

**Compliance Guideline for Sampling Beef Trimmings for  
*Escherichia coli* O157:H7**

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**I. Introduction**

The purpose of this guidance document is to provide information about the design of sampling and testing programs for *Escherichia coli* O157:H7 (*E. coli* O157:H7) to manufacturers and users of boneless beef manufacturing trimmings and other raw ground beef components. For purposes of this document, all these raw beef source materials for use in making ground beef are collectively referred to as “trimmings.” This guidance document is intended to assist in the development of programs to assess the adequacy of process controls for *E. coli* O157:H7.

An effective sampling and testing program can provide considerable benefits to an establishment. If *E. coli* O157:H7 is found in trimmings that have left an establishment, there is likely to be considerable expense in removing the product from commerce. Consequently, sampling plans that provide high probabilities or high confidence of finding product containing *E. coli* O157:H7 are cost-effective, and the likelihood of finding *E. coli* O157:H7 is greater. Establishments should have sampling and testing programs designed to find *E. coli* O157:H7.

Sampling and careful evaluation of test results can lead to a reduction of *E. coli* O157:H7 contamination over time. For each positive result, there should be an investigation of its cause. Once a possible cause is identified, then appropriate action should be taken to make corrections and to eliminate the cause. Doing so will bring a steady decline in the percentage of positive results. Feedback of sampling and testing results to the supplier of the source materials should be provided as a matter of good manufacturing practice. Importantly, there should be an affirmative following-up on each and every positive test result with the supplier, whether you produced the trimmings or procured the source materials from another supplier. Without reporting, the sampling and testing program is merely a “test-and-divert” program. Test-and-divert programs will not prevent, eliminate, or reduce to a non-detectable level *E. coli* O157:H7 in raw beef.

In evaluating sampling and testing results, it is important to distinguish the occasional or sporadic positive from a series of positive results that indicate a systemic cause or breakdown of the process controls (“high event days”). This determination is important for decisions regarding the disposition of product and necessary corrective and preventive actions. To help make this determination, FSIS is providing guidance regarding statistical procedures that can be used to determine whether a process is either in or out of control. The approach that FSIS is recommending is based on the principles of Statistical Process Control (SPC).

***Principles of SPC Related to E. coli O157:H7 Control in Trimmings:***

- Because of the presence of *E. coli* O157:H7, the process of removing the hide and intestinal tract from cattle requires care, and even under good manufacturing practice, occasional contamination of the carcass will occur.

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- Slaughter and dressing procedures should be designed to minimize, to the maximum extent practical, cross-contamination of carcasses with the contents of the hide and intestinal tract contents.
- The decontamination and antimicrobial treatments applied during the slaughter and dressing operation should be designed to remove, to the maximum extent practical, contamination with *E. coli* O157:H7. Each establishment should know the limits of capability of its slaughter and dressing operation for reducing microbial contamination as evidenced by objective validation data such as for aerobic plate counts (APCs) and other indicator organisms of process control.
- Sampling and testing of trimmings for *E. coli* O157:H7 should occur at a frequency sufficient to find evidence of contamination surviving the capability of the slaughter and dressing operation. Optimally, every production lot should be sampled and tested before leaving the supplier and again before use at the receiver. Results of the testing program should be conveyed back to supplier in order for the supplier to assess the adequacy of its slaughter and dressing program and the sampling and testing program for trimmings. 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.
- Prevalence of *E. coli* O157:H7 is known to be higher in cattle coming to slaughter during the warmer months (April through October – the “high prevalence season”) than the colder months. Thus, event days should be anticipated during the high prevalence season and steps should be implemented to increase confidence that contaminated product is not released into commerce for use in raw beef. Such steps could include more frequent monitoring and verification procedures as part of both slaughter and dressing and sampling and testing programs. Additionally during event days, unless primal and sub-primal cuts are effectively treated with antimicrobials after trimming, these cuts and food contact surfaces should be assessed for the presence of *E. coli* O157:H7.
- Establishments should use their historical data, as part of SPC, to ascertain their process capability regarding the incidence of positive testing results over time. The incidence could be used to reflect the difference between sporadic positive results and multiple positive results associated with a systemic breakdown of the process (e.g., event days). If there is statistical evidence of an increase in the incidence, then the process should be considered as “out-of-control,” and there should be a more intensive (in-depth) investigation of the contributing cause, as compared to the follow-up investigation that occurs with the sporadic positive test result findings.. The finding of an “out-of-control” process for one production lot would likely implicate product in other production lots (i.e., a negative test result could be viewed by FSIS as a false negative). FSIS provides guidelines for this within this document.

In the recent years, the incidence of positives has not decreased as quickly as FSIS had hoped. This document is meant to help establishments define and implement sampling and testing programs that will lead to reductions of contaminated product reaching consumers.

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***Testing Results for Trimmings:***

FSIS recently conducted a nationwide baseline survey of trimmings and found 0.68% of the samples that it collected to be positive for *E. coli* O157:H7. The method of sampling used by FSIS is called N60, which involves collecting 60 thin exterior slices of trimmings likely exposed during the slaughter and dressing operations. The samples were composited into one sample and analyzed for the presence of *E. coli* O157:H7. However, some proportion of the test samples that FSIS collected were from production lots already pre-tested by establishments and found negative for *E. coli* O157:H7. Thus the percent positive rate found by FSIS is expected to be lower than that found by many establishments in pre-tested trimmings prior to releasing production lots for use in raw ground beef.

Since FSIS currently samples trimmings available for use in raw ground beef (i.e., from production lots of trimmings that cleared pre-shipment review), FSIS doesn't have actual data on the percent positive rate for pre-tested trimmings prior to release of the trimmings by the establishments. In the absence of such information, FSIS is, therefore, setting a percent positive guidance value of pre-tested trimmings at 1.5%. The 1.5% value is mid-way between the 1 to 2% positive rate that industry has anecdotally shared with FSIS as the annual average percent positive rate for pre-tested trimmings. The 1.5% positive rate for pre-tested trimmings is not a regulatory limit. FSIS is using this 1.5% value to identify a statistical framework for identifying when a process, during some period of time, is producing product such that the process is not adequate to control the occurrence of *E. coli* O157:H7<sup>1</sup>. During such event days, any negative test results might also be considered false negatives. The finding of too many positive test results should trigger the establishment to take intensified efforts to find a cause for the positive results and to ensure that adulterated product is not released for raw beef production. Event days are viewed by FSIS as potential evidence of production of product under insanitary conditions whereby all associated raw beef product may be adulterated, including primal and sub-primal cuts. Further details, regarding how this evaluation related to event days could be made are given within.

***Labeling Claims for Raw Beef Tested for E. coli O157:H7:***

This document does not discuss issues explicitly related to labeling of product as being tested negative for *E. coli* O157:H7. FSIS is developing guidance to labeling regarding testing. These are expected to be made available shortly after the release of this sampling guidance document.

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<sup>1</sup> For example, if 4 or more results are positive for *E. coli* O157:H7 within 91 N60 samples, then there is 95% confidence that the process was producing a percent positive rate not less than 1.5%, based on statistical assumptions that are commonly used (binomial distribution). Examples of QC sampling rules for determining whether the percentage of 1.5% is exceeded are provided in section VIII. FSIS recognizes that some establishments use laboratory screening methodologies that identify *E. coli* O157:H7 as one of several organisms that might be present in the sample without confirming that *E. coli* O157:H7 specifically is present. Such operations generally treat a screen positive sample as if it were confirmed positive for *E. coli* O157:H7. In such circumstances, the percent positive rate might be higher than the 1.5% positive rate value identified by FSIS. Thus, more than 4 positives might occur and not be potential evidence of event days. Such establishments are expected to have a rationale on file at the establishment to discern what the percent positive rate is for that establishment that distinguishes sporadic positives from high event days.

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

FSIS believes that contamination of beef carcasses with *E. coli* O157:H7 and other pathogens is reasonably likely to occur during the slaughter and dressing procedures. 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 during slaughter, fabrication, grinding and processing (e.g., primals, sub-primals, and mechanically tenderized or enhanced beef). Specifically, establishments are recommended to have in place procedures that are validated to reduce microbial contamination. There should at least be one slaughter intervention included as a critical control point (CCP) in the HACCP plan.

Since 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 testing of source material both at the point of production (e.g., fabrication of primals and sub-primals) and prior to use of the trimmings for use in the production of ground beef. In addition, as a further verification activity, 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, the product might have become contaminated after it was sampled, or the *E. coli* O157:H7 cells within the actual sample tested might not have been detected because their numbers at the time of testing were below the limit of detection.

FSIS recommends that establishments conduct verification testing directly for *E. coli* O157:H7. However, it also is acceptable to conduct verification testing for associated organisms that include this pathogen (e.g., a screen methodology for *pathogenic E. coli*) and maintain records of results as a quality control (QC) activity. Measurements of ubiquitous organisms such as aerobic plate counts (APC)<sup>2</sup> or generic *E. coli* can be used to evaluate the effectiveness of process controls designed to limit or eliminate microbial contamination. 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.

This document includes examples of sampling plans. Defining a sampling plan involves establishing the procedures the establishment will use, including how it will take a sample, the size of the units it will collect, the number of samples it will collect, the frequency with which it will sample, and the procedure it will use to analyze the sample.

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<sup>2</sup> Measuring the level of APC on pre-eviscerated carcasses can serve as an indication of the efficacy of the slaughter and dressing procedures, including the antimicrobial interventions. APC levels less than 4-log<sub>10</sub> versus greater than 4-log<sub>10</sub> on pre-eviscerated carcasses have been shown to be a good indication that the carcasses are more likely to be contaminated with *E. coli* O157:H7 than not being contaminated with the pathogen (see T. A. Arthur, et al., 2004, J Food Protection 67(4): 958-665).

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Trimnings 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. During high prevalence season months (from April through October), the frequency of such testing should be increased compared to that of the other months (e.g., if testing of incoming trimnings is conducted quarterly for each supplier, increase the frequency to at least once monthly). Be sure to always define the production lot size before sampling. Do not redefine it during testing or after test results are known. Record the results obtained from the testing over time in order to monitor the process, as part of its SPC-QC program (see Section VIII for recommendations).

A sampling plan together with criteria for signaling an out-of-control process is referred to as a Quality Control (QC) sampling plan. For a QC sampling plan, the number of samples before an out-of-control signal is called the “run length.” A typical operating characteristic measure of the QC sampling plan is based on the distribution of the run lengths that would arise under specified operating conditions (e.g., an assumed steady state percentage of positive samples); in particular, the average run length (ARL) is used to characterize the QC sampling plan. For a process in control, the ARL should be relatively large. For a process clearly not in control, ARL should be small. More details regarding the design of QC sampling plans are given in the last section of this document.

### **III. Frequency of Sampling for Small and Very Small Establishments**

FSIS recognizes that extensive, high frequency, sampling might be cost prohibitive for small and very small establishments. FSIS considers it very important, however, that small and very small plants test product on an on-going basis. To assist small and very small plants in designing an appropriate sampling plan, the Agency offers the following minimum sampling frequencies as guidelines for small and very small establishments for testing trimnings or finished ground product. It is important to note that FSIS recommends that establishments producing finished ground beef product from trimnings purchased from other establishments should have control procedures in place to know details about the design of the sampling and testing program of their suppliers for the trimnings. The minimum frequencies recommended below assume that all lots of purchased source trimnings have been tested. If the trimnings have not been tested (e.g., in-house trimnings), sampling frequencies for finished ground beef should be much higher than those given below. FSIS recommends that any product that is released should have been subjected to sampling and testing at least once.

1. More than 250,000 pounds produced daily—sample more than once per month (>12 times annually);
2. More than 50,000 pounds but less than or equal 250,000 pounds daily—sample at least once every month (12 times annually);
3. More than 1,000 pounds but less than or equal 50,000 pounds daily—sample at least once every 2<sup>nd</sup> month (6 times annually);
4. Less than or equal 1,000 pounds daily—sample at least once every 3<sup>rd</sup> month (4 times annually)

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FSIS recommends that the sampling rates referenced above be adjusted to take into consideration seasonality. In warmer months, from about April through October, FSIS has found higher percentages of samples with *E. coli* O157:H7 than in the other months. Hence, it recommends that during the higher prevalence season months (from April through October), the frequency of testing be increased (e. g. by a factor of 2) over the frequency an establishment uses in months with lower expected prevalence.

**NOTE:** Establishments, especially small and very small establishments, can seek guidance from Extension Service specialists that are within the state that the establishment is located on how to design sampling plans using this guidance document, or recommendations for laboratories that can test beef samples for *E. coli* O157:H7. Extension Services may be found in universities.

Since FSIS expects to test each establishment that produces trimmings and ground beef at least once a quarter, establishments can take the FSIS verification test results into account in documenting that their food safety systems are operating properly. Because of the sporadic and low level contamination associated with *E. coli* O157:H7, FSIS recommends that all establishments, those that produce trimmings as well as those that grind, conduct additional ongoing verification testing for *E. coli* O157:H7.

#### **IV. 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. It is lots that are sampled, and the results (positive or negative for the presence of *E. coli* O157:H7) determine the disposition of the product within the tested lot and possibly other product as well, depending on how the lots are defined. For sampling purposes, lots should be defined so that if a positive result is found on one lot, the product in other lots is not implicated. FSIS has stated (FR Oct 7, 2002) that when one lot 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 independent.

Suggestions for defining independent lots are:

1. Product from different carcasses can be considered as independent lots provided the meat from the carcasses from each lot was handled so as to not cross-contaminate one another. This includes having assurances that the carcasses were not co-mingled.
2. Defining lots by supplier would be acceptable if the product from one supplier could not have cross-contaminated the product from the other. For example, following the grinding of product from one supplier, the lines and equipment are sanitized before the product from the next supplier is processed.

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Before it is possible to discuss the design of a sampling plan, a few terms must be defined.

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

Sample: A sample consists of selected product from a lot that is to be tested for the presence of *E. coli* O157:H7 cells.

Portions: Often the sample is a composite of smaller portions of product that are selected throughout the lot. The selected portions are combined to form the sample. The resulting sample is called a “composite sample” but often is just referred to as the sample. Sampling plans are often designated by the number of portions that are selected and comprise a sample, e.g., N60 refers to a sampling plan with 60 portions.

Subsamples: In the laboratory, the materials of the sample might be divided into subsamples that are analyzed separately.

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

1. products to be tested
2. size of the lot (amount of product being sampled)
3. size of portions that are selected from the product
4. number of portions that comprise a composite sample that is tested in the laboratory
5. number of samples to be collected per lot (typically 1)
6. use of an acceptable aseptic sampling procedure
7. the amount of product that would actually be tested in the laboratory
8. testing methods used
9. actions to take when samples are positive
10. frequency of sampling

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

- A. What products are to be tested?
  1. Trimmings that are supplied to grinders, including cheek meat and head meat (see Directive 10,010.1).
  2. For grinding establishments, source material (e.g. trimmings) and final ground product
    - Note: testing both types of product is more effective than testing only one type alone. FSIS recommends that any product released should have been tested at least once throughout its production cycle.
- B. What amount of product (i.e., the lot) is to be represented by the sample?
  1. The establishment should define how much product is going to be grouped together to constitute a “lot” (e.g., combo bins of



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trimmings; boxes of packaged ground beef in a pallet load; ground beef produced within specified time (15 minutes (m), 20 m, 1 hour (h))).

2. The establishments should consider creating lots that consist of product from one supplier produced in one shift. Lots formed in this way are at significantly lower risk of being implicated in verification testing than lots that consist of product ground from several suppliers or in several shifts.
3. FSIS strongly recommends that the lot definition not be re-defined based on the results of testing (see F.7. below).

C. How is product going to be sampled?

1. *E. coli* O157:H7, when present, is not evenly distributed throughout a production lot. Therefore, a sampling plan that samples product at different sites within the lot (e.g., combo bins, pallets) or different production times is more likely to detect pockets of contamination than a sampling plan that samples at less sites or production times.
2. For trimmings, potential contaminants will most likely be on the surface of the product. Therefore, sampling 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. Some establishments freeze combo bins of trimmings before being scheduled for grinding. Freezing and thawing may kill or injure some *E. coli* O157:H7 and reduce the sensitivity of testing.
  - If product is sampled in a non-frozen state, it should be maintained under refrigeration (7 °C – 10°C) until testing is conducted.
  - If product is sampled in a frozen state, it should be maintained in a frozen state until it is prepared for testing by the laboratory. If it is not so maintained, the test results might not be valid.
  - Samples should be analyzed as soon as possible (i.e., ideally on the same day as collected or within 24 hours of collection).
4. For ground beef, portions can consist of product taken from several packages of finished ground product and combined to form a sample, representing the lot, which is sent to the laboratory selected by the establishment for testing; or
5. Portions can also consist of small amounts of ground beef product collected periodically during grinding of a lot. Examples:
  - A lot consisting of about 1 hour of production and 13 portions of at least 25-grams, each are collected about every 5 minutes, and combined into a sample of 325 grams for testing by the laboratory.

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- A lot of about 1 hour of production and 4 portions of about 95 grams are collected every ¼ hour and combined into a sample of about 375 grams for testing by the laboratory.
6. For trimmings, samples can be collected by:
- Slice sampling, where a number of exterior surface portions of a certain thickness from representative combo bins or packages combined and composited for testing (e.g., An “N60” sampling plan implies that 60 surface samples have been collected from different combo bins of product)
  - “Plug” sampling, where samples are collected by inserting a specially designed “tube” between pieces of meat so as to excise the trim (surface areas) of adjacent pieces. This procedure is performed many times, by inserting the tube at randomly selected locations within the combo bin, to ensure that a certain minimum number of exterior surface pieces are sampled.
  - Picking pieces of trimmings, randomly, from trim in combo bins
  - Core sampling collected from trim at several places in the combo bins. Core samples can be taken from fresh or frozen trim.
  - From frozen trimmings, samples are collected by using a sanitized band saw from 12 points around the edges of a 60 pound frozen block. To make up N60, five randomly selected frozen blocks would be sampled similarly.

With all these sampling procedures, specifications should be provided designed to ensure that a high percentage of the sample that is to be used for testing consists of exterior surface tissue.

**D. How much of the sample 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, sub-samples are formed and analyzed in the laboratory. Thus, to analyze the entire sample, multiple analyses may be needed. Not analyzing the entire sample could lead to a significant increase in false negative rates compared to when the entire sample is analyzed, so that sampling results could be misleading. It is possible that the probability of finding contaminated product would decrease in proportion to the proportion of the sample analyzed.
2. To evaluate a sampling plan, the establishment should consider how much of the sample is represented in the tested material. For example: An N60 sampling plan involving analysis of a 375-gram composite sample means that the weight of each of the 60 portions that is ‘represented’ in the tested material needs to be about 6.25 grams (375 grams/60 portions = 6.25 grams per portion).

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- If a larger than 25-gram composite sample for testing is to be used, FSIS recommends that there be validation that demonstrates that the analysis maintains the same degree of sensitivity with respect to the colony forming units (CFU) per test portion (i.e., not CFU/gram) compared to that that is obtained by typical analysis that uses 25-grams (otherwise, the theoretical benefit of the larger test portion may be negated).

E. How effective is the testing method?

1. FSIS recommends that the establishment understand and have documentation regarding how the laboratory is testing the product, both in terms of tested sample portions and the methods that are used.
  - This guidance document should be shared with the laboratory to ensure it understands the testing needs.
2. FSIS recommends that laboratory methods be properly validated to ensure detection of very low levels of potentially “sublethally” injured *E. coli* O157:H7 that may be present. Particularly, FSIS recommends that
  - Given the current state of testing technology, methods be used to provide high sensitivity.
  - Methods be validated by a recognized government or independent body (e.g., FSIS, FDA, AOAC, AFNOR, ISO, etc.)
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, there is a limit to the number of samples that can be pooled before sensitivity is reduced.
  - FSIS recommends that the probability of detecting a positive for a sample not be reduced by pooling with other, possibly negative, samples.
4. As the size of the enrichment sample increases, it becomes increasingly important to pre-warm the enrichment broth to the incubation temperature prior to incubation to help ensure the greatest sensitivity.
5. Some enrichment-based methods have been found to be effective with a reduced ratio of broth to test portion (i.e., to make the sample more manageable in the laboratory). Although the typical sample dilution is 1:10, methods may be effective at reduced volumes (1:3, 1:5 etc).
6. In circumstances when a pooled sample is positive, it may be appropriate to re-test the individual enrichment samples, in an attempt to more accurately identify contaminated product. In such a procedure, it is important that the storage of the enrichment

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samples not cause a decrease in the sensitivity of the test as compared to the test on the pooled sample.

- F. What actions should an establishment take when there are positive results?
1. If an establishment finds product positive for *E. coli* O157:H7, it needs to assess the significance of the finding.
    - a. If the establishment addressed *E. coli* O157:H7 in its HACCP plan, it would need to assess whether:
      - i) The positive was a random occurrence, or whether there was a loss of control in its HACCP system;
      - ii) If there was a loss of control, the establishment needs to take corrective action to bring the critical control point (CCP) that was involved under control;
      - iii) If there was a loss of control, the establishments would need to take measures to prevent recurrence; and
      - iv) However the positive occurred, the establishment needs to ensure that no product that is injurious to health or otherwise adulterated enters commerce.
    - b. If the establishment did not address *E. coli* O157:H7 in its HACCP plan, and the establishment finds that the positive for *E. coli* O157:H7 was more than a random occurrence, the establishment needs to:
      - i) segregate and hold the affected product;
      - ii) take action to ensure that no product that is injurious to health or otherwise adulterated enters commerce; and
      - iv) evaluate the process to determine whether *E. coli* O157:H7 should be addressed in the HACCP plan.
    - c. The regulations require that corrective actions be documented in records subject to HACCP verification requirements (9 CFR 417.4(a)(2)(iii)) and HACCP recordkeeping requirements (9 CFR 417.5).
  2. A positive result implicates not only the lot from which the positive sample came but also other lots that might have common source material with that of the positive lot.
  3. All the product in the lot is implicated and must be disposed of as outlined in FSIS Directive 10010.1 “Microbiological Testing Program and Other Verification Activities For *Escherichia Coli* O157:H7 In Raw Ground Beef Products And Raw Ground Beef Components And Beef Patty Components.”
  4. The establishment should review its purchase specifications, cleaning and sanitizing procedures, process control procedures, lot size determination, and sampling plans to determine if these need to be improved or tightened in order to reduce or eliminate positive products.

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5. The establishment may increase the sampling frequency or increase the sample size, until the source of the problem can be found.
6. Establishment may find it useful to record the producer or supplier of source materials that test positive. This type of record may be useful in establishing a pattern of positive results associated with a particular supplier.
7. FSIS strongly discourages establishments from defining sub-lots that the establishment would want to argue could be considered to be a lot, and thus released, if the overall original lot tested positive but the portions from that “sub-lot” test negative in further testing (see E.6 above). To do this would create a relatively high probability that contaminated product would be released.
  - In light of a positive result, to counter the presumption of contamination of a given sub-lot, a much larger number of portions than originally tested from that sub-lot would need to be tested and found negative.
  - FSIS recommends that before such a procedure is used, the establishment collect and analyze data in order to demonstrate that conditions exist for which such a procedure would provide a decrease of the probability of contaminated product leaving the establishment.
8. The establishment should define specific Quality Control rules to aid it in evaluating the effectiveness of its process controls (see sections VI for further discussion) and determine the extent of product disposition when there is a positive result.

**V. Factors Affecting the Design of Sampling Plans**

There are several factors that can guide establishments in making decisions in designing their sampling plans.

- A. Level and percentage of positive samples of *E. coli* O157:H7 in the product
  - The percentage of positive samples is expressed as a percentage, determined as the number of positive samples for the pathogen per the total number of samples tested, multiplied by 100. The expected value of this percentage in this document is called “the percent positive rate.” Based on FSIS sampling covering recent years, (2005-2007), FSIS recommends that establishments use a percent positive rate of not more than 1.5% for trim beef and not more than 0.20% for ground beef.
  - The distribution of cells of *E. coli* O157:H7 will depend on the levels on the carcasses and effectiveness of the control measures used by the establishment during slaughter, dressing, fabrication and grinding (e.g. intervention treatments, temperature, sanitation). An establishment that has verified that its control measures (e.g., its organic acid spray wash, its control of incoming materials, or its sanitation program during grinding) are effective in reducing contamination by the pathogen should have lower

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levels and incidence of *E. coli* O157:H7, and thus, FSIS recommends, should use a lower percent positive rate.

- If the process produces product with a relative high frequency of *E. coli* O157:H7 positive results then, besides improving process controls, it would be prudent for the producer to decrease the number of combo bins or packages per lot, increase the number of samples tested per lot, or increase the number of analyses per sample, in order to minimize the likelihood of product containing *E. coli* O157:H7 cells being released.
- Seasonality: FSIS has found that there is an increase in the percentage of positive tests for *E. coli* O157:H7 during the warmer months (April to October). Without effective process control, higher temperatures and higher humidity can lead to more growth and thus result in a greater likelihood of the pathogen being on the product. Therefore, FSIS recommends that establishments increase their sampling and verification testing during these months unless they have established that they have effective process control.

**B. Number of suppliers of source materials**

- Placing product from a single supplier in one lot will facilitate tracking of that product and will make it easier to catalogue the interventions used during slaughter and fabrication. If products from several suppliers are placed in one lot, tracking products will be hampered significantly regarding discriminating the most likely source of the contaminant.

**C. Degree of confidence desired**

- The percentage of portions of product that will test positive is likely to be small. Thus, a large number of portions of product would needed to be tested to determine with high confidence that a specified lot has a low incidence of *E. coli* O157:H7 cells. The following table shows the number of portions that would need to be taken to have 95% confidence of detecting *E. coli* O157:H7 in the composite sample consisting of a random sample of n portions, assuming a specified true percentage of portions within the lot.

Table 1: Number of portions constituting a composite sample needed to have at least 95% confidence of finding a positive, assuming a specified percentage of positive portions within the lot. Calculations used to derive the number of portions given in the table assume the possibility of false negatives is zero.

Percentage positive portions	0.5%	1%	2%	5%	7.5%	10%	15%	23%
Number of portions needed	598	299	149	59	39	29	19	12

- Because it is expected that *E. coli* O157:H7 cells when present would be distributed unevenly in clumps, in constructing composite samples it is advisable to use many small sample portions rather than few larger portions.

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Table 1 shows that about 60 selected portions are needed to have a 95% confidence that contamination will be detected when the percentage of potential portions (that could have been selected) that are contaminated is equal to 5%. Selecting 12 sub-sample portions (such as would happen if the N60 sample is collected from each of 5 combo bins) only provides the same degree of confidence of finding a positive when the true percentage of contamination is about 23%. FSIS strongly recommends that establishments collect the N60 sample from one combo bin rather than from across multiple combo bins.

## **VI. Product Disposition When There is a Positive Result**

When a sample is positive for *E. coli* O157:H7, the product within the lot and all other lots that are not independent from the positive lot are deemed to be adulterated. In accordance with FSIS Directive 10,010.1 “Microbiological Testing Program And Other Verification Activities For *Escherichia Coli* O157:H7 In Raw Ground Beef Products And Raw Ground Beef Components And Beef Patty Components,” the implicated product must be cooked before it leaves the establishment and be sold, be moved off-site for proper disposition under appropriate controls, which may include being moved under seal, or be destroyed.

It is critical that a full investigation be made of the process surrounding the positive result. In summary thus:

- a. If the process can be considered to be in-control (the positive result is just a sporadic occurrence, and there is no systematic cause of it) based the on-going (historical) QC statistical evaluation of the test results and on an investigation of the process as a result of the positive result, then lots, even with product produced from meat from common carcasses with those used for the positive lot, can be released without further testing.
- b. However, if this determination cannot confidently be made, then FSIS strongly recommends further testing of product from lots that are not independent or that were produced on the same day or shift before the product can be released.
- c. If the process is found to be out-of -control either because of too many positive results with too few samples, or because the investigation reveals that there is a systemic cause that can cause contamination throughout the day or shift, the product for that day or shift should not be released but rather disposed of according to FSIS Directive 10,010.1.

## **VII. Examples of Sampling Plans**

### **Example of a Robust Sampling Plan: The N60 Method for Beef Manufacturing Trimmings**

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A sampling plan called N60 is often used for monitoring incidence of *E. coli* O157:H7 in beef trim products manufactured by the industry. The '60' refers to the number of portions that are used in constructing the composite sample. The portion size is small and not burdensome to collect. The portions are collected randomly from strata that partition the lot in order to help ensure a good 'representative' sample from the product within the lot.

The sampling plan is as follows:

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

Sample size: a sample consisting of 60 pieces of product sliced from the surface of the meat, 12 from each combo bin

Portion size: each portion consists of a slice from the surface of the meat of about 6.25 grams and 1/8 inch thickness

Test sample size: 375 grams, composited from the 60 slices or portions.

1. Take 12 portions of product, randomly selected from each combo bin, such that each portion consists of slices of product of more than 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 are more likely to reside) as feasible. As a guide, the dimensions of the sample can be about 4 inches in length and 2 inches in width.
2. If, for some reason, there are less than 5 combo bins from which samples are to be taken, 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) portions for every lot – the combined 60 portions is referred to as a composite sample.
4. Store the sample at about 7 to 10 °C (44 to 50 °F), and send to the laboratory. Samples should be analyzed within 24 hours after collection.
5. At the laboratory the sample must be mixed before selecting the material to be analyzed. It is important that an approximate equal amount of material from every portion be included in the material that is being analyzed.
6. At the laboratory, if necessary, create subsamples to be analyzed (typically 5 75-gram subsamples), though some procedures allow for the whole 375 gram sample to be analyzed.
7. Incubate each subsample to ensure adequate growth of any *E coli* O157:H7 cells.
8. Analyze each subsample for the presence of *E coli* O157:H7 - Confirm all presumptive positive results for *E coli* O157:H7.
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 lot and all other implicated product, in accordance with FSIS Directive 10010.1.
11. If more 4 or more positive results occur within 91 samples (see section VIII, Table 2), then the process should be considered to be out-of-control, and a



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thorough investigation should be made to identify and prevent causes for the positive. Sampling should be intensified so that instead of 12 slices per combo bin, 60 slices per lot would be selected and a composite sample formed from these 60 slices.

The method of analysis should be of equal or better sensitivity than that of the method that the FSIS laboratories use in its recently completed beef trim baseline survey, (See: <http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/18-07.pdf>)

Some variations of N60:

A. The N60 sampling plan is being used by most establishments, where 5 combo bins constitute a lot. This plan was designed to detect contamination incidence of 5%; lower rates, averaged over the lot would not so readily be detected. In particular, N60 was not designed to detect contamination in individual combo bins. The results from Table 1 indicate a possible reason for this, namely the number of samples 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. That is, for the N60 sampling plan, as described above, for each combo bin there would be 60 surface samples collected. If the combo bin-specific sample 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.

Using one combo bin as a lot may increase the cost of analysis. One way to help reduce the costs of analyses when testing each combo bin over the present costs associated with 5 combo bins per lot, the N60 samples from each combo bin would be enriched individually at the laboratory, and aliquots of individual enrichments from five N60-sampled combo bins can be pooled for analysis. Care would need to be taken to ensure that the enrichment procedure (time and temperature of incubation) is adequate so as to not decrease the sensitivity of the test compared to the test used by FSIS. However, FSIS believes that the testing of pooled samples from 5 individual enriched samples can be made without losing sensitivity<sup>3</sup>. In this situation, if the laboratory pooled sample is positive, then the laboratory would separately analyze the 5 enriched samples that were used to constitute the pooled sample, to ascertain which of the combo bins represented in the laboratory pooled sample likely contributed to the positive pooled sample result. If the enrichment is done properly, at least one of the 5 enriched samples would be found positive. If none of the individually analyzed N60 enriched samples were found positive, then this might indicate a problem with the enrichment procedure or with the sample handling. However, in such a case, all products within the 5 combo bins, even though individually tested negative, would need to be diverted and re-cooked in order to be released. The above procedure would provide a significantly greater likelihood of finding contaminated product, even though still, negative results would not imply that tested product is not contaminated.

B. Increasing the scope and sensitivity of testing. The N60 sampling and testing procedure was designed to detect *E coli* O157:H7 cells. However, a variation of the

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<sup>3</sup> Study is needed to ascertain this fact.

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testing procedure described above has been developed that is more sensitive and thus more protective of the public health. Basically, a lot is defined to be 1 combo bin, with about 60 samples from each combo bin enriched. Aliquots of the enrichment from 5 combo bins are pooled and tested for an initial reactive result for *E. coli* O157:H7. However, product is not released based on a negative result on this screening test. Rather the importance of the screening test is determining the nature of tests for the individual samples. Whether the screening test for the pooled enrichment sample is positive or negative, individually enriched samples from each combo bin are further tested (in terms of identifying more signals indicative of a pathogenic *E. coli* strain) using immunomagnetic bead assay or multiplex PCR assays. Release or disposition of the combo bin is dependent on the result of the further confirmation testing of each individual enrichment sample.

Following are examples of sampling plans for small and very small establishments:

Example 1

A small establishment that receives combo bins of trim for grinding has purchase specifications for suppliers that include having intervention procedures for eliminating *E. coli* O17:H7 cells and verification testing methods that includes a N60 testing program for each trim beef lot. The suppliers assure (presents evidence) the processor that their process controls are effective and the percentage of contaminated product is low. Since it is receiving trim from several suppliers, it separates the trim from each supplier into one lot. In addition to its purchase specification as control, the establishment has decided to test both the incoming trim and the finished ground beef product using the following sampling plan:

Product to be tested: beef trim and finished ground beef product

Size of the lot: For trim, up-to 5 combo bins from each supplier;

For ground beef: product ground from each lot of trim

Samples and portions to be collected and material tested:

Trim combo bins – 12 surface portions (1/8 inch thick) per combo bin for a total of 60 samples composited into 1 sample or 375 grams to be tested (N60 method).

Finished ground beef – grab portions every 15-30 minutes of processing (depending on size of lot) combined into 1 composite sample to be tested (375 grams per composite sample).

These are both robust sampling plans.

Frequency of sampling: twice a month.

Testing method: The establishment sends the samples to a laboratory for testing which uses a testing method equivalent to the FSIS testing method.

Evaluation: The processor should keep track of the results from the twice a month testing. Because the rate of positive is assumed low, there should not be more than 1 positive result within 1 year (24 samples, see Table 1 and Table 2, below). After a positive result, the supplier for which the positive sample is associated with should be informed of the positive result, and the supplier should investigate and take corrective action if necessary. If 2 positive results occur within 24 samples of product from the

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same supplier, the processor may wish to discuss the results of its investigation with the supplier and discuss actions to prevent contamination in the future.

Example 2

A very small establishment frequently processes a trim product from a supplier (the only one it receives product from) into ground beef. This establishment also has a purchase specification for the supplier of the trim to have an intervention method effective in controlling *E. coli* O157:H7 and verified by continuous testing implying that all trim product has been tested using N60. Because the test results from the supplier show very low incidence of positive findings, the establishment has decided not to test the trim before grinding but rather test the finished product.

Product to be tested: finished ground beef product

Size of the lot: product ground from the supplier's trim product.

Samples and portions to be collected and material tested:

The establishment determines that the supplier's trim product can be processed in 2 hours. Subsamples of ground product portions of about 65-75 grams are taken every 24 minutes of grinding. The five consecutive portions (representing 2 h of production) are combined into 1 composite sample to be tested (at least 325 grams to reflect the FSIS method; the beef industry method typically uses at least 375 grams per composite sample).

Frequency of sampling: once a month.

Testing method: The establishment sends the samples to a laboratory for testing which uses a testing method equivalent to the FSIS testing method.

Evaluation: The processor should keep track of the test results over time. Because the rate of positive is assumed low, there should not be more than 1 positive result within two years (see Table 1 and Table 2, below). If 2 or more positive results within a year did occur, then the processor would be warranted to request documentation from the supplier regarding the results of its investigation and identification of possible causes of contamination, and actions to prevent contamination in the future.

## **VIII. Process Control (PC)**

As mentioned in section I, PC sampling is performed in order to evaluate the process controls over time, to help ensure that they are working properly. This section discusses possible criteria for judging that a process is out of control, based on the frequency of positive results over a series of consecutive samples or shifts.

In the following two sections, the statistic of concern is the sample-specific incidence of *E. coli* O157:H7. A sample is classified as positive and is called a 'positive sample', when any of the subsamples for the sample tested positive. The percent positive rate then is the expected or mean value of the percentage of positive samples.

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The occurrence of a single positive sample for *E. coli* O17:H7 calls for an investigation regarding the reason for that positive sample and possible corrective action to help prevent the recurrence of positive samples. However, the occurrence of two or more positive samples (in ‘too few’ samples, as defined as part of the PC sampling plan specifications) could indicate more systemic control problems so that the process could be considered as out-of-control. FSIS is aware of cases where many positive results occur within a day or a shift. Cross contamination can spread *E. coli* O157:H7 bacteria throughout the product, which in turn would cause many consecutive samples to be positive. Such events should necessarily be construed as a process control failure.

Consequently, when one of these events occur, FSIS recommends more in depth investigation be instituted with respect to sources of material and processing, and the sensitivity of the sampling plan be increased. To help ensure that contaminated product does not be leave the establishment, the establishment should consider more intensive sampling, for example defining lots to be 1 combo bin instead of 5 combo bins for the N60 sampling plan.

In deriving the operating characteristics given in the following tables, it is assumed that the samples are randomly selected from a large population of possible samples and that past results could be used to make inferences on product that would be produced in the future.

It is further assumed that the sampling plan will not find all contaminated product. Therefore, if the incoming product has a high percent positive rate (i.e., low or poor incoming quality), it is possible that the finished product would have a relatively high percent positive rate as well (low outgoing quality). A process in control would have a good incoming quality (low percent positive rate). The QC sampling plan thus is designed to evaluate the incoming quality to verify that it is good (as could be expected). If it is determined that the incoming quality is poor so that the process would be considered as being out-of-control, then, to increase the confidence of detecting a high percentage of contaminated product and thus to reduce percent positive rate of the outgoing product, the lot size should be decreased (smaller number of combo bins or packages per lot for each sample), or the sample size increased.

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 positive and takes corrective action to prevent the positive 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.

Important: PC percent positive rates used to assess control are affected by the sampling plan used. If under one sampling plan less material is sampled or analyzed than under another plan, given everything else being equal, a lower percent positive rate would be expected for the former plan and thus a lower PC percent positive rate should be set.

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As stated above, FSIS believes that establishments should be concerned if their sampling of trimmings produce a positive rate of 1.5% or greater. For ground beef, based on FSIS sampling covering recent years, (2005-2007), FSIS recommends that establishments should be concerned by a percent positive rate in plant testing that is greater than 0.2%.

These rates are considered as upper bounds of what would be considered acceptable. Thus, for an establishment using the FSIS sampling plan and analytical procedures, FSIS recommends that modest statistical evidence (95% confidence over a series of samples) suggesting that the rate is not being met should be considered as a (presumptive) signal that the establishment's process is out of control, and, as a result, the establishment should investigate whether its process is out of control.

FSIS believes that processes that are consistently showing percent positive rates above the percent positive rates mentioned above are not in control and are processes that can be improved (assuming that the establishment is using the FSIS sampling and measurement procedures). However, the establishment-specific process percent positive rate could be different than the FSIS rate (assuming that the sampling plan and analyses are described as above). Consequently, a specified percent positive rate for a given establishment should be identified and justified if other than that stated by FSIS if past results indicate that a different percent positive rate was being achieved consistently and product has low likelihood of being adulterated.

Deviations from previously obtained percent positive rates 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. SPC, using a rate based on process capability, implies, in particular, that the rate an establishment uses to assess its control during the traditional high prevalence season (April – October) should be no higher than the rate of the traditional low prevalence season (November – March).

Percent positive rates of *E. coli* O157:H7 in the product:

- FSIS recommends that establishments monitor the percentage of positive findings. If the observed percentage suggests that the process percent positive rate is higher than expected, then the processor should review process control measures and intervention measures used during slaughter, dressing, fabrication, and grinding.
- The following table can be used to help gauge whether the process percent positive rate might be higher than expected.

Table 2: Number of samples (entries in table) in which, if at least the corresponding number of positive results were obtained, there would be at least a 95% confidence that the true rate is greater than percent given in column heading.

Number positive	percent positive rate							
	0.10%	0.20%	0.50%	0.68%	0.75%	1.0%	1.5%	2.0%
2	355	178	71	52	47	35	24	18
3	818	409	164	120	109	82	55	41
4	1367	683	274	201	182	137	91	69

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5	1971	986	395	290	263	198	132	99
6	2614	1307	523	385	349	262	175	131
7	3286	1644	658	484	439	329	220	165

For example, if 4 or more positive results occurred within 91 samples, based on the assumption given above, there would be at least a 95% confidence that the true process percent positive rate exceeded 1.5%; if 4 or more positive results occurred within 201 samples, there would be at least 95% confidence the true process percent positive rate exceeded 0.68%.

The implication of the results in the above table is that, multiple positive results within a day or shift indicates that during that period of processing there existed some systematic cause of the positive results, so that all product the day or shift would be suspect regarding contamination. For example, if an establishment tested 100 lots in a day and obtained 4 or more positive results, then the other lots, even if independent of the positive lots, should be further tested to ensure that there is not some unknown systematic factor, such as cross contamination between lots, that would cause contamination for the product within the negatively tested lots.