow Agar



Agglutination Tests



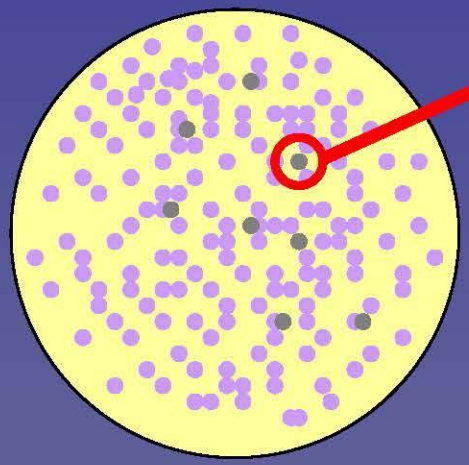
O157 positive
Card agglutination

O26 positive
Slide agglutination

STEC Analytical Procedure Confirmation

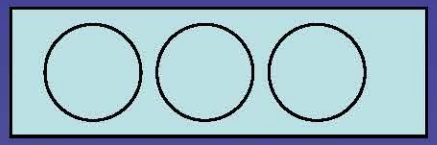
Day 3

Rainbow Agar



= Typical

= Non Typical



Latex Agglutination

Presumptive Positive

OR

= Conf. Negative

Analysis Complete

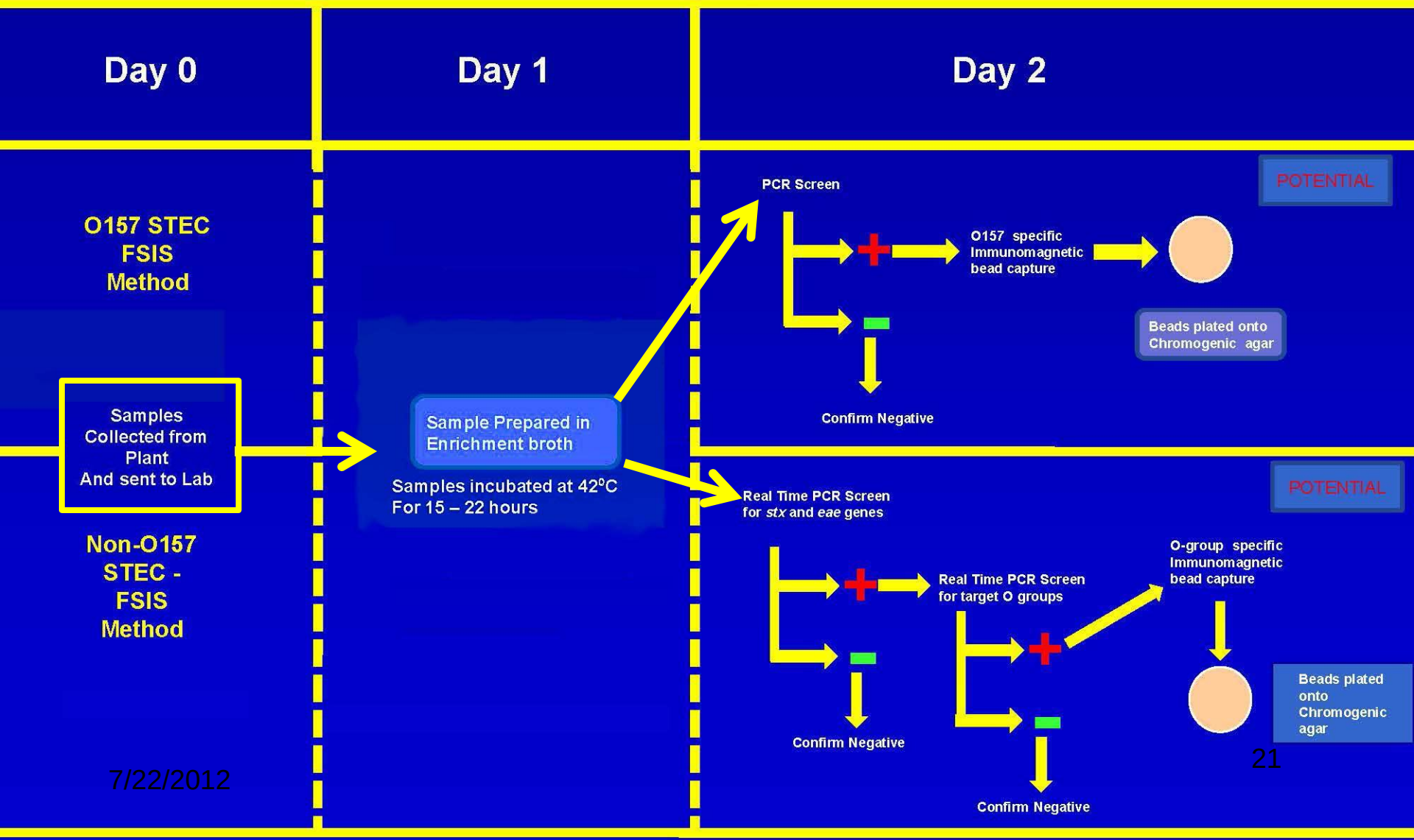
Day 4

Confirmed Positive

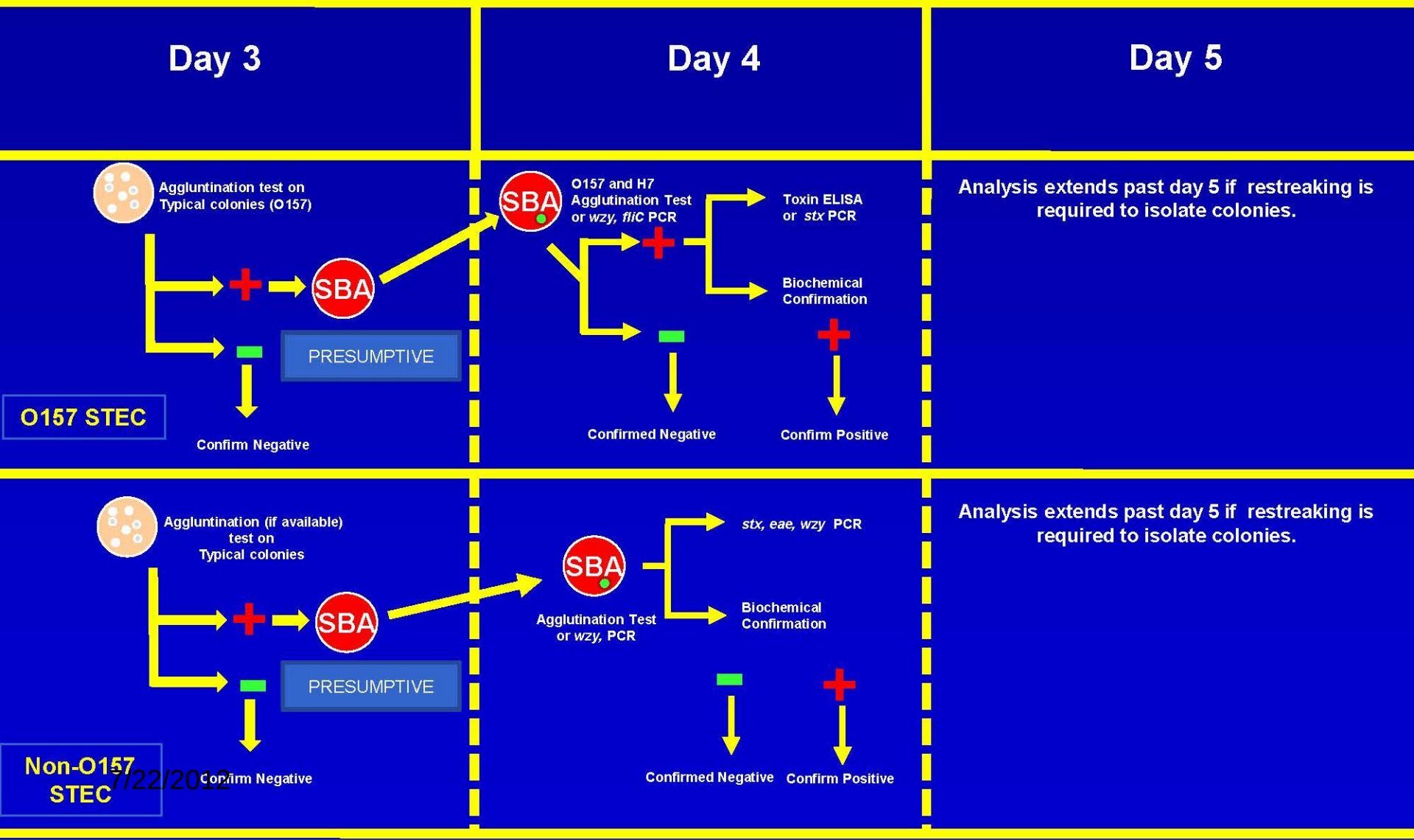
Biochemical Analysis

Confirmatory PCR
(stx, eae, serogroup)

FSIS E. coli O157:H7 and Non-O157 STEC Method Comparison



FSIS E. coli O157:H7 and Non-O157 STEC Method



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

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

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 closeto 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

Table 2: Non-O157 STEC (by serogroup) and *E. coli* O157:H7

Non-O157 STEC (by serogroup) and *E. coli* O157:H7 YTD[1]				
Raw Ground Beef Components (RGBC)[2]				
	Federal Plants			Import
Target STEC[3]	Trim Verification Percent Positive (Number)	Follow-up to RGB Positive at Supplier Percent Positive (Number)	Follow-up to RGBC Positive Percent Positive (Number)	Verification/ Intensified Percent Positive (Number)
O157:H7	0.53% (4/759)	0.00% (0/79)	1.72% (1/58)	1.05% (5/478)
Total non-O157 STEC	2.73% (3/110)	0.00% (0/0)	20.00% (1/5)	0.00% (0/6)
O26	0.00% (0/110)	0.00% (0/0)	0.00% (0/5)	0.00% (0/6)
O45	0.91% (1/110)	0.00% (0/0)	0.00% (0/5)	0.00% (0/6)
O103	0.91% (1/110)	0.00% (0/0)	20.00% (1/5)	0.00% (0/6)
O111	0.00% (0/110)	0.00% (0/0)	0.00% (0/5)	0.00% (0/6)
O121	0.00% (0/110)	0.00% (0/0)	0.00% (0/5)	0.00% (0/6)
O145	0.91% (1/110)	0.00% (0/0)	0.00% (0/5)	0.00% (0/6)

[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: http://www.fsis.usda.gov/Science/RGBC_STEC_Results/index.asp

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).
- A summary table of no-objection letters issued by FSIS for non-O157 STEC test methods can be found at: http://www.fsis.usda.gov/Regulations_&_Policies/NTT_STEC_NOL/index.asp.

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

AskFSIS Q&As

- 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...
  Date Updated: 06/06/2012