UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
WASHINGTON, DC

FSIS NOTICE

40-12 | 6/4/12

FSIS VERIFICATION TESTING FOR NON-O157 SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (NON-O157 STEC) UNDER MT60, MT52, AND MT53 SAMPLING PROGRAMS

I. PURPOSE

A. This notice re-issues the content of notice 29-12, FSIS Verification Testing for Non-O157 Shiga toxin-producing *Escherichia coli* (Non-O157 STEC) under MT60, MT52, and MT53 Sampling Programs and explains that, beginning 90 days after FSIS' implementation of sampling and testing of beef manufacturing trimmings for non-O157 STEC on June 4, 2012, establishments will be required to reassess their HACCP systems in response to FSIS or establishment non-O157 STEC positive test results, if they have not already addressed the hazard in their HACCP system.

B. This notice provides inspection program personnel (IPP) with information on non-O157 Shiga toxin-producing *Escherichia coli* (STEC) laboratory tests that will be performed on samples of beef manufacturing trimmings collected under certain existing *Escherichia coli* (*E. coli)* O157:H7 verification sampling programs. This testing applies only to samples of beef manufacturing trimmings from cattle slaughtered on-site on or after June 4, 2012. A separate FSIS Notice addresses sampling and testing of beef manufacturing trimmings at port-of-entry sites. FSIS will test beef manufacturing trimmings (but not ground beef, bench trim, or other components of ground beef such as cheek meat, head meat, or other components described in FSIS Directive 10,010.1) for the six relevant non-O157 STEC serogroups (O26, O45, O103, O111, O121, and O145) in addition to *E. coli* O157:H7 under the following sampling programs:

1. MT60 (formerly MT50), Routine Testing of Domestic Raw Beef Manufacturing Trimmings;

2. MT52 (beef manufacturing trimmings only), Testing of Beef Manufacturing Trimmings or Other Components From Originating Slaughter Suppliers, Based on an MT43 Positive Result, at Federal Establishments; and

3. MT53 (beef manufacturing trimmings only) Follow-up Testing of Positives From Routine Testing of Beef Manufacturing Trimmings (MT60); or Positive Follow-up Testing at Suppliers (Positive MT52 Samples).

C. MT52 and MT53 samples that contain components or materials other than beef manufacturing trimmings will be tested for *E. coli* O157:H7 but not the non-O157 STEC serogroups.

D. At a later date, FSIS laboratories will implement non-O157 STEC testing in other sampling programs. FSIS will issue both a *Federal Register* notice and an FSIS Notice reflecting these changes before FSIS begins testing these additional raw beef products for the six non-O157 STECs.

II. BACKGROUND

A. Because of the public health concern regarding the non-O157 STEC serogroups, in 2011, FSIS announced to the public in a *Federal Register* notice its intent to declare at least six non-O157 STECs (O26, O45, O103, O111, O121, and O145) adulterants in non-intact raw beef products and product components (http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2010-0023.pdf). On June 4, 2012, FSIS will begin testing beef manufacturing trimmings (but not other components of ground beef, such as cheek meat, head meat, or other components described in FSIS Directive 10,010.1) collected from cattle slaughtered on-site on or after June 4, 2012, for the six non-O157 STECs. Beef manufacturing trimmings are produced from cattle slaughtered on-site and include traditional boxed beef products (e.g., two-piece chucks, primal cuts, and subprimal cuts) intended for non-intact use. To provide establishments time to prepare for the Agency's new testing, production lots containing any amount of beef manufacturing trimmings collected from cattle slaughtered on-site before June 4, 2012, will be analyzed for *E. coli* O157:H7 only.

NOTE: A separate FSIS Notice will be issued for import inspection personnel regarding imported beef manufacturing trimmings.

III. IPP RESPONSIBILITIES FOR ESTABLISHMENT AWARENESS MEETING

IPP are to discuss non-O157 STEC testing with establishment personnel during the next weekly meeting and document the meeting in a Memorandum of Interview (MOI) as instructed in FSIS PHIS Directive 5000.1. IPP are to share the following information with the establishment:

1. Samples of raw beef manufacturing trimmings that are from cattle slaughtered on-site on or after June 4, 2012, collected under the MT60, MT52, and MT53 sampling programs will be tested by FSIS for non-O157 STECs as well as *E. coli* O157:H7. Explain the following key points:

    a. Starting June 4, 2012, if the raw beef manufacturing trimmings in the production lot that is sampled by FSIS are from cattle slaughtered on-site on or after June 4, 2012, FSIS laboratories will perform analyses for the six non-O157 STECs (O26, O45, O103, O111, O121, and O145) in addition to *E. coli* O157:H7.

    b. If the slaughter date of any amount of the raw beef manufacturing trimmings in the production lot is prior to June 4, 2012, FSIS laboratories will only analyze these samples for *E. coli* O157:H7.

c. FSIS will analyze any follow up sample (MT52 and MT53) of raw ground beef components other than beef manufacturing trimmings for only *E. coli* O157:H7.

d. FSIS will analyze ground beef and other raw ground beef components for only *E. coli* O157:H7.

2. FSIS does not expect establishments to reassess their HACCP plans because of non-O157 STEC testing implementation. No noncompliances (NRs) or Food Safety Assessments (FSAs) will result should an establishment not reassess its HACCP plan.

3. Background information is in Attachment 1.

4. Initially, FSIS will not schedule a for-cause FSA in an establishment in response to an FSIS non-O157 STEC positive sample result. FSIS recognizes that establishments will begin taking steps to address non-O157 STECs in their HACCP systems and performing activities to gather data to validate that their food safety systems are adequately designed to control non-O157 STECs. Establishments are to document and identify in their initial validation activity plans the time frame in which they will have accumulated sufficient data to conclude that their food safety systems are demonstrated to be adequate to control for the relevant non-O157 STECs. IPP are to verify that establishments are adhering to the controls that they identified in their food safety systems. FSIS recognizes that establishments may initially or permanently modify their Certificates of Analysis (COA) and Letters of Guarantee (LOG) to identify that the relevant non-O157 STECs are being controlled using the same controls as for *E. coli* O157:H7. Approximately 90 days after June 4, 2012, FSIS will provide instructions to IPP as to how to verify that establishments have validated their HACCP plans, and that they are controlling for the relevant non-O157 STECs in beef manufacturing trimmings.

5. Except for not requiring establishments to reassess their HACCP systems if they have not yet addressed the hazard, and not initially scheduling for-cause FSAs in response to non-O157 STEC positive test results for 90 days after June 4, 2012, when FSIS finds beef manufacturing trimmings positive for any of the relevant non-O157 STEC, the Agency's actions will be consistent with its actions in response to *E. coli* O157:H7 positive results (see Section VI below), including performing follow-up sampling as described in Directive 10,010.3 Rev 3.

6. Establishments may differentiate production lots containing beef manufacturing trimmings from cattle slaughtered on or after June 4, 2012, from production lots of beef manufacturing trimmings from cattle slaughtered prior to this date. FSIS does not anticipate that establishments will change their lotting practices or other normal operational practices (including lot composition) in response to non-O157 STEC testing.

IV. IPP RESPONSIBILITIES FOR COLLECTING AND SUBMITTING SAMPLES

A. When IPP receive a sample request for MT60, MT52, and MT53, they are to continue to follow the instructions in FSIS Directive 10,010.1, Chapter II and Chapter III, for collecting samples. There are no changes to product sampling eligibility or sample collection procedures for the MT60, MT52, or MT53 sampling programs.

B. For each sample of beef manufacturing trimmings collected in the MT60, M52, and MT53 sampling programs, IPP are to answer the following questions in the Public Health Information System (PHIS) sampling collection task. IPP's responses to the questions are important as they determine which analyses the FSIS laboratories will perform. (Only when the answer to all below questions is "yes" will the sample be analyzed for non-O157 STEC.)

Following some of the answer choices is guidance to assist IPP with answer selection.

1. Does the sampled lot contain beef manufacturing trimmings from cattle slaughtered only on-site at this establishment?

a. Yes

b. No

2. Does the sampled lot contain beef manufacturing trimmings from cattle slaughtered only on or after June 4, 2012?

a. Yes

(IPP are to select 'Yes' when they have verified that the entire sampled lot includes only beef manufacturing trimmings from cattle slaughtered on site on or after June 4, 2012, and does not contain any amount of beef manufacturing trimmings from cattle slaughtered before June 4, 2012.)

b. No

(IPP are to select 'No' when the sampled lot includes any amount of beef manufacturing trimmings from cattle slaughtered prior to June 4, 2012.)

3. Does the sampled lot contain only beef manufacturing trimmings and no other components? (**FOR MT52 AND MT53 SAMPLES ONLY)**

a. Yes

(IPP are to select 'Yes' when they have verified that the entire sampled lot is composed only of beef manufacturing trimmings and does not contain any amount of other components or other material.)

b. No

(IPP are to select 'No' when the sampled lot contains any amount of material other than beef manufacturing trimmings)

C. Should IPP have concerns about an establishment changing its lotting practices or

other normal operational practices (including lot composition) to limit FSIS from testing beef manufacturing trimmings samples for the relevant non-O157 STECs, IPP are to consult their supervisory chain of command for further instructions.

V. OBTAINING SAMPLE RESULTS

A. IPP are to access LEARN for sample results. The laboratories will report the results for both the *E. coli* O157:H7 and non-O157 STECs together for each sample in LEARN according to the Sample Request Form Number. IPP are to obtain sample results for both *E. coli* O157:H7 and the six non-O157 STECs by accessing LEARN for each sample submitted.

B. "Not acceptable" positive test results for *E. coli* O157:H7 or any of the six non-O157 STECs are reported in LEARN as soon as each analysis is completed and reviewed. If the sample confirms positive for a non-O157 STEC, LEARN will display the specific non-O157 STEC serogroups that are positive. "Acceptable" test results are not reported in LEARN until after all sample analyses are completed, and the results comply with FSIS regulatory requirements.

NOTE: Results for non-O157 STEC testing are not expected to take longer than for *E. coli* O157:H7 testing.

VI. IPP AND EIAO ACTIONS FOLLOWING A POSITIVE FSIS TEST RESULT

A. IPP are to follow the same actions for each non-O157 STEC analysis positive result as outlined in FSIS Directive 10,010.1, Chapter III, as they do for *E. coli* O157:H7, with the following changes:

1. IPP are to assess the sanitary dressing procedures and process controls that cattle slaughter establishments employ in their food safety systems, in the manner described in Directive 6410.1. Such controls are likely to include decontamination and antimicrobial intervention treatments. IPP are to especially focus on how the establishment is preventing visible contamination on the carcass at all stages of the hide removal process, not just after the hide is completely removed.

2. When verifying adequate corrective actions, IPP are first to determine whether the establishment identified non-O157 STEC as a hazard in its hazard analysis. If the establishment identified non-O157 STEC, IPP are to verify that the establishment takes corrective action in accordance with 9 CFR 417.3(a). If the establishment did not identify non-O157 STEC in its hazard analysis, IPP are to verify that the establishment takes corrective action in accordance with 9 CFR 417.3(b). When verifying compliance with 9 CFR 417.3(b), IPP are not to expect the establishment to initiate a testing program for non-O157 STECs if it does not already have one at this time. Additionally, when verifying compliance with 9 CFR

417.3(b), IPP are not to expect the establishment to perform reassessment for the first 90 days after June 4, 2012. Beginning 90 days after June 4, 2012, when verifying compliance with 9 CFR 417.3(b), IPP are to verify that the establishment has reassessed its HACCP system for non-O157 STEC or maintains support demonstrating that its existing controls for *E. coli* O157:H7 effectively control the non-O157 STEC. When an establishment takes corrective actions in accordance with 9 CFR 417.3(b), IPP are to evaluate whether the establishment properly implemented existing controls and sanitary dressing procedures.

B. Enforcement, Investigations and Analysis Officers (EIAOs) are to follow the same instructions for each non-O157 STEC analysis positive result as outlined in FSIS Directive 10,010.1, Chapter III, as they do for *E. coli* O157:H7, with the exception that a for-cause FSA would not be scheduled.

VII. IPP AND EIAO RESPONSIBILITIES RELATED TO AN ESTABLISHMENT'S CONTROLS FOR *E. coli* O157:H7 and Non-O157 STEC

A. On June 4, 2012, FSIS will not require establishments to adjust their existing testing programs for non-O157 STEC. Establishments are also not required to automatically implement additional specific requirements in their COAs, antimicrobial interventions, or other process controls specifically for the six non-O157 STECs. Establishments that produce non-intact raw beef products, such as ground beef, or the intact raw components of those products, typically operate HACCP systems that address *E. coli* O157:H7, and, as a result, many have already incorporated antimicrobial interventions, such as organic acid sprays, into their processing.

B. IPP are not to expect establishments to reassess their HACCP plans because of non-O157 STEC testing implementation, and IPP are not to issue an NR, nor is an FSA to be conducted should an establishment not reassess its HACCP plan.

C. When IPP review records as described in FSIS Directive 10,010.1, Chapter IV, and FSIS Directive 5000.2, IPP are to review such records for any non-O157 STEC testing the establishment is conducting in addition to any *E. coli* O157:H7 testing.

D. EIAOs are to review supporting documentation that the establishment has for its sampling and testing programs, as described in FSIS Directive 10,010.1, Chapter VI, as part of a Raw, Intact HACCP verification task (for trim and other component sampling) and a Raw, Non-intact HACCP verification task (for raw ground beef or patty sampling). Supporting documentation may include information regarding the establishment's test method compared to the FSIS test method (refer to FSIS Directive 5100.1, Attachment 1 for guidance). An example of such documentation includes referring to a test method reviewed by FSIS, and for which FSIS has issued a "letter of no objection," and verifying that the establishment is following the parameters for test use as stated in the "letter of no objection." When performing a routine or "for-cause" (for reasons other than non-O157 STEC) FSA in an establishment producing beef manufacturing trimmings, EIAOs are to review and assess supporting documentation and establishment decision-making regarding non-O157 STECs and are to document this information in the FSA, but they are not to recommend issuing any NRs .

VIII. DATA ANALYSIS

The Office of Public Health Science (OPHS), the Office of Policy and Program Development (OPPD), and the Data Analysis and Integration Group (DAIG) within the Office of Data Integration and Food Protection (ODIFP) will analyze sample results for non-O157 STECs collected through the routine sampling programs. Specifically, OPHS will produce a weekly report on sample findings, and OPPD will produce an annual summary report that will be published on the FSIS Web site. Approximately a year after the start of this program, DAIG will analyze the sampling data to identify trends (e.g., geographical, seasonal) and to evaluate program effectiveness (e.g. sample scheduling and collection rates). Ad hoc analyses may be conducted within a year to address specific Agency questions.

IX. ASKFSIS QUESTIONS

FSIS has published askFSIS Questions and Answers on non-O157 STEC issues. IPP can view the questions by searching the questions database on the askFSIS website.

IPP are to refer questions regarding this notice to the Risk, Innovations & Management Division through askFSIS at http://askfsis.custhelp.com or by telephone at 1-800-233-3935 (press 1 and 4).

Acting Assistant Administrator
Office of Policy and Program Development

Attachment 1

Background on FSIS non-O157 STEC Testing

The Centers for Disease Control and Prevention (CDC) estimates that there are approximately 175,905 domestically acquired foodborne illnesses associated with all Shiga toxin-producing *E. coli* (STEC) annually (Scallan et al, 2011)[1]. *E. coli* O157:H7 is the most well known STEC and, according to the CDC, annually is responsible for approximately 63,153 (36%) of the domestically acquired foodborne STEC illnesses. The remainder of the illnesses associated with STEC (112,752 or 64%) are caused by non-O157 STEC. While more than 50 non-O157 STEC serogroups have been associated with human illness, 70 to 80 percent of confirmed non-O157 STEC illnesses are caused by six STEC serogroups – O26, O45, O103, O111, O121, and O145. These illnesses can be equivalent in severity to those caused by *E. coli* O157:H7. In the U.S, at least one outbreak and several sporadic illnesses from non-O157 STEC serogroups have been associated with ground beef products.

Because of the public health concern regarding the non-O157 STEC serogroups, in 2011, FSIS announced to the public in a *Federal Register* notice its intent to declare at least six non-O157 STECs (O26, O45, O103, O111, O121, and O145) adulterants in non-intact raw beef products and product components (http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2010-0023.pdf). On June 4, 2012, FSIS will begin testing beef manufacturing trimmings from cattle slaughtered on-site on or after June 4, 2012 for the six non-O157 STEC in addition to *E. coli* O157:H7. To provide establishments time to comply with the new policy, FSIS announced that beef manufacturing trimmings collected from cattle slaughtered on-site before June 4, 2012 will be analyzed for *E. coli* O157:H7 only (http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2010-0023N.pdf). At a later date, FSIS laboratories will implement non-O157 STEC testing in other sampling programs

Non-O157 STEC testing results

FSIS, together with the Agricultural Research Service (ARS), has developed a laboratory method for detection and isolation of non-O157 STEC serogroups from raw beef. Further information regarding the current laboratory method is available on the FSIS website, at http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp. The FSIS methods for non-O157 STEC and *E. coli* O157:H7 differ, but both have similar stages when results are communicated by the laboratory (e.g., potential, presumptive, confirmed). Table 1 shows a side-by-side comparison of the non-O157 and *E. coli* O157:H7 stages for reporting results.

One difference between these two methods identified in Table 1 is that testing for non-O157 STEC involves a two-stage polymerase-chain-reaction (PCR) screening test, while the methodology for *E. coli* O157:H7 only includes a single-stage PCR screening test. In the non-O157 STEC screening test, the first stage will detect samples positive for the genes *stx* (Shiga toxin) and *eae* (intimin). In the second stage, samples will be

[1] Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, Jones JL, and Griffin PM. 2011. Foodborne illness acquired in the United States – major pathogens. Emerg Infect Dis. 17(1):7-15.

screened for the presence of one of the six target serogroups (O26, O45, O103, O111, O121, and O145). Both these PCRs are performed at the same time. A sample will be identified as ‘‘potential positive’’ when it tests positive for the *stx* gene and the *eae* gene and is also positive for one or more of the target serogroup genes. Samples identified as “potential positive” will continue through further testing to confirm whether they are positive for one or more of the six non-O157 STEC. Only samples that are confirmed positive for one of the six non-O157 STEC or that are confirmed positive for *E. coli* O157:H7 will be considered adulterated.

Table 1. Comparison between 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”

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 policy are needed.

Establishment Testing for non-O157 STEC

Many establishments that produce raw non-intact beef products, such as ground beef, incorporate antimicrobial interventions such as organic acid sprays in their processing. These methods should be effective in controlling non-O157 STEC. However, many firms will want to implement their own testing programs. A prudent establishment would use a test method that includes all hypothetical strains of *E. coli* O157:H7 and the target non-O157 STEC, typical or variant, that would be identified using FSIS' confirmatory testing procedures and criteria and that increases the likelihood of detecting low level contamination by these pathogens. FSIS recognizes that industry uses non-cultural methods that detect alternative target analytes for *E. coli* O157:H7 including, but not limited to, *eae* and *stx*. Establishments may increase the likelihood of detecting all hypothetical strains and low levels of contamination by these pathogens in a variety of ways, including but not limited to using a test method that is also used by a regulatory body or that is validated and certified by an independent body (i.e., AOAC, AFNOR, MicroVal, or NordVal). An establishment may also opt to use a test method that is subjected to a robust validation using the FSIS cultural method as a reference. Several companies have developed or are developing test kits to detect at least the six relevant STEC serogroups. Some kits have been submitted for review by validation bodies. In addition, some kits have been submitted for review by FSIS and have received "letters of no objection" from the Agency. FSIS continues to review other test kits submitted for review.

For establishment testing or testing on behalf of an establishment, FSIS recognizes that other criteria, while not used specifically by FSIS for identification of a non-O157 STEC, may be a significant and expedient indicator of the presence of non-O157 STEC in products. Such tests might be applied as rapid screening procedures to expedite analyses. If an establishment uses or contracts with a laboratory that uses such rapid screening procedures, and product is found positive by that test, FSIS expects the establishment to take appropriate corrective action and to ensure the proper disposition of adulterated products following a positive test result (9 CFR 417.3). The establishment will need to define and support the criteria it uses to define non-complying product.

Questions and Answers on non-O157 STEC Testing

Additional information on non-O157 STEC testing may be found in askFSIS at http://askfsis.custhelp.com/ (first select "General Inspection Policy" on the "Limit by product" drop-down menu, then select "Sampling" on the "Limit by category" drop-down menu, and then enter "non-O157" in the "Find the answer to your question" field).