Compliance Guideline for Establishments Sampling Beef Trimmings for Shiga Toxin-Producing *Escherichia coli* (STEC) Organisms or Virulence Markers

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Table of Contents and Section Links:

I. Introduction and Background Discussion

This Compliance Guideline is meant to help beef slaughter/fabrication establishments that manufacture beef trimmings develop and implement statistical process control procedures to assess the effectiveness of their controls for preventing contamination during the slaughter operation. These procedures are especially applicable for verification activities that include "N60" excision sampling and testing programs for shiga toxin-producing *Escherichia coli (E. coli)* (STEC) organisms, particularly *E. coli* O157:H7, or their associated virulence markers (e.g., *eae* and *stx* genes*)*. These procedures can also be supplemented with verification activities associated with production of other raw ground beef and patty components.

Most establishment testing methods include an enrichment step followed by differential screening specific to STEC or their virulence markers. Positive results during screening tests require further testing to detect *E. coli* O157:H7. If the establishment does not perform further testing, it should treat positive screen results as confirmed positive. FSIS will consider those results positive for *E. coli* O157:H7.

FSIS recognizes that many establishments test for other STEC or their associated virulence markers and treat those positive screen results as positive for *E. coli* O157:H7. Establishments can apply the guidance in this document to such positive screen results. Therefore, much of the discussion in this document refers to "STEC organisms or virulence markers," in addition to *E. coli* O157:H7.

This guideline reflects comments received on the Agency's Draft Compliance Guideline for Sampling Beef Trimmings for *Escherichia coli* O157:H7 issued on August 12, 2008. On October 14 and 15, 2008, FSIS also held a public meeting to discuss the guidance and other topics concerning *E. coli* O157:H7 (http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2008-0035.pdf).

The major change in this updated document is revised guidance that establishments can use to determine if they are experiencing a "high event period" (HEP) situation. HEPs are periods in which slaughter establishments experience a high rate of positive results for *E. coli* O157:H7 (or STEC or virulence markers) in trim samples from production lots containing the same source materials. That is, the trim was produced from one or more carcasses slaughtered and dressed consecutively or intermittently within a defined period of time (e.g., shift). A HEP situation may mean that a systemic breakdown of the slaughter dressing operation has occurred and has created an insanitary condition applicable to all parts of the beef carcass (e.g., primal cuts in addition to the beef manufacturing trimmings and other raw ground beef and patty components). FSIS recommends that establishments identify HEP criteria so that they can determine whether they need to withhold product from commerce when a HEP has occurred because the presence of a HEP may indicate more

widespread adulteration of product, beyond the product found positive. If establishments identify and respond to HEPs, they will minimize the chance that they release adulterated product into commerce.

The 2008 guidance specified that an *E. coli* O157:H7 percent positive of greater than 1.5%, for samples collected using the N60 method, indicated that a process may be out of control and thus in a HEP situation. Based on that percent positive, the prior guidance recommended that four positive *E. coli* O157:H7 results out of 91 consecutive N60 samples should be seen as indicating a loss of control.

This revised guidance recommends two distinct HEP situation criteria: one for a localized out-of-control situation, and a second for a systemic break-down situation. In both situations, FSIS believes that establishments should be concerned if their sampling of trimmings produce a positive rate statistically significantly greater than 5%, rather than 1.5% positive, as discussed in the 2008 draft guidance. In such cases the processor should review process control measures and intervention measures used during slaughter, dressing, fabrication, and grinding. During a systemic break-down situation, establishments may identify more product that needs to be assessed to determine whether it may be adulterated than in a localized HEP. A localized HEP may affect only the production of one lot, while a systemic break-down may affect more product. Also, a localized HEP may indicate an isolated problem (such as improper application of an antimicrobial in one lot); a systemic HEP may indicate a broader problem (systemic failure to prevent cross contamination among carcasses). When either of these trigger criteria is reached, the establishment may determine that production lots of beef manufacturing trimmings containing same source materials that were sampled, tested, and found negative should be considered as having a false negative result, depending on the reason for the HEP. If the establishment makes that determination, such product should be diverted to a full lethality treatment or otherwise destroyed.

FSIS is recommending a higher target value for two primary reasons. First, FSIS recognizes that many establishments treat a potential or presumptive positive sample result as if the sample was confirmed to contain viable *E. coli* O157:H7. These practices result in a higher positive rate than FSIS verification testing that is specifically for viable *E. coli* O157:H7. Second, FSIS made this change to a higher target value to increase confidence that an insanitary condition likely occurred during the slaughter/dressing operation. With a higher target value of 5%, the establishment and the Agency can be more certain that the food safety system is truly out of control than compared to its confidence using a target of 1.5%. FSIS does not expect such HEP situations to happen often during any 12-month period when an establishment's slaughter dressing operation is properly functioning. Establishments may choose to use the earlier guidance based on 1.5%. As is discussed below, establishments may always

choose to develop stricter HEP criteria than FSIS is recommending. By choosing the stricter HEP criteria, the establishment reduces its vulnerability for releasing product into commerce that could test positive at a subsequent point in processing or that could be associated with illness.

The HEP situation guidance provided here applies mainly to beef slaughter/fabrication establishments that manufacture 50,000 pounds or more of trimmings daily because such establishments are likely to conduct sufficient verification testing on same source materials to be able to determine whether a HEP occurred. Establishment verification testing results on trimmings are likely the best available objective information a slaughter establishment can use to determine the effectiveness of its slaughter/dressing operation. Although this document also provides general information for non-slaughter establishments that produce or receive trimmings, non-slaughter establishments will not know if problems with slaughter and dressing procedures have contributed to a HEP situation unless provided with that information by the supplier as part of a purchase specification program arrangement.

Although the HEP guidance applies mainly to beef slaughter/fabrication establishments that manufacture 50,000 pounds or more of trimmings daily, this guidance includes some general discussion at the end of chapter III regarding how smaller establishments may choose to define HEPs.

Contamination events generally are not perceptible visually. For this reason, and because they may significantly reduce the safety margin afforded by antimicrobial treatments, FSIS recommends that slaughter/fabrication establishments conduct sampling and testing of trim at a frequency sufficient to find evidence of contamination surviving the slaughter and dressing operation (optimally every production lot) in an effort to ensure that adulterated product does not enter commerce. Establishments grouping five combo bins of trimmings into production lots represented by one N60 sample may be less capable of discerning a HEP situation than establishments that collect an N60 sample from one combo bin production lot. However, establishments that group multiple combo bins into a production lot may have a scientific basis for selecting samples or grouping samples that allows them to identify a HEP effectively; they may have had a contract study conducted for them based on their own in-plant conditions that supports their lotting practices and shows that their sampling and testing has a high probability of detecting positives when present. In addition, establishments that exclude exterior fatty trimmings from calculation of a HEP situation may be less capable of identifying a HEP situation.
Additional assistance and information on these matters can be found in publications of the Beef Industry Food Safety Council, at:
http://www.bifsco.org/groundbeef.aspx

This guideline represents current FSIS thinking and is usable now. FSIS will update the guideline as needed to reflect the most current information available

to FSIS and stakeholders. This document provides recommendations rather than regulatory requirements.

This Compliance Guideline follows the procedures for guidance documents in the Office of Management and Budget's (OMB) "Final Bulletin for Agency Good Guidance Practices" (GGP). More information on the bulletin can be found on the FSIS Web page:

www.fsis.usda.gov/Significant_Guidance/index.asp.

Request for comments:

FSIS requests that all interested persons submit comments regarding any aspect of this document, including but not limited to: content, readability, applicability, and accessibility. The comment period will be 60 days. The document will be updated in response to comments.

Comments may be submitted by either of the following methods:

Federal eRulemaking Portal: This Web site provides the ability to type short comments directly into the comment field on this Web page or attach a file for lengthier comments. Go to http://www.regulations.gov. Follow the online instructions at that site for submitting comments.

Mail, including CD-ROMs, etc.: Send to Docket Clerk, U.S. Department of Agriculture, Food Safety and Inspection Service, Docket Clerk, Patriots Plaza 3, 1400 Independence Avenue SW, Mailstop 3782, Room 8-163A, Washington, DC 20250-3700.

Hand- or courier-delivered submittals: Deliver to Patriots Plaza 3, 355 E. Street SW, Room 8-163A, Washington, DC 20250-3700.

Instructions: All items submitted by mail or electronic mail must include the Agency name and docket number FSIS-2011-0009. Comments received in response to this docket will be made available for public inspection and posted without change, including any personal information, to http://www.regulations.gov.

Background Discussion

An establishment may incur considerable expense if it becomes necessary to recall contaminated product from commerce[1]. This action becomes necessary when trimmings that have been subjected to antimicrobial interventions are later found positive for *E. coli* O157:H7, or when other production lots from "same source" materials – i.e., fabricated from a single, common source rather than

[1] FSIS has estimated that a recall can cost government and industry $3-5 million.

multiple, commingled sources – are found positive. For these reasons, robust sampling and testing programs that can find product containing *E. coli* O157:H7 can be highly cost-effective. Extensive sampling of trimmings and careful evaluation of test results can help identify areas of poor processing for corrective action. FSIS recommends that establishments continually strive to decrease *E. coli* O157:H7 (or STEC organisms or virulence markers) positive percentages, and we expect establishments that investigate and correct problems to improve processes and decrease positive percentages over time. FSIS is making this guidance available to establishments so that they can better identify HEPs that indicate more widespread adulteration of product, beyond any product found positive for the pathogen. By following this guidance and withholding adulterated product from commerce during HEPs, establishments are more likely to be able to avoid costly recalls.

Under 9 CFR 417.3, establishments are required to identify corrective actions in response to every deviation from a critical limit or a deviation not covered by specified corrective action. An *E. coli* O157:H7 postive would fall into one of these two categories that require corrective actions. Corrective actions required in the regulations include identifying and eliminating the cause of the deviation (if O157:H7 is addressed in the HACCP plan) or reassessing the HACCP plan and determining whether changes to it are necessary (if O157:H7 is not addressed in HACCP plan).

Process control of STEC *E. coli* O157:H7 can be evaluated by tracking past sample results, enabling establishments to tell the difference between an occasional, sporadic, positive result and a loss of process control as indicated by many positive results over time. If past sample results lead establishment management to believe the process is out of control, the establishment should carefully investigate to find all contributing causes. This type of investigation would be more involved than a follow-up investigation when an occasional positive result is found. The finding of an out-of-control process may implicate product in other production lots produced during the period that the process was out of control or from the same source material.

It is important to note that a HEP situation likely means that insanitary conditions occurred during the slaughter/dressing operation such that contamination is widespread across production lots. When a HEP situation occurs, negative test results from the production lots of trimmings made from the same source materials as trimmings found positive during the HEP may not be reliable.[2] Therefore, those production lots that tested negative may not be microbiologically independent of those directly associated with the HEP. In other words, even though an N60 product sample from a production lot tested negative, trimmings

[2] The statement does not imply that the usual N-60 sampling and testing is not reliable regarding their sensitivity and specificity. However, it is reasonable to assume that during the HEP, the incidence and levels of contamination could be greater than normal.

produced from the same source materials as the production lots directly associated with the HEP are also potentially contaminated.

When a HEP situation occurs, establishments should take appropriate precautionary steps to ensure adulterated lots of raw beef are not released into commerce. The establishment needs specifically to consider whether negative-tested lots of trimmings are affected and whether intact primal and sub-primal product produced from the same source materials as the trimmings may be positive for *E. coli* O157:H7.

Generally, if primals are not commingled before packaging, and the establishment prevents cross contamination among primals, primals can be considered independent lots. Normally, FSIS does not consider primal cuts designated for intact use to be adulterated if contaminated with *E. coli* O157:H7. During a HEP situation, however, unless the establishment has controls in place to ensure that the primals are not used for non-intact purposes, such primals may be considered adulterated because they were prepared under insanitary conditions. Establishments that subject primals to an antimicrobial treatment as part of a routine production process may be able to demonstrate that the primals are not adulterated, provided they have on-going verification testing results to affirm that contamination was not evident. It should be noted that a recent large-scale recall of beef that included primal cuts was associated with a HEP (see http://www.fsis.usda.gov/News_&_Events/Recall_034_2009_Expanded/index.asp and

http://www.fsis.usda.gov/News_&_Events/Recall_034_2009_Release/index.asp). Although all or most trim was diverted to cooking, including trim that tested negative for *E. coli* O157:H7 (or STEC organisms or virulence markers), primal cuts that had not been treated with an antimicrobial entered commerce. Illnesses were associated with the trim derived from the untreated primal cuts.

II. High Event Periods (HEPs):

FSIS conduted a baseline study in 2005-2006. The Agency collected 1900 N60 samples in “high” volume establishments producing 50,000 pounds of trimmings or more daily and analyzed the samples for the presence of *E. coli* O157:H7. Thirteen of the 1900 samples were positive (0.68%). From these results, FSIS estimated a volume weighted prevalence for *E. coli* O157:H7 in all beef trimmings of 0.39 %, with an upper 97.5% confidence bound of 0.73%.

These results reflect only confirmed *E. coli* O157:H7 positive test results rather than initial, unconfirmed (presumptive or potential) positive results. The samples were collected by FSIS from production lots that likely had already been tested by the establishments and found negative. Because of this, FSIS believes that the percentage of pre-tested percent positive product is greater than what FSIS estimated in this baseline. In addition, based on periodic review of

establishment test results and the FSIS survey of inspectors at large beef slaughter establishments discussed below, industry typically bases its decisions regarding identifying HEPs on presumptive positive results (or what FSIS terms potential positive results) from initial screening tests that produce a higher percentage of false positives.[3] Percentages of presumptive positive results are greater than percentages of confirmed results. FSIS wanted to identify a target that was in accordance with today's present industry standard.

To develop recommendations for identifying HEPs, FSIS examined industry data collected in 2010 by FSIS inspection personnel from the top 33 slaughter establishments, based on production volume (heads slaughtered).
Of the 33 establishments, 32 responses were received, 19 had clear definitions of a HEP, 2 had definitions that were incomplete because they did not specify a frame of time (which we interpreted to be a day), 10 had unclear definitions of a HEP, and 1 did not have a definition. Of the 21 establishments that had clear definitions (including the two we interpreted), 7 were using a 5% threshold definition; there were 3 that had definitions greater than 10%.

Based on these results, FSIS selected a target of 5%. FSIS, did not want to define HEP criteria that would be as, or more, rigorous than those of a large number of establishments, and, therefore, did not select a lower target. FSIS intended to identify criteria that would indicate exceptional events of poor processing. FSIS did not select a higher target (e.g.,10%) because such a target we believe could result in many cases where poor processing, as defined by most the industry, would not be detected as a HEP.

FSIS recommends that establishments evaluate their testing results for both long term (systemic) and short term (local) periods for which the positive rate is substantially greater than expected within the production day or shift. Establishments can evaluate both periods by applying a set of criteria within a moving window of testing results. If the establishment exceeds either HEP criterion, FSIS believes there is a high degree of confidence that a particular event occurred that indicates poor processing or poor food safety controls.

For the purpose of this document there are two types of a HEP that may indicate out-of-control situations:

1. A HEP that indicates a localized out-of-control event in which some specific occurrence or event causes a clustering of *E. coli* O157:H7 (or STEC organisms or virulence markers) contamination in product.
2. A HEP that indicates a systemic break-down or inherent weakness of the process or food safety system.

[3] The FSIS analysis for the presence of *E coli* O157:H7 has two screening stages before confirmation. See FSIS Microbiology Laboratory Guidebook methods MLG 5 and 5A. Also see: http://www.fsis.usda.gov/PDF/MLG_ECOLI_Flowchart.pdf for a flow chart of FSIS's procedures.

Below are sample criteria establishments may use for determining whether they have experienced a HEP. The criteria will be most useful to establishments that have rigorous testing programs. As noted above, beef slaughter/fabrication establishments that manufacture 50,000 pounds or more of trimmings daily are likely to conduct sufficient verification testing on same source materials to be able to determine whether a HEP occurred based on these criteria.

1. For a local HEP: 3 or more *E. coli* O157:H7 (or STEC or virulence markers) positive results out of 10 consecutive samples from production lots containing same-source materials; that is, the trim was produced from one or more carcasses slaughtered and dressed consecutively or intermittently within a defined period of time (e.g., shift); and

2. For a systemic HEP:

A. 7 or more *E. coli* O157:H7 (or STEC organisms or virulence markers) positive results out of 30 consecutive samples from production lots containing same-source materials.
B. At establishments that test more than 60 samples per day from production lots containing same-source materials, the number of positive samples below within the samples tested in the table:

Unacceptable # Positives	Within Samples Tested
8	61
9	74
10	86
11	100
12	113
13	127
14	141
15	155
16	169
17	184
18	198
19	213
20	228

The number of positive results within these windows would typically provide a high degree of confidence of poor processing or poor food safety controls. The moving window method, such as the 10 or 30 consecutive samples suggested here, is a simple and useful tracking procedure.

FSIS established the HEP sample criteria above based on an establishment percent positive that exceeds 5%. FSIS chose a very high degree of confidence that results indicate correctly a systemic HEP: about 99.95% confidence that the percent positive during the period is not less than 5%. For the systemic HEP based on daily testing of at least 60 samples, the table provides numbers that would result in a 99 percent confidence that the 5% target would have been exceeded. For the local HEP guidance, FSIS used close to 99 % confidence (= 98.849644%) for making the assertion.

FSIS is not providing a tolerance for an acceptable number of *E. coli* O157:H7 (or shiga toxin-producing *E. coli* (STEC) organisms or virulence markers) positives. Rather, FSIS is providing guidance on when the number of positive results within a certain number of samples indicates a HEP occurence. In such situations, negative tested production lots are possibly contaminated because they were likely produced under insanitary conditions. In this situation, the establishment would need to determine whether the lots are releasable.

The establishment–specific process percent positive may differ from the pecent positive used to construct the above example (assuming that the sampling plan and analyses are described as above). These percent positives may also differ depending on the time of year and increase during high prevalence seasons. Consequently, a specified percent positive for a given establishment at a given time should be identified by indicating that a different percent positive was being achieved consistently and product has low likelihood of being adulterated. Deviations from previously obtained percent positive should be construed as presumptive evidence that the process is out of control and would warrant investigation to find and eliminate any potential causes for the positive results. As part of their supporting documentation for their hazard analysis, FSIS recommends that establishments document their criteria for identifying a HEP.

One example for how an establishment might develop their own criteria would be to determine an upper bound process percent positive and then determine how many actual sample results they will use to show whether they have exceeded that upper bound[4]. FSIS expects that slaughter/fabrication establishments are subjecting 100% of production lots of trim to N60 verification testing. In addition, FSIS recommends that establishments have a more rigorous verification testing program during the high prevalence season (from spring into early autumn) in order to have greater confidence that increased contamination is not passing through the slaughter/dressing operation into the trim production lots. More rigorous verification testing programs might reflect more restrictive HEP criteria. In addition, small establishments or those that produce product infrequently might choose a different set of criteria from those provided by FSIS. See the end of Chapter III below for specific suggestions.

[4] There are other tracking procedures, such as calculating and graphing cumulative sums of differences of results from a specified target (CUSUM) and exponentially weighted moving averages (EWMA), which do not require such determinations.

III. Suggested HEP Numerical Criteria

The following tables are provided to help establishments derive parameters for determining whether they have experienced a HEP. The tables provide specified numbers of positive results (given in the first column) occurring within a specified number of samples (entries within the remaining columns), that would indicate that the true percent positive of *E. coli* O157:H7 findings would be greater than or equal to the specified percent positive given in the column headings, for the following percent confidence intervals:

- with 95% confidence (Table 1);
- close to 99% confidence (Table 2); and
- close to 99.95% (Table 3).

In the tables below, note that the test result from one composite sample of multiple slices (e.g., N60 sample) is considered one positive or negative result.

Table 1: True positive percent of *E. coli* O157:H7 (or STEC organisms or virulence markers) findings is greater than corresponding lower bound percentage in column with 95% confidence, given the number of positive results (rows) within corresponding number of samples (interior table entries)

	Lower Bounds of Percent Positive, based on number of samples tested								
Number Positive	0.50%	0.68%	0.75%	1.0%	1.5%	2.0%	3.0%	3.5%	5.0%
2	71	52	47	35	24	18	12	10	7
3	164	120	109	82	55	41	27	23	16
4	274	201	182	137	91	69	46	39	28
5	395	290	263	198	132	99	66	57	40
6	523	385	349	262	175	131	88	75	53
7	658	484	439	329	220	165	110	95	67
8	797	586	532	399	266	200	134	115	81
9	940	692	627	471	314	236	158	135	95
10	1086	799	725	544	363	273	182	156	110
11	1235	909	824	618	413	310	207	178	125

Based on Table 1, if there were 4 or more positive results within 69 samples, then there would be 95% confidence that the process positive percent was not less than 2%.

Table 2: True positive percent of *E. coli* O157:H7 (or STEC organisms or virulence markers) findings is greater than corresponding lower bound percentage in column with about 98.85% confidence, given the number of positive results (rows) within corresponding number of samples (interior table entries)

	Lower Bound of Percent Positive, based on number of samples tested								
Number Positive	0.50%	0.68%	0.75%	1.0%	1.5%	2.0%	3.0%	3.5%	5.0%
2	32	23	21	16	11	8	5	5	3
3	92	68	62	46	31	23	16	13	10
4	172	127	115	86	58	44	29	25	18
5	266	196	178	133	89	67	45	39	27
6	369	272	247	185	124	93	62	54	38
7	481	354	321	241	161	121	81	70	49
8	598	440	399	300	200	151	101	87	61
9	720	530	481	361	241	181	121	104	74
10	846	623	565	424	283	213	143	123	86
11	976	718	652	489	327	246	164	141	100

Table 3: True positive percent of *E. coli* O157:H7 (or STEC organisms or virulence markers) findings is greater than corresponding lower bound percentage in column with about 99.95% confidence, given the number of positive results (rows) within corresponding number of samples (interior table entries) .

	Lower Bound of Percent Positive, based on number of samples tested								
Number Positive	0.50%	0.68%	0.75%	1.0%	1.5%	2.0%	3.0%	3.5%	5.0%
3	32	24	21	16	11	8	6	5	4
4	75	55	50	38	25	19	13	11	8
5	132	97	88	66	45	34	23	20	14
6	200	148	134	101	68	51	35	30	21
7	278	205	186	140	94	71	48	41	30
8	363	268	243	183	123	92	62	54	38
9	455	335	304	229	153	116	78	67	48
10	552	407	369	277	186	140	94	81	58
11	654	482	437	329	220	166	111	96	68

Based on Table 3, if 5 positive results occur within the set of 20 samples, then there is about 99.95% confidence that the positive percent would exceed 3.5%. The establishment may decide that if its percent positive exceeds 3.5%, then the establishment has experienced a high event period.

Establishments might have reason to collect a number of samples representing product processed under similar conditions and thus indicative of the processing during a set period. For example, an establishment might run product during a given time, or for a given day (shift), and take 20 samples of that product. In that case, the tables can be used for deciding how many positive results within sets of 20 samples would indicate a percent positive greater than that expected.

Small establishments that test infrequently might decide to develop other criteria for determining whether they have experienced a high event period. For example, a small slaughter establishment may test 5 samples and find 2 of them

positive. For a small establishment that does not test frequently, two positive results (or even 1) might indicate a lack of control in the production of that product and thus could be considered as a high event period.

If Tables 1-3 above do not meet an establishment's needs for determining high event criteria appropriate for the establishment, the establishment should contact *askFSIS* at http://askfsis.custhelp.com/ and categorize their question as "Sampling" within the system. Through the *askFSIS* system, establishments can obtain expert advice on the design of high event criteria.

IV. Action in a High Event Period

In a robust testing program, negative results normally indicate that product may be released in commerce. However, when a high event period occurs, the establishment needs to consider whether negative tested lots of trimmings are releasable, and whether primal and sub-primal product produced from the same source materials as the trimmings may be positive for *E. coli* O157:H7.

The actions taken in response to a high event period could depend upon the findings of the investigation of the positive results. If slaughter establishments experience a high event period, they should assess what happened during the slaughter and dressing process and take appropriate action that would ensure only unadulterated product is released into commerce. Studies have shown that *E. coli* O157:H7 is present in the hides and intestinal contents of cattle and therefore can contaminate the surface of the carcass, trimmings, ground beef, and other beef products (e.g., primals, sub-primals, and mechanically tenderized or enhanced beef) during slaughter, fabrication, grinding and processing.

The process of removing the hide and intestinal tract requires care, and even under good manufacturing practices, occasional contamination of the carcass meat will occur from direct contact of the hide to the carcass, contact of the hide to equipment, hand-to-hide-to-carcass contact, aerosolization when removing the hide, or puncture of the intestinal tract. Slaughter and dressing procedures should be designed to minimize, to the maximum extent practical, cross-contamination of carcasses with the contaminants from the hide and intestinal tract.

As FSIS stated in FSIS PHIS Directive 6410.1, Rev. 1, Verifying Sanitary Dressing and Process Control Procedures by Off-line Inspection Program Personnel in Slaughter Operations of Cattle of any Age, the Agency expects that establishments will slaughter and process cattle in a manner designed to prevent contamination from occurring at any step in the process and will use decontamination and antimicrobial interventions treatments as necessary to address any contamination that (a) may result from the implementation of the slaughter process or (b) otherwise occur on the carcasses.

If a slaughter establishment believes a HEP may be occurring, FSIS recommends that the processor review process control measures and intervention measures used during slaughter, dressing, fabrication, and grinding. Such controls may include measures to reduce the pathogen load on incoming animals, measures to ensure that contamination of the carcass does not occur during slaughter or dressing procedures, decontamination or antimicrobial treatments, and measures to minimize carcass-to-carcass contact and cross contamination. Ensuring and verifying that such controls are indeed working is crucial to preventing future HEPs.

The actions taken in response to an out-of-control signal could depend upon the findings of the investigation of the positive results. If the establishment finds the cause for the HEP and takes corrective action to prevent positive results from recurring, then an increase in the sampling rate would not be needed. However, the establishment needs to have a high degree of confidence that the corrective actions will be effective before reducing the intensity of its testing. Until such a high degree of confidence is obtained, FSIS recommends that the establishment conduct increased testing when it experiences a HEP. For example, the establishment could increase sampling rates by either defining smaller lots of trimmings (1 combo bin instead of 5 combo bins) or selecting additional samples from the 5 combo bin lots.

During systemic HEPs, FSIS recommends that primal and sub-primal cuts be sampled and tested, even if treated with an antimicrobial treatment. In addition, during systemic HEPs, FSIS recommends that establishments test food contact surfaces for the presence of *E. coli* O157:H7 (or STEC organisms or virulence markers). If they detect the pathogen, establishments should consider product that came into contact with those surfaces to be adulterated.

Note that these recommendations are not regulatory requirements. However, by taking these additional steps, establishments will be able to better ensure that they do not release adulterated product into commerce. Therefore, these additional steps may reduce the likelihood of costly recalls.
During local HEPs, FSIS recognizes that establishments may determine that less product may be affected or implicated by the positive results than in systemic HEPs. Establishments may not need to sample, test, or hold primals and sub-primals during local HEPs.

The prevalence of *E. coli* O157:H7 has been greater in cattle coming to slaughter during the warmer months (from spring into early autumn – the “high prevalence season”) than the colder months. Thus, HEPs should be especially anticipated during the high prevalence season. Extra steps should be implemented to increase confidence that contaminated product is not released into commerce for use in raw beef during the high prevalence season compared to the low prevalence season. Such steps could include more frequent monitoring and verification of both slaughter and dressing procedures, additional antimicrobial

reduction treatments, or sampling and testing additional product. FSIS also recommends increasing sampling and verification testing programs during the high prevalence season.

V. General Guidance for Verification Testing of *E. coli* O157:H7

Because microbial contamination is not visible to the naked eye, microbiological testing is needed to verify that the slaughter and dressing procedures that are designed to prevent microbial contamination are effective. Consequently, FSIS recommends that both slaughter establishments and receiving establishments test source product, including trimmings, for *E. coli* O157:H7 (or STEC organisms or virulence markers). FSIS recommends testing of finished product even if the source material has been tested and found negative. The reason for this recommendation is that negative test results on samples of product do not imply that product is free of *E. coli* O157:H7 cells for the following reasons: there may have been pockets of contamination in the product that were not in the actual sample tested at the slaughter establishment, the product might have become contaminated after it was sampled at the slaughter establishment, or the *E. coli* O157:H7 (or STEC organisms or virulence markers) cells within the actual sample tested might not have been detected at the slaughter establishment because their numbers at the time of testing were below the limit of detection. If a receiving establishment finds incoming product intended for grinding or other non-intact use positive for *E. coli* O157:H7 (or positive in a screening test but not confirmed negative), that product is adulterated, although it may be treated to eliminate the pathogen. The receiving establishment should inform the supplier of the positive test results.

It is useful to conduct verification testing for associated organisms that include *E. coli* O157:H7 (e.g., a screen methodology for pathogenic *E. coli*) and maintain records of results. Measurements of ubiquitous organisms such as *Enterobacteriaceae*, aerobic plate counts (APC),[5] or generic *E. coli* can be used to evaluate the effectiveness of process controls designed to limit or eliminate microbial contamination. Frequent measurement of APC counts may capture a short-term trend, which would be useful for quality control, both before and after the sanitary dressing processes. However, such measurements, while helpful for ensuring microbial process control, cannot be used as a substitute for determining the actual presence or absence of *E. coli* O157:H7 in the final product.

Section VI of this document includes examples of sampling plans. Defining a sampling plan involves establishing the procedures the establishment will use, including how it will collect a sample, the size of the units it will collect, the

[5] Measuring the level of APC on pre-eviscerated carcasses might be useful for evaluating the effectiveness of a sampling program and antimicrobial interventions (see T. A. Arthur, et al., 2004, J Food Protection 67(4): 958-665).

number of samples it will collect, the frequency with which it will collect a sample, and the procedure it will use to analyze the sample.

During the high prevalence season months (from spring into early autumn), the frequency of such testing should be increased compared to that of the other months in order to have increased confidence that contamination is not affecting the food safety system.

The decontamination and antimicrobial treatments applied during the slaughter and dressing operation should be designed to remove, to the maximum extent practical, contamination with pathogens. Each establishment should know the limits of capability of its slaughter and dressing operation for reducing microbial contamination as evidenced by objective data, such as for aerobic plate counts (APCs) and other indicator organisms of process control on the carcass immediately after hide removal, before washing, and other antimicrobial intervention treatments.

Sampling and testing of trimmings for *E. coli* O157:H7 (or STEC organisms or virulence markers) should occur at a frequency sufficient to find evidence of contamination surviving the slaughter and dressing operation. Optimally, every production lot should be sampled and tested before leaving the slaughter establishment and again before use at the receiver. Establishments that do not slaughter but produce trimmings should report their test results back to the slaughter supplier in order for the supplier to assess the adequacy of its slaughter and dressing practices, as well as antimicrobial treatment programs. Through this feedback, an investigation of the possible reasons for the contamination getting through the slaughter and dressing operation can be conducted and could lead to the identification and correction of possible deficiencies.

VI. Designing Sampling Plans for Verifying Control of *E. coli* O157:H7

Designing a sampling plan involves identifying many factors, including among others, the lot size and the amount of product from each lot that is to be sampled and analyzed. Perhaps the most important step in designing a sampling plan is the definition of a lot of product. The results (positive or negative for the presence of *E. coli* O157:H7, STEC organisms, or virulence markers) may determine the disposition of the product within the selected lot and possibly other product as well, depending on how the lots are defined.

Trimmings from each supplier should be tested separately. Limiting product in a lot to that from a single supplier could help decrease the extent of product that would be recalled or sent for cooking when a positive test result is obtained. Be sure to always define the production lot size before sampling. Do not redefine it during testing or after results are known.

Lots should be defined so that if a positive result is found from one lot, the product in other lots is microbiologically independent and is not implicated. FSIS has stated (FR Oct 7, 2002) that when one lot of trimmings tests positive, lots constructed from the same source material would likely be implicated. "FSIS would expect the establishment to have a scientific basis that justifies why any raw ground product produced from those source materials should not be considered to be adulterated" (p. 62333). One way to avoid the results for one lot implicating another is to ensure that the lots are microbiologically independent.

Note that the establishment is responsible for determining the lot of product represented by the collected product and should have a sound basis for defining the lot.

Suggestions for defining microbiologically independent lots are:

1. Product from different carcasses can be considered as independent lots provided the meat from the carcasses was handled so as not to cross-contaminate one another.

2. Defining lots based on microbiological testing would be acceptable if the sample collection and testing method is designed to have a high confidence of detecting positive results when *E. coli* O157:H7 is present in a production lot.

3. Processing interventions that limit or control *E. coli* O157:H7 contamination can help to define the lot.

4. Beef manufacturing trimmings and raw beef components or rework carried over from one production period to another may expand the implicated lot in the event of a positive result.

5. Sanitation Standard Operating Procedures (Sanitation SOPs) or any other prerequisite programs used to control the spread of *E. coli* O157:H7 cross-contamination among raw beef components during production can help to define the lot. Note that the following may lead to cross-contamination of raw beef components during production and may expand the implicated lot in the event of a positive result:
   - improper sanitary dressing procedures
   - insanitary product contact surfaces on equipment, such as machinery and employee hand tools
   - improper employee hygiene

Below are some defined terms used in the discussion of designing sampling plans below.

Lot size: The amount of product (pounds) within a lot.

Frame: The population of lots that is available for sampling.

Sample Design: The specified procedures used to select samples. This involves procedures for defining lots, determining which ones would be sampled, and collecting samples from the selected lots.

Lot Sample: The lots that are selected for sampling from the frame. FSIS is recommending generally that all lots be sampled.

Sample: The product collected from a lot that is to be tested for the presence of *E. coli* O157:H7 cells (or STEC organisms or virulence markers). Samples should be collected using a method that ensures the collected product statistically "represents" the product in the lot as best feasible.

Slices: The smallest unit of contiguous product collected as part of the sample. The selected slices are combined to form the sample. The resulting sample is sometimes referred to as a "composite sample." Collection methods are often designated by the number of slices that are selected and comprise a sample, e.g., N60 refers to a sample collection method with 60 slices.

Sub-samples: In the laboratory, a sample might be divided into parts, which are referred to as subsamples, wherein one or more are analyzed separately. FSIS recommends that all subsamples be analyzed.

Pooled samples: Combined aliquots of samples after they have been enriched.

Size: The weight or some other appropriate dimensional measurement; e.g., sample size refers to the weight of the sample; slice size refers to the surface area and thickness of the slice.

A sampling plan used to verify process controls should address the following:

1. Products to be tested;

2. Lot size (usually in pounds and number of combo bins);

3. Statistical sampling method for selecting lots; percentage of lots that are sampled (Lot sample);

4. Slice size (dimensions) and number of slices that comprise a sample;

5. Collection method for selecting samples and slices from a selected lot;

6. Procedures for preparing a sample for analysis;

7. (Sub) sample size analyzed in a laboratory;

8. Laboratory testing methods used (including sample size analyzed, enrichment procedures and size of portions analyzed);

9. Actions to take when samples are positive;

In designing a sampling plan, an establishment should consider the following questions:

A. What products are to be tested?

   Trimmings or other source materials that are supplied to grinders, including cheek meat and head meat (see FSIS Directive 10,010.1).

B. The size of the lot: what amount of product (i.e., the lot) is to be represented by a sample?

   The establishment should define how much product is going to be grouped together to constitute a "lot" (e.g., combo bins of trimmings; boxes of packaged headmeat or cheekmeat).

   Note: FSIS strongly recommends that the lot definition not be redefined. It is unacceptable to change the lot definition based on the results of testing.

C. How is sample going to be collected?

   1. *E. coli* O157:H7 (or STEC organisms or virulence markers), when present, is not evenly distributed throughout a production lot. Therefore, a collection method that selects product at multiple sites within the lot or multiple production intervals within a given lot is more likely to detect pockets of contamination than a sampling plan that samples at fewer sites or production intervals.

   2. For trimmings, potential contaminants will be on the exterior surface of the product that was exposed during the slaughter and dressing process. Therefore, collection methods that provide more surface area for the test increase the sensitivity of the sampling (i.e., collect thin slices of the exterior exposed fat and lean tissue).

   3. For trimmings, samples can be collected by:

- Obtaining 60 slices from exterior surface of product within the lot that are as thin as possible resulting in the desired sample size (grams) to be collected.

- "Plug" collection, where product is collected by inserting a specially designed "tube" between pieces of meat so as to excise the trim (exterior areas) of adjacent pieces. This procedure is performed many times, by inserting the tube at randomly selected locations, to ensure that a certain minimum number of exterior surface pieces are collected and achieving the proper weight for the sample.

- Randomly selecting slices of trimmings from trim in combo bins.

- Core drilling, where product is collected at several places in the combo bins by drilling a hole, approximately 25 mm in diameter, into the surface of meat through a template. The product is thus extracted through a coring tube, and can be taken from fresh or frozen trim.

- For frozen trimmings, using a sanitized band saw at 12 points around the edges of a 60 pound frozen block. To make up N60, five randomly selected frozen blocks would be collected similarly.

With all these collection methods, specifications should be designed to ensure that a high percentage of the collected product that is to be used for testing consists of exterior surface tissue.

D. How much of the collected product is analyzed in the laboratory?

1. FSIS recommends that the entire sample be analyzed. To accommodate laboratory testing methods that limit the amount of material per analysis, subsamples could be formed and each subsample analyzed in the laboratory. Thus, multiple analyses may be needed. Not analyzing the entire sample could lead to a significant increase in false negative results (negative results found when the product is actually positive) compared to when the entire amount is analyzed, so that results could be misleading. Laboratory Methods used should be effective in detecting the pathogen.

NOTE: A sampling plan using the N60 collection method and analyzing a 325-375 gram composite sample means that the weight of each of the 60 slices that is 'represented' in the tested material needs to be about 6.25 grams (375 grams/60 slices = 6.25 grams per slice).

E. How effective is the testing method?

1. FSIS recommends that the establishment understand and have written documentation regarding how the laboratory is testing the sample, in regard to the size of the sample analyzed and the analytical method that is used.

2. FSIS recommends that laboratory methods be “fit for purpose” and ensure detection of very low levels of *E. coli* O157:H7 (or STEC organisms or virulence markers) that may have survived lethality treatments. FSIS recommends that methods be approved or used by a recognized government or independent body (e.g., FSIS, FDA, AOAC, AFNOR, ISO.)

3. In some circumstances, multiple samples may be “pooled” after enrichment to save costs for testing. Because negative broths can dilute positive broths in the pooled test broth, “wet-pooling” analytical methods should ensure that sensitivity is not compromised. Wet-pooling refers to combining multiple samples for a single screening test after the samples have been enriched; i.e., incubated overnight in a broth as the first stage for detecting a pathogen.

4. It is important for testing laboratories to follow the testing protocol as written to ensure the method will perform as expected. This includes pre-warming the enrichment broth to the incubation temperature before incubation to help ensure the greatest sensitivity, particularly for methods using enrichment periods less than 15 hours.

5. In circumstances when a test result for pooled samples is positive, it may be appropriate to re-test the individual sample-specific enrichments, in an attempt to identify contaminated product more accurately. In such a procedure, it is important that the storage of the enrichments not cause a decrease in the sensitivity of the individual test as compared to the pooled test.

VII. Factors Affecting the Design of Sampling

Several factors can guide establishments in designing their sampling plans. Two of them are discussed here, including the percentage of positive samples in the product and degree of confidence desired for a given sample to test positive. A critical limiting factor is the maximum sample size that the laboratory can analyze. Given this maximum sample size, the sample is characterized by the number of slices and the slice size. Because the contaminant occurs on the

surface of the meat, slices should be as thin as possible. Because it is expected that *E. coli* O157:H7 cells (or STEC organisms or virulence markers) when present would be distributed unevenly in clumps, in constructing samples it is advisable to use many small sample slices rather than few larger slices (all slices should be of the same thickness). Using many small slices provides a more "representative" sample of the lot and greater likelihood of finding contamination. However, the limiting factor here is the time to collect many slices. At present, an N60 sample involves collecting 60 slices of a specified dimension. An N120 sample with slices ½ the surface area of those used for N60 would provide a greater likelihood of finding positive results, given everything else being equal; however, the time needed to collect an N120 sample might be twice as long as needed to collect an N60 sample. Over the years, the N60 sample has become the standard sample for beef trimmings. It is important to remember that changing the slice size of samples or the number of slices for a sample could have an impact on the expected percentage of positive findings.

A. Percentage of positive samples of *E. coli* O157:H7 (or STEC organisms or virulence markers) in the product

- The percentage of positive samples is determined as the number of positive samples for the pathogen divided by the total number of samples tested, multiplied by 100. The process percent positive is the expected percentage of positive samples over time.

- The distribution of cells of *E. coli* O157:H7 (or STEC organisms or virulence markers) will depend on the levels on the carcasses and effectiveness of the control measures used by the establishment during slaughter, dressing, and fabrication (e.g., sanitary dressing procedures, intervention treatments, temperature, and sanitation). An establishment that has verified that its control measures (e.g., organic acid spray wash or control of incoming materials) are effective in reducing contamination by the pathogen should have lower levels and incidence of *E. coli* O157:H7 (or STEC organisms or virulence markers) and thus, should have a lower process percent positive.

B. Degree of confidence desired for a given sample to test positive

- The percentage of contaminated slices within a contaminated lot might likely be small. Thus, large numbers of slices for a sample are needed to determine with high confidence that a sampled specified lot has *E. coli* O157:H7 (or STEC organisms or virulence markers) cells. Table 4 shows the number of slices that would need to be taken to have 95% confidence of detecting *E. coli* O157:H7 (or STEC organisms or virulence markers) in the sample

consisting of a random sample of n slices, assuming a specified true percentage of contaminated slices within the lot.

Table 4: Number of slices needed (second row) for 95% probability that contamination will be detected given the percentage of contaminated potential slices (first row). Calculations used to derive the number of slices given in the table assume that the 'sizes' of the slices are the same.

Percentage positive slices	0.5%	1%	1.7%	2.5%	5%	7.5%	10%	15%	23%
Number of slices needed	598	299	178	119	59	39	29	19	12

Table 4 shows that about 60 selected slices are needed to have a 95% confidence that contamination will be detected when the percentage of potential slices (available for selection) that are contaminated is equal to 5%. Selecting 12 slices only provides the same degree of confidence of finding a positive when the true percentage of contamination is about 23%.

The above table suggests that if sensitivity greater than that of N60 sampling is desired, more slices (i.e., more surface area) would be needed. Since each slice varies in thickness, and thus in weight, the entire N60 sample is portioned into a 325g analytical portion size, which is a requirement of the method. It is possible to obtain more sensitivity by taking larger samples. For example, two N60 samples per lot could be collected, for a total of 120 slices (of the same size). From Table 4, this would provide about 95% confidence of detecting contamination if 2.5% of the slices within the lot were contaminated. The costs of such sampling, however, could be double that of N60 sampling, assuming that all lots were to be tested, because the time to collect the samples could be doubled, and two samples rather than one sample would be analyzed. To help mitigate the latter cost, the wet-pooled procedure for testing could be used.

VIII. Examples of Sampling Plans

A collection method known as N60 (mentioned above) is often used for monitoring incidence of *E. coli* O157:H7 (or STEC organisms or virulence markers) in beef trim products manufactured by the industry. The '60' refers to the number of slices that are used in constructing the composite sample. The slices are collected randomly from the lot in order to help ensure a good 'representative' sample from the product within the lot.

The collection method may be as follows:

Lot size: 5 combo bins consisting of 2,000 pounds each, for a total of 10,000 pounds trim.

Number of slices: 60 slices of product sliced from the surface of the meat, 12 from each combo bin.

Slice size: each slice is about 6.25 grams and 1/8 inch thickness.

Sample size: 375 grams, composited from the 60 slices.

1. Take 12 slices of product, randomly selected from each combo bin, such that each consists of product of about 6.25 grams with thickness of no more than 1/8 inch, to help ensure that the sample will consist of as much surface area (where the *E. coli* O157:H7, STEC organisms, or virulence markers are more likely to reside) as feasible. As a guide, the dimensions of the sample can be about 3 inches in length and 1 inch in width.

2. If for some reason, there are less than 5 combo bins from which product is to be collected, a total of 60 surface slices from the available combo bins would still be taken. For example, if there are 2 combo bins to be used for grinding, 30 surface slices from each combo bin to make a total of 60 surface slices would be taken; if there were 3 combo bins, 20 slices, and so forth, would be taken.

3. Combine (composite) slices for every lot – the combined 60 slices is referred to as a composite sample.

4. Store the sample at temperatures between 7 and 10 ºC (44 - 50 °F), and send to the laboratory. The sample should be analyzed within 24 hours of collection.

5. At the laboratory, the sample must be mixed before selecting the material to be analyzed. It is important that an approximately equal amount of material from every slice be included in the material that is being analyzed.

6. At the laboratory, if necessary, create sub-samples to be analyzed separately (typically five 75-gram sub-samples), though some procedures allow for the whole 375 gram sample to be analyzed.

7. Incubate (enrich) each sub-sample to ensure adequate growth of any *E. coli* O157:H7 cells.

8. Analyze each sub-sample for the presence of *E. coli* O157:H7 – confirm as positive or negative all presumptive positive results for *E.*

coli O157:H7 or assume presumptive results are positive.

9. Investigate possible sources of the contamination, the process, and the controls that have been designed to prevent contamination if a result is positive.

10. Dispose of the positive lot and all other implicated product or send for full lethality.

The method of analysis should be of equal to or better sensitivity than that of the method that the FSIS laboratories use as cited in the Microbiological Laboratory Guidebook (MLG) (see: http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp).

Some Variations of N60:

The N60 collection method being used by most establishments involves 5 combo bins defining a lot. This method was designed to detect contamination slice-specific incidence at a within-lot contamination of 5%; lower percentages, averaged over the lot, would not so readily be detected. The results from Table 4 indicate a possible reason for this, namely the number of slices per combo bin (12 for N60) is too small to detect contaminated combo bins. Consequently, FSIS recommends establishments decrease the production lot size from 5 combo bins to 1 combo bin in order to provide greater assurance that contamination is detected within combo bins. That is, for the N60 method, as described above, for each combo bin there would be 60 surface slices collected. If the combo bin-specific test is positive, the product in the combo bin is sent for cooking; if negative, the product from the combo bin is sent for grinding, provided that there is no evidence that the process is out of control, based on the percent of positive results for neighboring lots that had been tested, or for any other reason known that could permit contamination not to be removed as effectively as normal.

Wet-Pooling of Samples

Using one combo bin as a lot may increase the cost of analysis. One way to help reduce the laboratory costs of analyses when testing each combo bin would be to enrich each N60 sample, and then pool aliquots of the individual enrichments from the five sampled combo bins. This means that the pooled aliquot represents five N60 samples. This sample method should ensure that a single positive sample pooled with multiple negative samples does not compromise the sensitivity of the testing method (the sensitivity of the test compared to the test used by FSIS).

Diagram 1: The variation of N60 sampling shown (below) is an example of a wet pooled sample. In this situation, if the laboratory pooled sample is positive, then the laboratory would separately analyze the 5 enriched samples (each representing an N60 sample from each of the combo bins) to ascertain which of the combo bins represented in the laboratory pooled sample likely contributed to the positive pooled aliquot sample result. If the enrichment step is done properly, at least one of the 5 enriched samples would be found positive. The establishment would divert the one combo bin represented by the positive sample to further processing to destroy the pathogen, such as cooking. In such a procedure, it is important that the storage of the enrichment samples does not cause a decrease in the sensitivity of the individual sample test as compared to the test on the pooled sample.

If none of the individually analyzed N60 enriched samples was found positive, then this might indicate a problem with the enrichment procedure or with the sample handling. In such a case, all product within the 5 combo bins, even though they individually tested negative, would need to be cooked or disposed of because the testing did not identify the positive bin.

Diagram 1: Variation of N60 Sampling

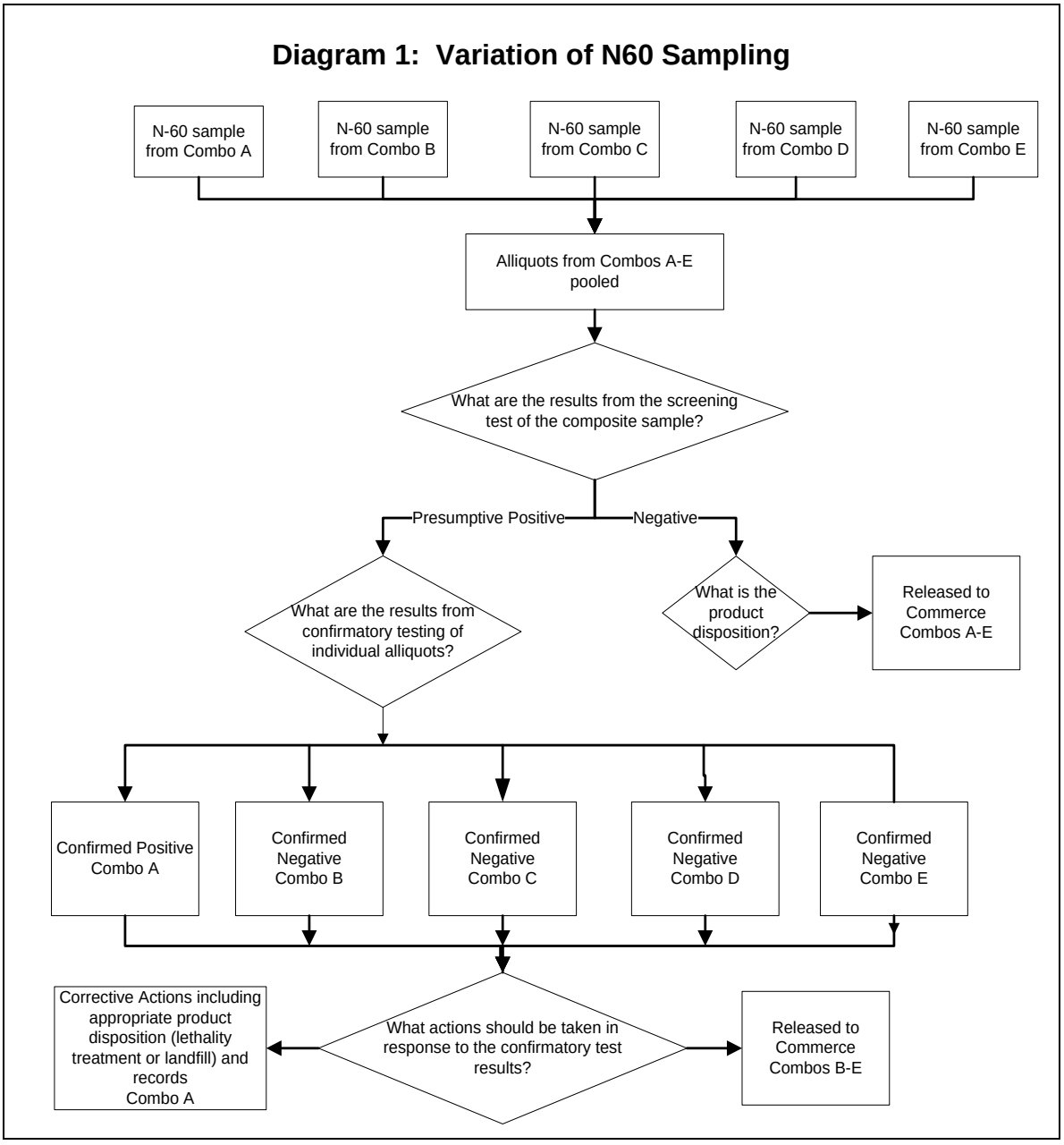

Diagram 2: Variation of N75 Sampling (below) depicts another variation of a *E. coli* O157:H7 testing program with wet pooling. In this example, a lot is defined to be 5 combo bins, with 15 portions from each of 5 combo bins enriched. Aliquots of the enrichment from 5 combo bins are pooled and tested for an initial screening test for *E. coli* O157:H7. If the screening test for the pooled composite sample is positive, then each of the individual N15 aliquots are tested with screening tests to determine which combo may be the source of contamination.