

Sampling Requirements to Demonstrate Process Control in Slaughter Operations

Objectives

To demonstrate mastery of pathogen reduction the trainee will:

1. Explain why generic *E. coli* sampling and analysis is performed in livestock slaughter operations.
2. Explain why microbiological sampling and analysis is performed in poultry slaughter (other than ratite) operations.
3. Identify who is responsible for selecting and analyzing livestock samples for generic *E. coli*.
4. Identify who is responsible for selecting raw poultry samples for microbiological analysis.
5. Explain the purpose of performance criteria and statistical process control.
6. Verify the regulatory requirements for generic *E. coli* testing by conducting the Generic *E. coli* verification task.
7. Verify the regulatory requirements for microbiological sampling and analysis of raw poultry by conducting the appropriate PHIS verification task.
8. Identify appropriate enforcement actions to take when noncompliance is found while performing the Generic *E. coli* verification task.

Introduction

Under the Pathogen Reduction; Hazard Analysis and Critical Control Points (HACCP) System – Final Rule (Fed Reg Docket No. 93-016F; 1996) requirements were set in place for livestock and poultry slaughter establishments to test for generic *Escherichia coli* (*E. coli*) to verify their process controls according to 9 CFR 310.25(a) and 381.94(a), respectively.

On August 21, 2014, FSIS published the Modernization of Poultry Slaughter Inspection – Final Rule. This rule requires all poultry slaughter establishments (except those that slaughter ratites) to develop, implement, and maintain written procedures to prevent contamination of carcasses and parts by enteric pathogens (e.g., *Salmonella* and *Campylobacter*) and fecal material throughout the entire slaughter and dressing operation (9 CFR 381.65(g)). At a minimum, these procedures must include sampling and analysis for microorganisms. Each establishment must incorporate their written procedures, including their microbiological sampling plans into the HACCP system, e.g., HACCP plan, or Sanitation SOP, or other prerequisite program. The rule also requires each establishment to maintained daily records to document the implementation and monitoring of their procedures.

With implementation of the Modernization of Poultry Slaughter Inspection rule, FSIS rescinded 9 CFR 381.94(a) that required poultry establishments to test carcasses (removes reference for all poultry species, except ratites) for generic *E. coli* to monitor control of the slaughter process. The generic *E. coli* testing requirements were replaced by the new testing requirements described in 9CFR 381.65. The Agency also removed the codified *Salmonella* pathogen reduction performance standards for poultry as per 381.94(b). FSIS will use new performance standards for *Salmonella* and *Campylobacter* established in 2011 to effectively manage these pathogens that will be discussed later in this training.

In summary, establishments that slaughter livestock and ratites will continue testing samples for generic E. coli as an indicator for process control in accordance to 9 CFR 310.25(a). In contrast, those establishments that slaughter poultry (other than ratites) are required to meet the new regulatory requirements, as per 9 CFR 381.65, to demonstrate the effectiveness of their process control procedures.

Generic *E.coli* Testing for Livestock and Ratite Slaughter Operations

Each official establishment that slaughters livestock or ratites is required to test for *Escherichia coli* Biotype I or generic *E. coli*. An establishment employee selects the samples for generic *E. coli* testing. The purpose of generic *E. coli* testing is to verify the effectiveness of sanitation and process control in slaughter establishments. FSIS verifies that the establishment meets the regulatory requirements for generic *E. coli* testing.

Fecal contamination is one of the principal sources of pathogenic organisms that contaminate livestock carcasses. *Escherichia coli*, Biotype I, also called generic *E. coli*, is an indicator of fecal contamination because it is common in the intestinal tract of food animals. The intestinal tract is also the primary pathway for contamination of meat and poultry with pathogens such as *E. coli* O157:H7, *Salmonella*, and *Campylobacter*. Ongoing *E. coli* testing by livestock and ratite slaughter establishments helps them determine whether the slaughter process is under control or whether carcasses are being contaminated with feces. In other words, generic *E. coli* testing is a process control indicator for fecal contamination.

Sections 310.25(a) of the meat regulations and 381.94(a) of the poultry regulations addresses the regulatory requirements that establishments need to meet for generic *E. coli* testing. Slaughtered livestock that will not receive the FSIS mark of inspection (such as custom exempt livestock) are exempt from generic *E. coli* testing.

Performance Criteria

FSIS has developed performance criteria for livestock using the excision sampling technique. Generic *E. coli* performance criteria are not enforceable regulatory standards. Performance criteria are numbers published in the regulations that represent the highest expected microbial loads on carcasses when the slaughter process is under control. They give livestock slaughter establishments guidance about the effectiveness of their slaughter process in preventing fecal contamination. Test results that meet the criteria in the regulations provide evidence that the establishment is maintaining adequate process control for fecal contamination and sanitary dressing.

Furthermore, the generic *E. coli* baseline results (statistical process control criteria) published in the Federal Register Notice on February 17, 2005 (Docket Number 02-046N), using the sponging sampling technique, can serve as a valuable support to establishments that slaughter cattle and swine in assessing the effectiveness of their process, using their own test results.

NOTE: Establishments must use statistical process control to evaluate their test results when they slaughter species or use sampling techniques for which the Agency has not developed performance criteria.

Inspection Program Personnel (IPP) Responsibilities

FSIS responsibilities are outlined in FSIS PHIS Directive 5000.1. The IPP is responsible for understanding and properly performing the Generic *E. coli* verification task in the Public Health Information System (PHIS) as described in this Directive. The Generic *E. coli* verification task addresses the regulatory requirements 9 CFR 310.25(a) or 381.94(a) the establishment must meet when developing the written generic *E. coli* testing procedure.

IPP are to perform the Generic *E. coli* verification task on a **routine** basis (priority scale level 6) at the frequency specified in the establishment's task list. IPP are also to initiate a **directed** Generic *E. coli* verification task if they observe noncompliance with the generic *E. coli* testing requirements while performing other tasks or when instructed to do so by supervision or other policy issuances.

Generic *E. coli* Testing Verification

Establishments that slaughter livestock or ratites must develop a written sampling procedure that identifies the employees designated to collect samples, the locations of sampling, how randomness is achieved, and measures to ensure sample integrity as described in 9 CFR 310.25(a)(2)(i) and 381.94(a)(2)(i), respectively.

IPP verify that establishment meets the applicable regulatory requirements for generic *E. coli* testing by reviewing the establishment's written sampling procedure, observing the designated establishment's employee executing the written sample procedures and reviewing the establishment's records. IPP are to document the results of their tasks in PHIS, including any noncompliance, according to the instructions described in FSIS Directive 5000.1.

E. coli testing requirements are met if the establishment successfully executes the activities addressed in its written procedure, analyzes samples, and keeps records of test results. An *E. coli* Testing Summary Chart (Attachment 1 of this module) is provided as a reference for the species tested, testing frequencies, sample locations, sample sites, and sampling methods allowed by regulation. It is a quick and easy inspection aid when conducting the Generic *E. coli* verification task.

IPP must understand what each section of the regulation means in order to conduct the Generic *E. coli* verification task. The IPP addresses the requirements in 9 CFR 310.25(a) and 381.94(a) as follows:

1. Sample collection – livestock or ratite samples (paragraph (a)(1) of section 310.25 and 381.94)

E. coli testing must be done in establishments that slaughter any market class of cattle, swine, sheep, goats, horses, mules, equines, or ratites.

If a combination of types of livestock is slaughtered, the establishment samples only from the species it slaughters in the largest number. It is only necessary to sample one type of livestock to determine whether sanitary dressing controls are effective. *E. coli* tests measure the effectiveness of the process regardless of which species is slaughtered. This means, for example, if an establishment slaughters both swine and sheep, but mostly swine, they should be testing swine for generic *E. coli*.

NOTE: IPP are to judge which type of livestock is slaughtered in the greatest numbers based on historical slaughter numbers (e.g. the previous year's totals) unless the establishment can project that the majority type of animal will be different because of a change in operations.

Workshop: Generic *E. coli* Testing

From the species below, select those that are covered by the generic *E. coli* testing regulations (§310.25 and §381.94).

- | | |
|----------------|-----------------|
| _____ Cattle | _____ Ostriches |
| _____ Chickens | _____ Rabbits |
| _____ Ducks | _____ Rheas |
| _____ Emus | _____ Sheep |
| _____ Geese | _____ Squab |
| _____ Goats | _____ Swine |
| _____ Guineas | _____ Turkeys |
| _____ Horses | |
| _____ Mules | |

2. Sampling requirements – location and technique (paragraph (a)(1)(i) and paragraph (a)(2)(ii) of section 310.25 and 381.94)

The IPP should remember the following things when considering the sample location and technique.

- The location refers to the place within the establishment where the sample is collected.
- Livestock samples are collected after they have been in the cooler for a minimum of 12 hours. There is no maximum time limit. Carcasses can be selected while on the rail or after the final wash and set aside in a convenient spot in the cooler for testing after cooling. It is acceptable to select random samples before carcasses enter the cooler.
- Ratite samples are collected at the end of the chiller or drip line or at the last readily accessible point prior to packing or cut-up.
- Hot-boning operation samples are taken after the final wash prior to boning.

The sampling site refers to places on the carcass where samples are collected. There are two sampling methods an establishment may use to collect generic *E. coli* samples.

- Excision
- Sponging

Excision sampling is aseptically cutting a surface section from the livestock carcass and sending the tissue sample for laboratory analysis. Excising tissue from a carcass is, of course, a destructive method of sampling.

Sponging is aseptically swabbing the surface of the livestock carcass or ratite carcass with a sterile sponge and sending the sponge to the laboratory for analysis. Sponging is a nondestructive method of sampling.

The chart below provides an easy reference for species and the sampling methods allowed.

Excision	Sponge
Beef Swine	Beef Swine Equine Goats Sheep Ratites

Notice that beef and swine carcasses may be sampled by the excision or sponging method.

Samples must be taken from specific sites on livestock carcasses. The three sites from which excision samples on cattle or sponge samples on cattle, sheep, goat, and equine carcasses must be taken are the:

- Flank
- Brisket
- Rump

In the case of hide-on carcasses for the above species, the sponge samples must be taken from:

- Inside the flank
- Inside the brisket
- Inside the rump

NOTE: No excision samples are taken from hide-on carcasses.

For swine carcasses, three excision or sponge samples must be taken from the:

- Belly
- Ham
- Jowls

When the ratite carcass is sponge-tested (which is the usual method), samples must be taken from the:

- Back
- Thigh

Workshop: Carcass Sampling Sites

In the left column of species, enter the matching letter for the regulatory sample sites listed in the right column.

_____ Cattle	A. Flank, brisket, rump
_____ Goats	B. Belly, ham, jowls
_____ Hide on calves	C. Back and thigh
_____ Hide on Sheep	D. Inside flank, brisket, rump
_____ Horses	
_____ Swine	
_____ Ratites	

3. Sample requirements – frequency (paragraph (a)(1)(i) and paragraph(a)(2)(iii), (a)(2)(iv), or (a)(2)(v)) of section 310.25 and 381.94

For *E. coli* testing purposes, livestock and ratite slaughter establishments are divided into two categories: very low volume establishments (VLV) and greater than very low volume establishments (>VLV). The categories of establishments are based on the establishment's annual slaughter volume.

Very low volume establishments are described as follows (paragraph (a)(2)(v)):

- Cattle, goats, sheep, horses, or other equine: Annually slaughter fewer than 6,000 head
- Swine: Annually slaughter fewer than 20,000 swine
- Livestock combination: Annually slaughter fewer than a combination of 6,000 cattle, plus sheep, goats, horses, or equines that equal no more than 20,000 animals total
- Ratites: Annually slaughter fewer than 6,000

Very low volume establishments begin sampling the first full week they operate after June 1st. They continue collecting at least one sample per week in each week they operate until 13 samples are completed. The series of 13 tests must show process control before the series can be ended. If the 13th test indicates

that the sanitary dressing process is out of control, the establishment must continue to test until process control is regained.

The 13 samples should not be collected in one day or even one week. Sampling over a period of time provides a better indication of the process control of the establishment than taking all samples at once.

Seasonal VLV operations must complete all *E. coli* testing during whichever months it operates. For example, a seasonal goat slaughter establishment that operates from September through December must begin testing during its first full week of operations and complete 13 tests before operations end in December.

When a VLV establishment that has completed 13 tests for the year makes changes like remodeling, new equipment, new employees, or new procedures that affect how well the process control measures works, weekly testing must be resumed until another series of 13 tests can establish the effectiveness of the changed process. If FSIS determines there have been changes that affect the process control measures, the information must be provided to the company in writing. The establishment would then be required to resume *E. coli* testing to judge the process control.

Establishments slaughtering more than the numbers indicated above for VLV establishments are classified as greater than very low volume establishments (paragraph (a)(2)(iii)).

Greater than very low volume establishments use the following frequencies for testing.

Cattle, sheep, goats, horses, or equines	1 test per 300 carcasses
Swine	1 test per 1,000 carcasses
Ratites	1 test per 3,000 carcasses

Greater than very low volume establishments must sample at the above frequencies or a minimum of at least once per week, whichever is greater. For example, an establishment that slaughters 9,000 cattle per year must sample once per week (a total of 52 samples per year), not only 30 samples per year as indicated by the 1 test per 300 carcasses frequency (30 samples for 300 carcasses = 9,000 carcasses).

Slaughter volume does not always match frequency rates in the regulations. Establishments should account for extra slaughter volume. This can be done by conducting additional tests. For example, a swine slaughter establishment that slaughters 1,500 swine per day should test at least once a day at the 1,000 carcasses per test frequency. However, the remaining 500 carcasses should also be accounted for to monitor process control. To account for the extra slaughter

volume, the establishment could “carry over” the 500 extra carcasses to the next day’s volume and conduct two (2) *E. coli* tests on the second day.

Livestock and ratite establishments may substitute an alternative testing frequency for the one in the regulations by including *E. coli* testing in their HACCP plan (paragraph (a)(2)(iv)). The alternative frequency must be part of the establishment’s verification procedures for its HACCP plan. It may not change the regulatory performance criteria or the limits determined by statistical process control.

4. Sample requirements – random selection of carcasses (paragraph (a)(1)(i), (a)(2)(i), and/or (a)(2)(ii) of section 310.25 and 391.94

For generic *E. coli* testing the regulations require that livestock and ratite carcasses for sampling be selected at random (paragraph (a)(2)(i)). Different methods, like random number tables, computer-generated random numbers, or drawing cards, may be used. Whatever the establishment chooses to use must be written into the *E. coli* sampling procedure.

The random method selected by the establishment and written into its plan must be followed. The designated establishment employee must be familiar with the written random sampling method.

In cattle, each half-carcass represents one unit eligible for sampling. Both the “leading” and “trailing” sides of a carcass should have an equal chance of being selected within the designated time frame. In other livestock species, each whole carcass represents one unit eligible for sampling.

If more than one shift is operating at the establishment, the sample can be taken from either shift, provided the sample selection time is based on the appropriate sampling frequency.

5. Sample analysis – paragraphs (a)(1)(ii) and (a)(3) of section 310.25 and 381.94

Some establishments conduct their own analyses. FSIS assumes establishments following the "Guidelines for *E. coli* Testing for Process Control Verification in Cattle and Swine Slaughter Establishments" and the “Guidelines for *E. coli* Testing for Process Control Verification in Poultry Slaughter Establishments”, respectively, will conduct their sampling in a manner that does not jeopardize the integrity of the sample or the reliability of the test results. Because these guidelines are not regulatory requirements, the establishment may choose to use a comparable sampling technique and be in compliance.

Establishment laboratory employees might have a copy of the Association of Official Analytical Chemists (AOAC) procedures or articles from peer-reviewed scientific journals that describe their procedure.

IPP are to review the establishment's written programs and records to verify that the laboratory analyzes the samples using an AOAC Official Method or another method that meets the criteria in paragraph (a)(3) of 9 CFR 310.25 or 381.94. IPP are to determine whether the establishment has documentation to demonstrate that the laboratory method meets these criteria. When in doubt about whether a testing procedure is acceptable, IPP should go through the supervisory chain-of-command to the District Office for assistance.

6. Records of test results – paragraph (a)(1)(iii) and (a)(4) of section 310.25 and 381.94

Establishments are **required to keep a table or a chart of the results for at least the most recent 13 test results**. IPP should consider the length of operations. In cases where the establishment has not been operating long enough to have 13 test results, there is not noncompliance for a lack of testing.

Generic *E. coli* tests are reported as a quantity or bacterial concentration. Bacterial concentration can be reported using either the Colony Forming Unit (CFU) or the Most Probable Number (MPN) based laboratory methods of analysis to evaluate the generic *E. coli* testing. These methods provide an estimate of the number of unit viable cells per sample, and are acceptable as valid measurements for bacterial limits. It is important to understand that these methodologies (laboratory procedures for sample analysis) are different and should **not** be used interchangeably.

An establishment using the “**m**” and “**M**” criteria must record each test result in terms of colony forming units per square centimeter (CFU/cm²) for excision and in colony forming units per milliliter (CFU/ml) for whole-bird rinses. Alternatively, an establishment using **statistical process control (SPC)** method may record results as CFU/cm² or MPN/cm² (sponge samples), and CFU/ml or MPN/ml (rinsate). IPP should match the units of measure with the testing technique used to ensure that results are reported correctly. They are to verify that the establishment records the results on a process control chart or table that shows at least the most recent 13 test results.

Establishments must keep records of the tables and charts with generic *E. coli* test results for 12 months. Establishments are not required to maintain a file of laboratory reports received from either an in-house laboratory or an outside laboratory.

7. Criteria for evaluation of test results – paragraph (a)(5)(i) and (a)(5)(ii) of section 310.25 and 381.94

IPP should refer to the generic *E. coli* testing regulations. If the Agency does not have performance criteria published for the species being sampled or for the sampling technique being used, the establishment must use **statistical process control** values to document generic *E. coli* test results (paragraph (a)(5)(ii)).

Livestock baseline studies conducted to arrive at the performance criteria published in the regulations were performed on cattle and swine only, using excision testing. Therefore, when the sponge method is selected for sampling any species, the performance criteria do not apply. For example, if a livestock slaughter establishment uses sponge sampling, statistical process control must be used to evaluate generic *E. coli* test results, not the m/M criteria.

Establishments that slaughter ratites must use statistical process control. There are no m/M criteria available for ratites.

Using Statistical Process Control (SPC) to Evaluation Test Results

SPC for generic *E. coli* is required with products that were not represented by the PR/HACCP Rule by a performance standard, because no relevant baseline studies were available at the time. As mentioned earlier, the generic *E. coli* results published in the Federal Register Notice (2005) can complement SPC by providing establishments with an additional measure of process control. The results below are for cattle and swine carcasses sampled using the sponge method of sample collection.

Class of product	Method	80th percentile	98th percentile
Cattle carcasses	sponge	0.0 CFU/cm ²	3.1 CFU/cm ²
Swine carcasses	sponge	0.46 FU/cm ²	400 CFU/cm ²

SPC provides a powerful mechanism for establishments to monitor and interpret the data collected for ongoing HACCP verification. SPC can provide establishments with an early warning that their process may not be functioning as designed. This warning can allow establishments to take corrective actions or make other process modifications to bring their process back into control without actually failing the desired performance.

SPC, used when the regulations do not cite performance criteria, begins when the establishment conducts a series of preliminary generic *E. coli* tests during its own slaughter operations. They chart the results in cfu/cm² or cfu/ml to determine the typical range of generic *E. coli* counts found at their establishment under

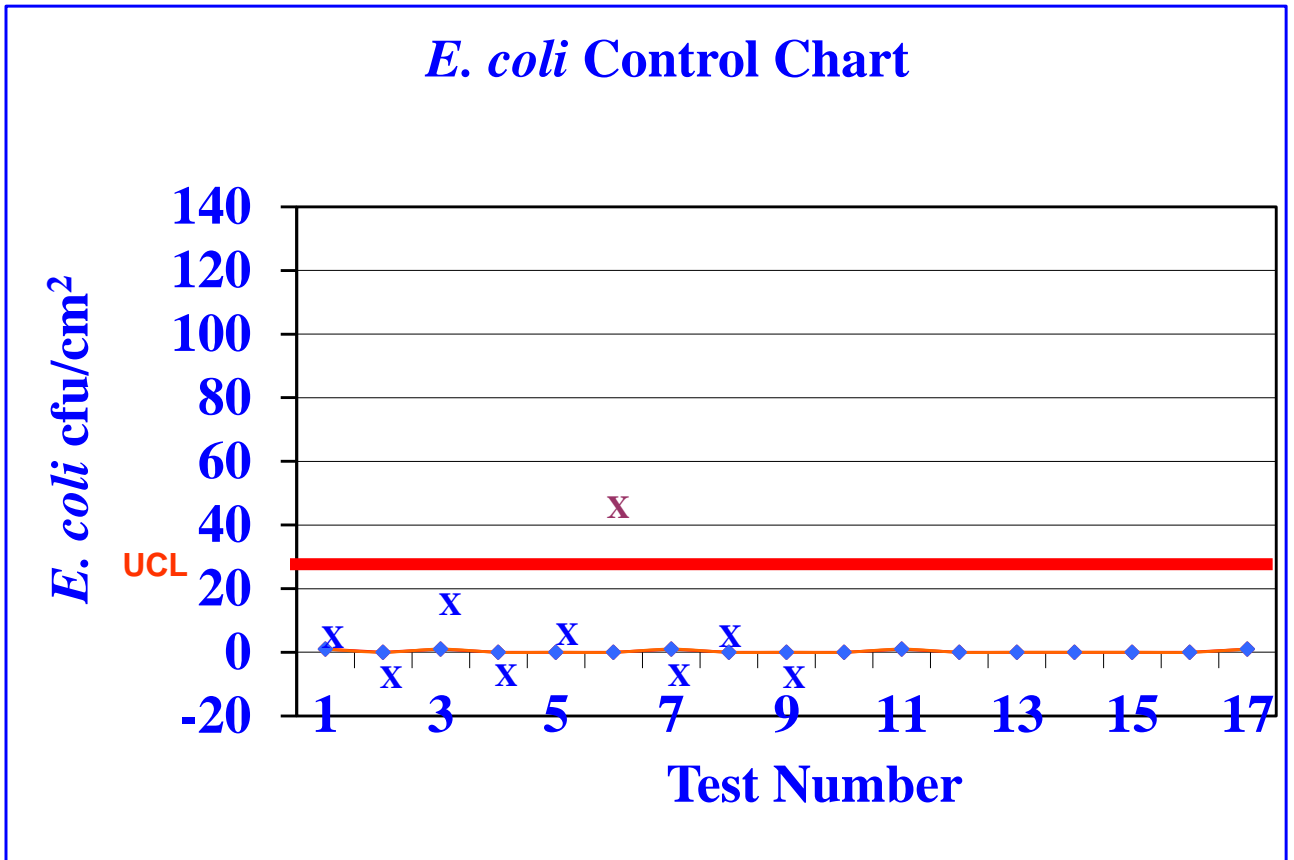
normal circumstances. After a company collects test results long enough to believe they have a true picture of their performance, they set an upper and lower control limit based on test results. There are no regulatory requirements for how statistical process controls are determined. Companies may use a variety of valid methods to determine limits for statistical process control. For example, establishments may calculate their own statistics, hire a consultant company, or use a software package to develop statistical process control values. Once the establishment determines the process control values and has set generic *E. coli* criteria to define process control, and as long as the data points on the company chart stay within the control limits set by the company, the process is considered in control.

An example of a method a company may use to develop a SPC program is as follows. The establishment:

- Conducts a series of preliminary generic *E. coli* tests during operations
- Charts the results in cfu/cm²
- Collects test results long enough to have a true picture of its performance (about 30 days usually).
- Determines the typical range of generic *E. coli* counts found normally
- Sets upper and lower control limits based on test results

IPP are to verify that the establishment is evaluating the test results using statistical process control techniques. In this context, IPP are to verify that an establishment that uses statistical process control has assessed the historical —normal performance of the slaughter process when it was in control and developed criteria that will indicate when the process may not be in control. IPP are to verify that the establishment uses generic *E. coli* testing results to identify times when the slaughter process is trending toward a loss of control and takes necessary actions to reestablish control. IPP **are not** to focus on the particular method the establishment uses to set process control criteria. Instead, they are to review the generic *E. coli* testing results and verify that the establishment has set generic *E. coli* criteria to define process control and responds to results outside those criteria.

The following example of a SPC chart plots test results in terms of test number, along the horizontal X-axis, against cfu/cm² on the Y-axis. This livestock slaughter establishment set a centerline value for its process control, which indicates the center point of the acceptable range of test results. The upper control limit (UCL) line marks the highest test result value considered acceptable by the company. The test result shown at test number 6 is above the upper control limit. The company recognized that this result was probably due to a variation in its process that needed to be identified, eliminated, and prevented from recurring. According to the chart, the establishment measures were effective because the following test result was back in the acceptable range.



Using Performance Criteria (m/M Values) to Evaluate Test Results

Cattle and swine establishments that choose excision of three sites must use the m/M performance criteria published in the regulations for evaluating test results when they are available. Regulatory m/M criteria apply only to swine and cattle sampling when the excision sampling technique is used. When performance criteria are published in the regulations, the *E. coli* test results are compared to the regulatory criteria and may fall into one of three categories: acceptable, marginal (represented by “m”), and unacceptable (represented by “M”).

- Marginal results (“m”) are those that fall within the worst 20% of overall industry performance in terms of *E. coli* counts (results taken from baseline study). More than three marginal results in the last 13 tests are unacceptable.
- Results in the worst 2% of overall industry performance (results taken from the baseline study) are called the maximum or “M” value. Any single test result exceeding “M” is unacceptable.

The m/M values taken from the regulations are applied to a moving window of the last 13-documented test results. That means that the establishment considers all of the last 13 test results when determining if the process is in control. Every time a new test result is added to their records, the oldest test is dropped and the new test becomes one of the most recent 13 results.

For the slaughter process to be judged in control no more than three sample results can be above the “m” marginal line. If four sample results are above “m”, the process is out of control.

If the test result of the most recent sample is above “M”, the process is automatically out of control, regardless of the previous test results. Once another test result is entered in the chart or table, the “M” test simply becomes another result considered to be above the “m” line. It no longer carries the consequence of causing “automatic” process control failure.

After the slaughter process is judged to be out of control, a subsequent test result below the “m” line indicates that the establishment did something to correct a problem and bring the process back into control (this correction does not have to be documented anywhere). However, the process is not judged totally in control until the window of 13 tests also shows process control.

The following table from the regulations shows the m/M values for *E. coli* performance criteria set by the Agency.

Species	Lower limit of marginal range (m)	Upper limit of marginal range (M)	Number of sample tested (n)	Maximum # permitted in marginal range (c)
Cattle	Negative	100 CFU/cm ²	13	3
Swine	10 CFU/cm ²	10,000CFU/cm ²	13	3

The previous table establishes performance criteria only for excision testing of cattle and swine.

An example of how to use the table is to consider a cattle slaughter establishment that uses the excision sampling method. An *E. coli* test result is:

- Acceptable if it comes back negative
- Marginal if the test result is positive but not above 100 cfu/cm²
- Unacceptable if it is above 100 cfu/cm²

The following table is an example of one method that may be used by establishments for record keeping.

Cattle Excision Test Results

Test Num.	Date	Test Result (cfu/cm ²)	Result unacceptable?	Result marginal?	Number marginal or unacceptable in last 13	Pass/Fail?
1	10-07	10	No	Yes	1	Pass
2	10-07	Negative	No	No	1	Pass
3	10-08	50	No	Yes	2	Pass
4	10-08	Negative	No	No	2	Pass
5	10-09	Negative	No	No	2	Pass
6	10-09	Negative	No	No	2	Pass
7	10-10	80	No	Yes	3	Pass
8	10-10	Negative	No	No	3	Pass
9	10-11	Negative	No	No	3	Pass
10	10-11	Negative	No	No	3	Pass
11	10-14	50	No	Yes	4	Fail
12	10-14	Negative	No	No	4	Fail
13	10-15	Negative	No	No	4	Fail
14	10-15	Negative	No	No	3	Pass
15	10-16	Negative	No	No	3	Pass
16	10-16	Negative	No	No	2	Pass
17	10-17	120	Yes	No	3	Fail

Looking at this establishment record the following determinations can be made.

1. Test number eleven, conducted on October 14th, documents the fourth test result in the marginal (“m”) range. Therefore, the establishment was in an unacceptable process control status because the fourth marginal result exceeds the limit of no more than three marginal results in the past 13 consecutive tests.

IPP should focus on dressing procedures and sanitation performance standard requirements when failing test results indicate the lack of slaughter process control.

2. Tests number twelve and thirteen are negative, and therefore in the acceptable range. However, considering the last 13 test results in the 13-test moving window, there are still more than three results in the marginal range. The company marked its record to show that it is still failing because there are four marginal test results. In reality this is not an unacceptable result because tests twelve and thirteen are negative, indicating the process is back in control, but there is evidence of problems in the recent past.
3. For test number fourteen the number of marginal results in the last thirteen tests window is reduced to three. The marginal result for test number one is dropped and replaced by an acceptable result as the 13-test window moves ahead one test.
4. The test result for test number seventeen exceeds 100 cfu/cm², the “M” value for cattle. Any result over 100 cfu/cm² is automatically unacceptable. It takes only one test in the “M” range to indicate the establishment may not have adequate process control.

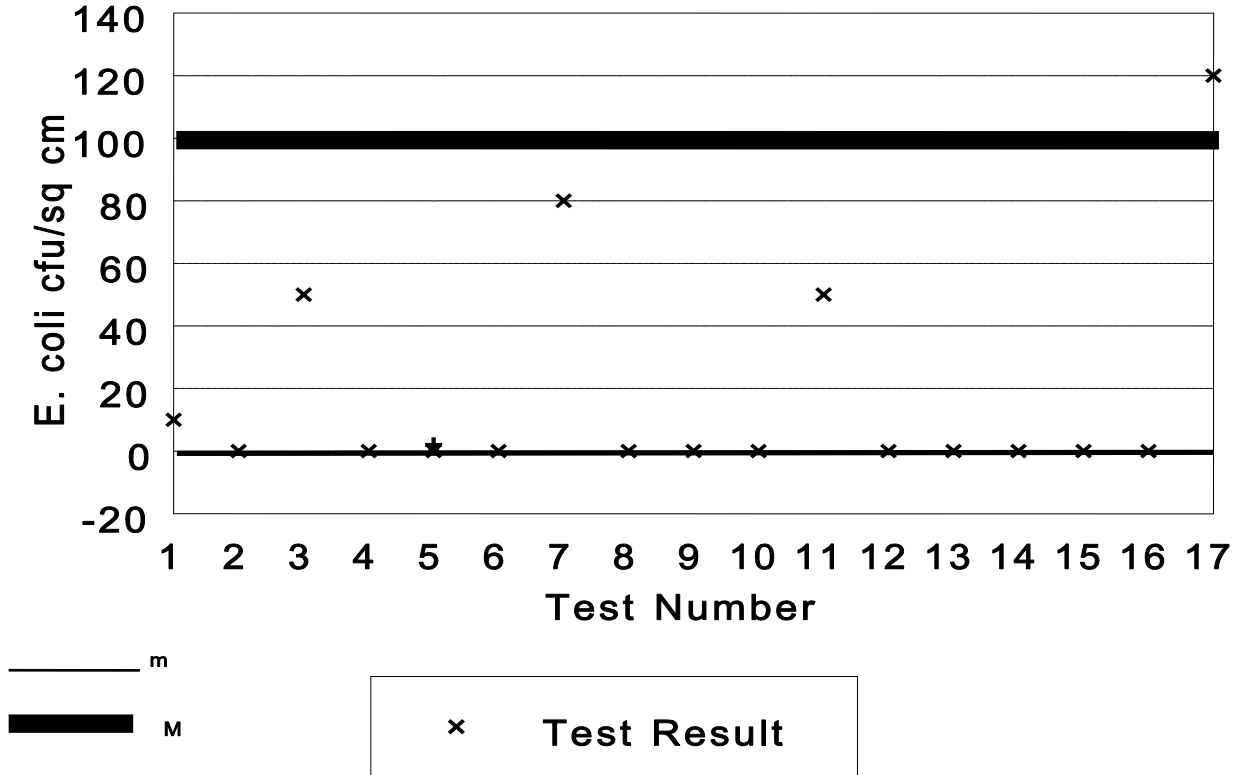
Inspection personnel reviewing this record should focus on sanitation performance standard requirements.

Another method the company may use to document its *E. coli* test results is a control chart. The seventeen test results written in the previous table are plotted on the following control chart.

The vertical Y-axis shows how many colony-forming units (cfu) of *E. coli* were found in a square centimeter (cm²) of the test sample analyzed at the laboratory. The horizontal X-axis indicates the test number. Marking an “X” at the point where the X and Y-axes converge (meets) reflects the test value or result for the particular test number. For ease of reading, the chart has a line to indicate the bottom limit of “m”, and a thicker line to indicate the upper limit of “M.” Any “X” plotted between the thin line and the thick line falls in the marginal range, we call “m.” Any “X” plotted above the thicker line is in the unacceptable range, or “M.”

E. Coli Control Chart

Figure 4



Action to Regain Process Control

Whenever a prudent livestock slaughter establishment determines that its *E. coli* test results do not meet m/M performance criteria or statistical process control values, it should take necessary actions to bring the slaughter process back into control.

Under the regulations, establishments are not required to take corrective actions or to document the necessary actions for *E. coli* test failures. However, when livestock or ratite slaughter establishments do not evaluate their test results (§381.94(a)(5) or §310.25(a)(5)), they might not be maintaining slaughter process controls sufficient to prevent fecal contamination.

Workshop: True or False

- ____ 1. As per 9CFR 310.25(a)(2), establishments that slaughter livestock are only categorized as very low volume.
- ____ 2. For generic *E. coli* testing, the regulation requires that livestock and ratite carcasses be selected at random for sampling.
- ____ 3. It is not necessary for the establishment to keep a table or chart showing the most recent 13 test results.
- ____ 4. If a livestock slaughter establishment uses the sponge sampling method, they must use the performance criteria published in 9 CFR 310.25 to evaluate generic *E. coli* test results.
- ____ 5. Establishments that slaughter livestock or ratite must keep records of the tables or charts with generic *E. coli* test results for 12 months.

8. Sample Integrity – paragraph (a)(2)(i) of section 310.25 and 381.94

According to this section of the regulations, sample integrity must be addressed in the establishment's written sample collection procedure and should be followed; but if it is not followed, it is not an enforceable issue. If IPP observe circumstances that seem to jeopardize sample integrity (e.g., freezing the sample, not shipping the sample on the same day it is collected), the District Office should be notified through supervisory channels. Further investigation of the situation and any enforcement actions will be directed from the District Office.

Microbiological Sampling for Poultry Slaughter (other than Ratite) Operations

The purpose of the new sampling requirements is to ensure that establishments monitor and evaluate the effectiveness of their procedures to prevent contamination of carcasses by enteric pathogens and visible fecal material on an ongoing basis. Fecal contamination is a principal source of pathogenic organisms that contaminate poultry carcasses. Under the Modernization of Poultry Slaughter Inspection final rule establishments that slaughter poultry, other than ratites, are required to perform microbiological sampling and analysis, for example, testing for *Salmonella*, *Campylobacter*, or indicator organisms such as aerobic plate count (APC), total coliform, Enterobacteriaceae, and *Escherichia coli*, Biotype I, also known as generic *E. coli*.

Because establishments have differences in their operations, each establishment has the flexibility to develop a sampling plan and determine the microbial organism that will accurately monitor the effectiveness of its process control procedures.

Microbiological test results that represent the level of microbiological contamination at key steps in the slaughter process are necessary for the establishment to provide comprehensive objective evidence to demonstrate process control. Process control consists of the programs and procedures that an establishment implements to ensure its process prevents contamination of poultry carcasses and parts, including contamination with pathogens and fecal material. Process control also ensures that the resulting product meets applicable standards or definitions.

Inspection Program Personnel (IPP) Responsibilities

In poultry slaughter establishments (other than ratite), IPP are to conduct verification tasks, as outlined in Directive 5000.1 following the verification instructions in Notice 64-14. The PHIS verification task that IPP perform depends on how the establishment has incorporated its written procedures for preventing contamination of carcasses and parts by enteric pathogens and fecal contamination throughout the entire slaughter and dressing operation in its HACCP system. For instance:

- If the establishment's written procedures are part of its HACCP plan, IPP are to verify HACCP regulatory requirements by performing the **Slaughter HACCP verification task** when it has been scheduled in PHIS.
- If the establishment's written procedures are part of its Sanitation SOPs, IPP are to verify that the establishment meets all Sanitation SOP regulatory requirements by performing the **Operational SSOP Review and Observation task** when it has been scheduled in PHIS.
- If the establishment's written procedures are part of another prerequisite program or other control measures, IPP are to verify the implementation of such program by performing the **Slaughter HACCP verification task** when it has been scheduled in PHIS.

IPP are to perform the appropriate PHIS verification task on a **routine** basis at the frequency specified in the establishment's task list. IPP are also to initiate a **directed** verification task if they observe noncompliance with the requirements in 381.65(g) and (h) while performing other tasks or when instructed to do so by supervision or other policy issuances.

IPP are to verify that the poultry slaughter establishment:

- Developed a written sampling program that identifies the specific microorganisms being tested and location/frequency where samples are collected,

- Incorporated its written sampling program for preventing contamination by enteric pathogens into its HACCP system,
- Implements and maintains its written sampling program,
- Maintains scientific and technical documentation to support the decisions that the establishment made in designing the sampling program,
- Maintains daily records documenting the implementation and monitoring of its procedures including sample results

Microbiological Sampling and Analysis Verification

Each poultry slaughter establishment's written procedures for preventing contamination of carcasses and parts with enteric pathogens and fecal material must include sampling and analysis for microbial organisms.

The regulations require each establishment to maintain scientific and technical documentation to support the judgments that the establishment made in designing the sampling program. The regulations prescribe the minimum requirements for the location and frequency of sampling, based on the establishment size and production volume. Each establishment must maintained daily records to document the implementation and monitoring of their procedures including records documenting the test results of its sampling plan.

Note: Establishments may use *Salmonella* Initiative Program (SIP) microbial data as part of their sampling plan to monitor their process control, provided they meet minimum frequencies and location requirements.

A Microbiological Testing of Raw Poultry Summary Chart (Attachment 2 of this handout) is provided as a reference for the establishment size, sampling frequencies, and sampling locations requirements. It is a quick and easy inspection aid when conducting the PHIS verification task.

IPP must understand what each statement of the regulation means in order to conduct the appropriate PHIS verification task. The IPP addresses the requirements of 9 CFR 381.65(g) and (h) as follows:

1. Sampling requirements – Microbial Indicator Organism paragraph (g) of section 381.65

Each establishment must develop its own sampling program/procedure that identifies the specific microbiological organisms (i.e., *Salmonella*, *Campylobacter*, or other enteric organisms) for which the establishment will test to monitor the effectiveness of its process control procedures that prevent contamination of carcasses and parts with enteric pathogens and fecal material.

Note: Very small and very low volume poultry slaughter establishments (as defined below) operating under **Traditional Inspection** can choose to continue conducting

generic *E. coli* testing at post-chill to meet the requirements under the Modernization of Poultry Slaughter Inspection final rule. FSIS considers the requirements under the former §381.94(a) regulations for generic *E. coli* testing of poultry to be scientifically validated “**safe harbor**” for monitoring process control.

2. Sampling requirements – location (paragraph (g)(1) and paragraphs (g)(1)(i) and (ii) of section 381.65) and technique

Poultry slaughter establishments are codified by size and annual slaughter volume, according to regulation 381.65(g)(1)(i) and (ii), and FSIS Notice 64-14.

- Very small establishments are establishments with fewer than 10 employees or annual sales of less than \$2.5 million.
- Very low volume (VLV) establishments annually slaughter no more than 440,000 chickens, 60,000 turkeys, 60,000 ducks, 60,000 geese, 60,000 guineas, 60,000 squabs or a combination of all types of poultry not exceeding 60,000 turkeys and 440,000 birds total.

The location refers to the place within the establishment where the sample is collected. Very small establishments and VLV establishments operating under **Traditional inspection** are required to collect samples for microbial organisms at the **post-chill point** in the process. All other establishments must collect samples at both the **pre-chill and post-chill** locations.

The pre-chill location for sampling is any point in the slaughter process from re-hang to just prior to the chiller. The post-chill location for sampling is a point in the slaughter process after the carcass exits the chiller and after all slaughter interventions are completed, which is the same point in the process that FSIS collects samples for *Salmonella* and *Campylobacter* verification testing.

Carcasses must be selected at the required points in the process (pre and post chill). At the post-chill site, samples should be collected after the final wash and the application of any final antimicrobial interventions. A drip time of at least 60 seconds should be observed before sample collection to prevent excessive antimicrobial carryover in the collected sample.

Note: Antimicrobials used during processing steps may make it harder to detect live bacteria in the collected sample if the carcass is not allowed adequate drip time before collecting the sample. Consequently, antimicrobial carryover (residual) can result in altered test results (lower bacterial counts), may invalidate the test results, and may not provide a true representation of the establishment’s process control.

The sampling methods for collecting carcass samples may include the nondestructive sponge technique for sample collection from turkeys and geese (back and thigh) and a

whole bird rinse technique for sample collection from chickens, guineas, ducks, geese, and squabs. All carcass samples should be taken using aseptic techniques.

The establishment must provide scientific or technical support for their sampling technique and sample site on the carcass. If IPP have concerns with the establishment's support, they should contact the District Office through supervisory channels.

3. Sampling requirements – frequency paragraphs (g)(2)(i) and (ii) of section 381.65

VLV establishments must collect and analyze samples at least once during each week of operation starting June 1 of every year. If, after consecutively collecting 13 weekly samples, a VLV establishment can demonstrate that it is effectively maintaining process control, it may modify its sampling plan. In this case the establishment would need to document the changes and maintain documentation showing that the changes allow the establishment to continue to effectively monitor process control.

Seasonal VLV operations must complete all microorganism testing during whichever months it operates. For example, a seasonal duck slaughter establishment that operates from September through December must begin testing during its first full week of operations and complete 13 tests before operations end in December.

All other establishments (including very small establishments) must collect and analyze a pair of samples, one at pre-chill and one at post-chill, at the following frequencies:

- Chickens: once per 22,000 carcasses but at a minimum of once during each week of operation;
- Turkeys, ducks, geese, guineas, and squabs: once per 3,000 carcasses but at a minimum once each week of operation.

Slaughter volume does not always match frequency rates in the regulations. Establishments should account for extra slaughter volume. This can be done by conducting additional microbiological tests. For example, a chicken establishment that slaughters 40,000 birds per day should test at least once a day at the 22,000 birds per test frequency. However, the remaining 18,000 birds should also be accounted for to monitor process control. To account for the extra slaughter volume, the establishment could “carry over” the 18,000 extra birds to the next day's volume and conduct two (2) microorganism tests on the second day.

4. Random selection of carcasses

Samples should be collected randomly at the frequency determined by the establishment as part of its sampling plan. At a minimum, the establishment must collect

samples at the frequency specified under 9 CFR 381.65(g)(2). If more than one shift is operating at the establishment, the sample can be taken on any shift. Different methods of selecting the specific carcass for sampling could be used, but the method used should include the use of random numbers to ensure that testing data is not biased. Examples of methods include random number tables, calculator or computer-generated random numbers, or drawing cards.

The carcass that is sampled should be selected at random from all eligible carcasses. If there are multiple lines or chillers, randomly select the line or chiller for sample collection for that interval. Each line or chiller should have an equal chance of being selected at each sampling interval within the relevant time frame (based on the sampling frequency for the plant).

The establishment must provide scientific or technical support the decisions it made in designing the sampling program.

5. Sample analysis and testing method

To obtain the most accurate results, samples should be analyzed as soon after collection as possible. If samples must be transported to an off-site laboratory, they should be refrigerated and then shipped refrigerated, on the same day they were collected, via an overnight delivery or courier service to the laboratory. A sample should arrive at the laboratory and be analyzed no later than the day after it is collected.

In addition, establishments should ensure that microbiological testing is reliable and meets its food safety needs. Each establishment needs to determine whether sample analysis will be performed by an outside or on-site laboratory. FSIS has available the compliance guideline "*Establishment Guidance for the Selection of a Commercial or Private Microbiological Testing Laboratory*" if the establishment decides to use an outside laboratory to analyze microbiological samples. This guidance document should be particularly useful to very small establishments when they are selecting a commercial or private laboratory to analyze establishment microbiological samples.

FSIS has also made available a list of *Foodborne Pathogen Test Kits Validated by Independent Organizations* for the detection of relevant foodborne pathogens (i.e., *Salmonella*, *Campylobacter*, *E. coli* O157:H7, and *Listeria spp.* including *L. monocytogenes*). This list is intended to be informational and is not an endorsement or approval of any particular testing method, regardless of its inclusion in the list.

Poultry slaughter establishments (other than ratite) must include the analysis of microbial organisms in their sampling procedures as part of their HACCP system (381.65(g)). Therefore, scientific and technical documentation must be provided to support the design of the sampling program. The Agency recommends that the industry follow the guidelines in the document titled "FSIS Compliance Guideline: HACCP Validation" published on May 2013. The documentation can be found in the FSIS website at:

http://www.fsis.usda.gov/wps/wcm/connect/a70bb780-e1ff-4a35-9a9a-3fb40c8fe584/HACCP_Systems_Validation.pdf?MOD=AJPERES

IPP are to review the establishment's written programs, scientific and technical support, and records to verify that the laboratory analyzes the samples using an AOAC Official Method or one validated by another recognized independent testing body. When in doubt about whether the laboratory testing procedure is acceptable, IPP should go through the supervisory chain-of-command to the District Office for assistance.

6. Records of test results – paragraphs (g)(2)(iii) and (h) of section 381.65

Official poultry slaughter establishments must maintain daily records documenting the implementation and monitoring of its procedures required under paragraph (g) including accurate records of all test results from its sampling plan for at least one year. These records can be maintained in an electronic format on a computer, provided there are measures in place to ensure the integrity of the electronic data. These records must be readily accessible for review by IPP upon request.

IPP are to verify that the establishment maintains daily records documenting the implementation and monitoring of its procedures, makes these records available for IPP to review and retains these records for one year, and implements appropriate controls to ensure the integrity of electronic data if records are maintained on computers

7. Criteria for evaluation of test results

Poultry slaughter establishments should use statistically valid approach or statistical process control (SPC) to interpret their microbiological test results as previously discussed in this handout. Establishments gather initial test results and set the upper control limit that is used to assess whether the slaughter process is under control. As long as the test results remain below the upper control limit, the slaughter process is considered under control.

In cases where an establishment does not have the resources or capacity to develop and implement their own statistical control limits or procedures, establishments can utilize the results from FSIS nationwide livestock or poultry surveys. The tables below demonstrate the indicator organism median values for chickens and turkeys.

Table 1 - Indicator Organism Median Values for Chickens

	Median (CFU/mL)			
	Generic E. coli	APC	Enterobacteriaceae	Total Coliform
Carcass – Rehang	540	28,000	1,600	940
Carcass – Post Chill	20	260	20	20

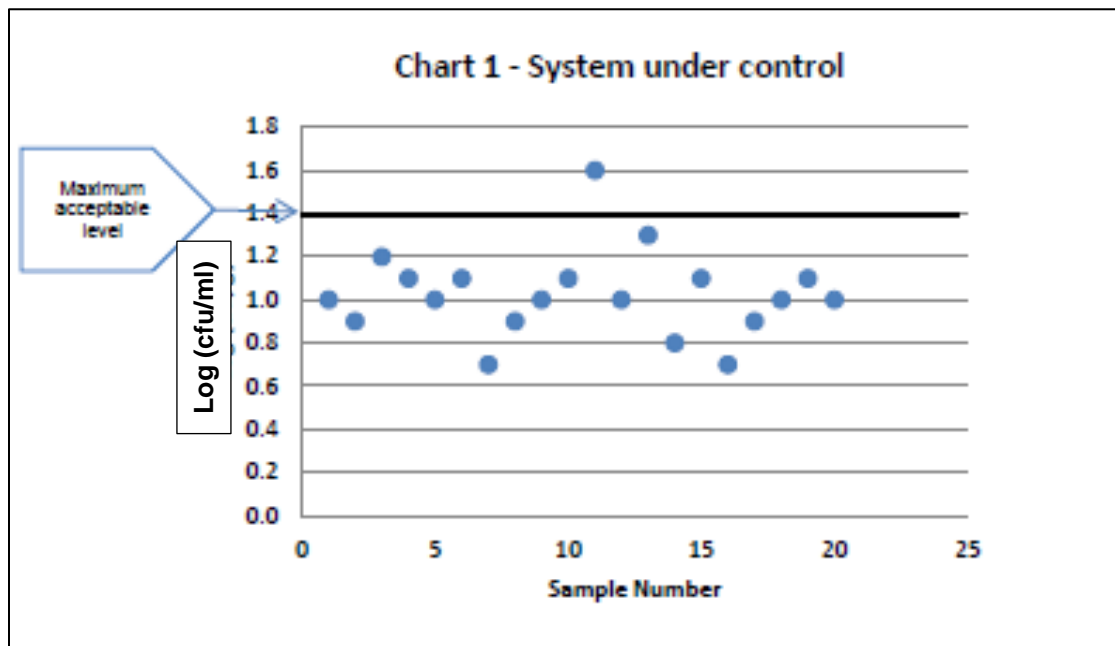
Table 2 - Indicator Organism Median Values for Turkeys

	Median (CFU/mL)			
	Generic E. coli	APC	Enterobacteriaceae	Total Coliform
Carcass – Rehang	22	1,800	50	40
Carcass – Post Chill	<1.2	18	<1.2	<1.2

An establishment sample value that is higher than the corresponding one listed in the table indicates the establishment may not be maintaining process control and may be less likely to meet applicable performance standards. Sample values lower than the one listed in the table indicate the establishment may be maintaining process.

SPC usually includes the use of a control chart, which plots data over time but also displays an upper control limit for specific measurements and a centerline (the average), above and below which there is an equal number of sample results. A sample result above the upper control limit would indicate the likely presence of a special cause of variation that should be addressed. Results within control limits indicate simply that the process is in control.

The example below shows a SPC chart for a poultry slaughter operation which plots test results for an indicator organism in terms of sample number, along the horizontal X-axis, against Log cfu/ml on the Y-axis. This chart illustrates a pattern of an indicator organism test results that would be seen in a well-controlled system. In a well control system, the majority of the test results will be clustered around a central value (the average). It is important to note that even a well-controlled system there is some frequency of isolated results above the acceptable level.



As part of its process control procedures, an establishment should define the actions it will take if the microbiological test results obtained through its sampling are above the limits it has set. The establishment should delineate what its actions will be, who will take each action, how the outcome of these actions will be documented, and how it will be verified.

FSIS has made available the *FSIS Compliance Guidelines for the Control of Salmonella and Campylobacter in Raw Poultry*. The guidelines summarize known control points for *Salmonella* and *Campylobacter* in the pre- and post-harvest production process. Establishments should use this compliance guide to improve management practices, to ensure effective dressing operations and to assist in investigating when there is a loss of control of the slaughter process.

When IPP review the establishment's records that document its microbiological test results, they should look for trends in the test results that indicate a loss of process control. For example, IPP are to look for:

- A significant number of test results that exceeded the establishment's upper control criteria, if the establishment has such criteria,
- Instances where the test results exceed the establishment's criteria by a large amount over a relatively short period of time (e.g., days or weeks); or
- Test results that show a trend of worsening performance over a relatively long period of time (e.g., days, months, seasonal).

Very Small or Very Low Volume Establishments that Slaughter Poultry under Traditional Inspection Using the Safe Harbors to Monitor Process Control

The Agency considers former provisions 381.94(a)(2)(i), (a)(3), and (a)(5)(i) as safe harbors if very small and very low volume establishments slaughter poultry under Traditional Inspection chooses to test for generic *E. coli* at post chill as the indicator microorganism. These establishments use the M/m values in the following table and a moving window of the last 13-documented test results to evaluate process control.

Type of poultry	Lower limit of marginal range (m)	Upper limit of marginal range (M)	Number of Samples tested (n)	Maximum number permitted in the Marginal range
Chickens	100 cfu/ml	1,000 cfu/ml	13	3

An establishment is operating within the criteria when the most recent generic *E. coli* test result does not exceed the upper limit (M), and the number of samples, if any, testing positive at levels above (m) is three or fewer out of the most recent 13 samples (n) taken.

Whenever a prudent poultry slaughter establishment determines that its generic *E. coli* test results do not meet m/M performance criteria, it should take necessary actions to bring the slaughter process back into control.

8. Sample Integrity

Even though the regulatory requirements in 9 CFR 381.65(g) for poultry slaughter microbiological testing programs do not specifically address the handling of the samples to ensure sample integrity, a prudent establishment should include a description of how samples are handled ensure the sample integrity. Remember, the regulation requires each poultry slaughter establishment to incorporate their written procedures in its HACCP system which must comply with the 9 CFR 416 or 417 regulations.

Documenting Inspection Results in PHIS

IPP are to follow instructions for documenting their inspection results in PHIS as described in FSIS Directive 5000.1, Chapter V. When the establishment is in compliance with the regulations, IPP select the mandatory regulations, any other regulation they verified on the “Regulations” tab and mark the task as ‘Inspection Completed’ at the bottom of the Inspection Results page. If IPP find noncompliance, they are to notify the establishment and document the noncompliance on an NR citing the appropriate regulation. IPP are to document noncompliance with 9 CFR 381.65(g) and 9 CFR 381.65(h) according to the methodology and steps outlined in both FSIS Directive 5000.1 and FSIS Notice 64-14.

Noncompliance in Livestock and Ratite Slaughter Establishments

The livestock or ratite slaughter establishment’s generic *E. coli* testing results cannot, by themselves, support a finding of noncompliance with 9 CFR 310.25(a) or 381.94(a). However, if the establishment’s testing results indicate a failure of process control, IPP are to verify the establishment’s sanitary dressing procedures. The IPP should use the findings from verifying the establishment’s sanitary dressing procedures in conjunction with other information, like zero tolerance failures and positive *E. coli* O157:H7 results (if applicable) in beef slaughter processing combination establishments and any other HACCP performance, in making determinations regarding the effectiveness of the food safety system.

Noncompliance occurs when the establishment is not meeting the prescribed regulatory requirements in 9 CFR 310.25(a) and 381.94(a).

The following findings are evidence that the establishment does not comply with 9 CFR 310.25(a) and 381.94(a).

1. The establishment is not conducting sampling at the required location.

2. The establishment is not using the required sampling technique or sampling at the required site on the carcass.
3. The establishment is not sampling the required frequency according to the establishment's production volume.
4. The laboratory is not using a quantitative method for analysis of generic *E. coli* that is approved as an Official Method of the AOAC International or approved and published by a scientific body.
5. Records are not available for FSIS access or not retained for 12 months

Noncompliance in Poultry Slaughter Establishments

The establishments test results by themselves do not necessarily indicate noncompliance. IPP are to consider all available information, including results from FSIS testing, to determine whether the establishment's microbiological sampling program enables the establishment to monitor its ability to maintain process control, including how the establishment is implementing its sampling program, any trends that are occurring in the test results, and how the establishment is reacting to its test results.

Noncompliance occurs when the establishment is not meeting the prescribed regulatory requirements in 9 CFR 381.65(g) and (h); is not following its written sampling and testing procedures; does not demonstrate that it is maintaining process control; or its corrective actions are not effective.

The following findings are evidence that the establishment does not comply with 9 CFR 381.65(g) and 9 CFR 381.65(h).

1. The establishment has not addressed minimizing contamination by enteric pathogens and fecal contamination at steps along the line in the slaughter process.
2. If the establishment is not implementing their written procedures.

Note: Additional regulatory noncompliance may need to be cited on the NR depending on where the establishment has incorporated its written procedures in the HACCP system. For example, if the written procedures for preventing contamination by enteric pathogens and fecal material throughout slaughter and dressing operations are in the SSOP, IPP should also cite 9 CFR 416.13(b) and (c) when the establishment is not implementing those procedures. Likewise, if the written procedures for preventing contamination by enteric pathogens and fecal material throughout slaughter and dressing operations are in a prerequisite program, IPP should also cite 417.5(a)(1) when the establishment is not implementing those procedures.

3. The procedures are not effective in preventing contamination, e.g., the system results in little or no reduction in visible fecal contamination, or the establishment's microbiological test results indicate that the establishment's procedures are not effective in maintaining process control,
4. The establishment is not conducting microbiological sampling at the required location or at the required frequency according to the establishment's size and production volume.
5. Records are not available for FSIS access or not retained for one year.
6. Sample integrity, e.g. randomness and handling of samples, is not maintained.

Note: If the establishment does not have written procedures to prevent enteric pathogen or visible fecal contamination throughout the slaughter process, or has not incorporated the procedures into its HACCP system, IPP are to perform the PHIS Slaughter HACCP verification task and issue a noncompliance record (NR) citing 9 CFR 381.65(g), 381.65(h), 417.2(a), and 417.5.

Enforcement

Livestock and Ratite Slaughter Establishments

FSIS generic *E. coli* criteria are guidelines, not regulatory standards. FSIS does not use company Generic *E. coli* test results to take regulatory action. Test results that show lack of process control should be considered in conjunction with other information, like sanitary dressing, SSOP and HACCP performance.

Further enforcement action might be necessary if the establishment repeatedly fails to implement appropriate immediate action or further planned action in response to NRs documenting noncompliance. In these cases, the inspector in charge (IIC) should notify the District Office through supervisory channels. The District Office will give instructions for additional enforcement action when necessary.

Poultry Slaughter Establishments

If the establishment has repetitive NRs, or the establishment's corrective actions are ineffective, IPP are to discuss with their immediate supervisor the need to take an enforcement action outlined in FSIS Directive 5000.1., Chapter V.

Attachment 1

GENERIC *E. COLI* TESTING SUMMARY CHART

SPECIES	TEST FREQUENCY	TEST LOCATION	SAMPLE SITES	SAMPLING METHOD
Cattle	1/300 carcasses or 1/wk. – whichever is greater	Carcass cooler >12 hrs. Hot boned: after final wash	Flank, brisket, rump	Excision* Sponging
Hide-on calves	Same	Same	Same	Sponging only
Swine	1/1000 carcasses or 1/wk. – whichever is greater	Carcass cooler >12 hrs. Hot boned: after final wash	Belly, ham, jowls	Excision* Sponging
Hide-on carcasses	1/300 carcasses or 1/wk. – whichever is greater	Chilled carcasses	Inside flank, inside brisket, inside rump	Sponging only
Horses, Mules, Other Equines	1/300 carcasses or 1/wk. – whichever is greater	Chilled carcasses Hot boned: after final wash	Flank, brisket, rump	Sponging
Sheep and Goats	1/300 carcasses or 1/wk. – whichever is greater	Chilled carcasses Hot boned: after final wash	Flank, brisket, rump	Sponging
Ratites	1/3000 carcasses or 1/wk. – whichever is greater	Chilled carcasses Hot boned: after final wash	Sponge back and thigh	Sponging

* These have applicable m/M values

Attachment 2

MICROBIOLOGICAL TESTING OF RAW POULTRY SUMMARY CHART

ESTABLISHMENT SIZE	DEFINED AS	MINIMUM SAMPLING EVENT FREQUENCY	MINIMUM SAMPLING LOCATION
Very small (VS)	Fewer than 10 employees or annual sales of less than \$2.5 million	At least once during each week of operation, starting June 1 of every year.	A sample at post-chill per sampling event
Very low volume (VLV)	Slaughter no more than 440,000 chickens, 60,000 turkeys, 60,000 ducks, 60,000 geese, 60,000 guineas, or 60,000 squabs or a combination of all types of poultry not exceeding 60,000 turkeys and 440,000 birds total annually.	If, after consecutively collecting 13 weekly samples and upon demonstrating effective process control, the sampling plan may be modified.	
Small	10 – 499 employees unless annual sales total less than \$2.5 million	Chickens: once per 22,000 carcasses, but at a minimum of once during each week of operation. Turkeys, ducks, geese, guineas, and squabs:	A sample at pre-chill and a sample at post-chill locations per sampling event
Large	500 or more employees	once per 3,000 carcasses but at a minimum once each week of operation	

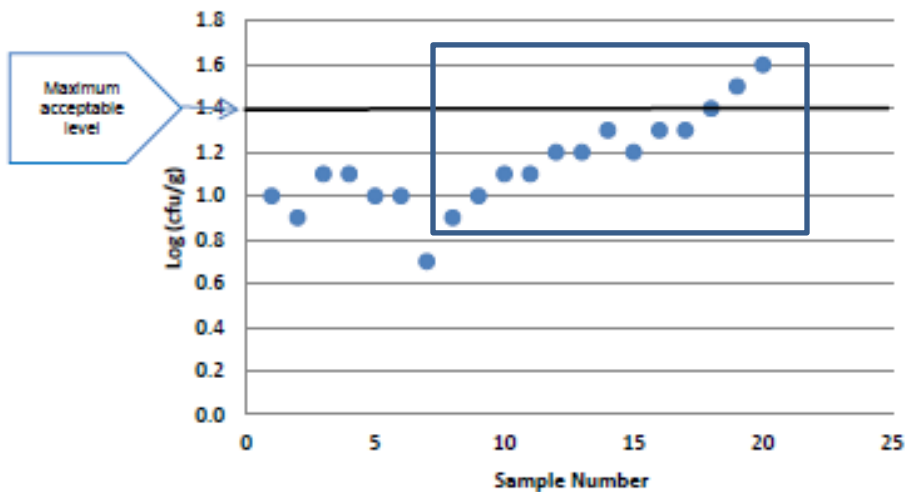
WORKSHOP

MICROBIAL INDICATOR ORGANISM IN POULTRY SLAUGHTER OPERATIONS (OTHER THAN RATITE)

An establishment that slaughters 22,000 young chickens daily has incorporated its written microbiological sampling procedure for analysis of Enterobacteriaceae into its Sanitation SOP. You scheduled an Operational SSOP Review and Observation task on your task calendar in PHIS for today.

Scenario

You review the written sampling procedure used to monitor the effectiveness of the procedures the establishment implements to prevent contamination of carcasses and parts with enteric pathogens in the QC office. In the sampling procedure, the establishment is collecting samples (carcass rinsate) at the required locations and frequencies in accordance with 9 CFR 681.65(g)(1)(2). You also review the company's process control chart for the Enterobacteriaceae analyses. The establishment uses a moving window of the thirteen most recent tests to evaluate process control. The chart shows the following results:



You conclude that the establishment is maintaining daily records sufficient to document the implementation and monitoring of the sampling procedures but note that the SSOP corrective action records for the sampling program do not reflect any action taken for the last two weeks. Based on the information given in the scenario, answer the following questions:

What do you conclude from the review of the establishment's process control chart with the most recent thirteen tests results?

Is this a noncompliance? If so, why and cite the noncompliant regulations.

GENERIC *E. COLI*/TESTING

General Instructions

Work through this workshop with at least one partner. Using the example generic *E. coli* written procedure, records, and the regulations provided, determine whether the establishment is in compliance with the generic *E. coli* testing requirements.

You have scheduled a generic *E. coli* verification task on your task calendar in PHIS for establishment number M44927 for today.

Generic *E. coli* verification task

The establishment collects samples from the type of livestock or poultry it slaughters in greatest numbers; selects carcasses randomly; selects carcass samples at required location in process, and by procedure specified in regulation.	310.25(a) or 381.94(a) FSIS Directive 5000.1	Observe sample collection and review procedures and records. Make determinations about compliance with regulatory requirements. Document failure(s) to comply with regulatory requirements on NR and, when appropriate, take other actions consistent with applicable directive(s).
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Scenario

From the random sample collection times provided to you by Irene Bossley, the QC technician, at the beginning of the shift, you decide to observe the second *E. coli* sample collection of the day. You observe the technician putting on sterile gloves and

randomly collecting one untrimmed half carcass in the cooler. Following the procedure, she changes sterile gloves, aseptically sponges' three sites (the flank, brisket and rump) of the selected beef carcass, following the guidelines for proper handling of the sponge. You follow her to the in-house microbiology laboratory where a qualified microbiology technician is waiting.

You discuss the testing procedure used in the on-site lab with the lab technician. She tells you that the analysis is completed using a test method she found in a peer-reviewed microbiology journal two years ago. She says she has memorized the technique and does not need to refer to the instructions in the article as she analyzes the sample. She does have a copy of the *E. coli* test procedure in her files and shares it with you.

Finally, you check the company's process control charts. There is a moving window of the thirteen most recent tests.

**OPEN BEEF, Inc.
M44927
8305 Hawthorne Way
Petaluma, CA**

***E. COLI* SPECIMEN COLLECTION PROCEDURE**

This is a one-shift establishment that slaughters **heifers and steers**. Each day of operation the Quality Control Manager, or his designee, will collect a half carcass in the cooler for each 300 steers slaughtered. When selecting a carcass in the cooler at the random time, the QC Manager, or his designee, will walk up to the selection point and count five half carcasses. He will then select the sixth half carcass.

Open Beef's average daily production volume is a combination of 500 heifers and steers. Based on this volume, one random sample will be taken during the shift the first day of sampling. Two samples are then taken for two days in a row. Then the three-day cycle begins again. This method is used to take into account the extra carcasses produced each day.

Before the beginning operations, the QC Manager, or his designee, will use a random selection computer program to select the time samples on the shift will be collected. If a random time occurs during a scheduled company break, it will be discarded. Only times within the hours of actual operation will be chosen. These times will be made available to FSIS personnel before operations begin.

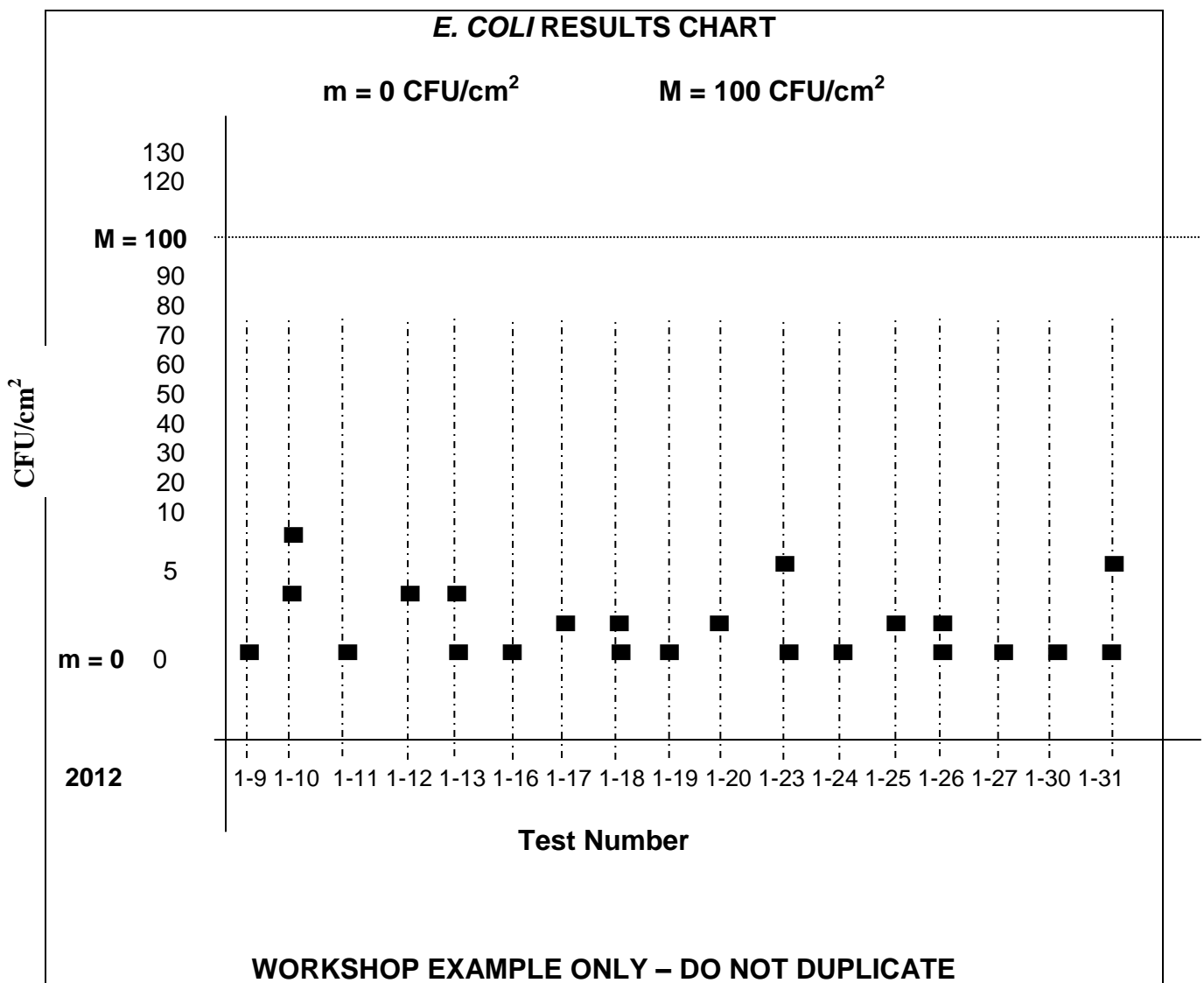
Aseptic sampling technique will be used to ensure sample integrity. The sponge method, as outlined in the "Guidelines for *E. coli* Testing for Process Control Verification in Cattle and Swine Slaughter Establishments" will be followed to ensure sample integrity. Samples will be taken to our own microbiology laboratory for immediate

analysis using an AOAC Official Testing Method. In the event our laboratory cannot conduct *E. coli* tests, the QC Manager, or his designee, will immediately refrigerate the sample. At the end of the shift, the refrigerated samples will be sent via overnight Federal Express to the Always Accurate Microbiology Laboratory in Rough and Ready, CA, for immediate analysis.

Ronald Lynn, Plant Manager

January 27, 2011

WORKSHOP EXAMPLE ONLY – DO NOT DUPLICATE



PHIS Instructions

Working independently, log into PHIS. You will:

- Add the Generic *E. coli* verification task to the task calendar,
- Document the results of the Generic *E. coli* verification task, and
- If noncompliance was found, document the noncompliance on an NR in PHIS


Use the following instructions as needed. If you need further instructions, consult the PHIS Quick Reference Guide.

Logging in to PHIS

1. Log-in as:

- User Name: **FSIS_user**
- Password: **FSIS**

2. If needed, start Internet Explorer using the Icon on upper left of the desktop:

- Double click the Internet explorer () icon
- PHIS comes up. Log In as:
 - User name: **Robert Allen**

Add the generic *E. coli* Verification Task to the Task Calendar

1. Left click on “**Task Calendar**” from the Navigation menu on the Home page, then left click the “**down arrow**” in the box next to “select establishment” and select “**Open Beef**”
2. Left click and hold on the slider bar to the right of the “**Establishment Task List**” panel, scroll through the list until you find a routine **Generic *E. coli* verification task** with the appropriate start and end dates
3. Find the “**Routine**” column for the task, and then left click on the “**Add**” link for the Generic *E. coli* verification task
4. When the calendar pop-up window appears, **Type a 1** in the box for today’s date, then left click on the “**Save**” button

Initiate/Claim the Generic *E. coli* Verification Task

1. Scroll down to the “**Task Calendar**” panel, left click the “**down arrow**” in the box next to “Establishment” that has the word “all” and select “**Open Beef**”
2. Find today’s date and the “**Generic *E. coli* verification task**” that you just added
3. Right click on the “**Generic *E. coli* verification task**” on the calendar
4. Highlight and left click “**Document**”
5. Left click on the “**Activity tab**”, and left click the “**radio button**” in front of the word “**Both**” for the verification activity
6. Left click on the “**Regulations tab**” and check the box next to the §310.25(a) regulation
7. Scroll down an left click on the “**save**” button
8. Left click on the “**close** button”

Documenting the Generic *E. coli* Verification Task Results

1. Scroll down to the “**Task Calendar**” panel, left click the “**down arrow**” in the box next to “Establishment” that has the word “all” and select “**Open Beef**”
2. Right click on the “**Generic *E. coli* verification task**” on the calendar
3. Highlight and left click “**Document**”
4. After the inspection results page opens, enter your inspection results
 - Enter the red meat generic *E. coli* §310.25(a) regulation you verified,
 - If noncompliance was found, document the noncompliance on an NR, and
 - Finalize the noncompliance

Livestock

Sec. 310.25 Contamination with microorganisms; process control verification criteria and testing; pathogen reduction standards

(a) Criteria for verifying process control; *E. coli* testing. (1) Each official establishment that slaughters livestock must test for *Escherichia coli* Biotype 1 (*E. coli*) Establishments that slaughter more than one type of livestock or both livestock and poultry shall test the type of livestock or poultry slaughtered in the greatest number. The establishment shall:

(i) Collect samples in accordance with the sampling techniques, methodology, and frequency requirements in paragraph (a)(2) of this section;

(ii) Obtain analytic results in accordance with paragraph (a)(3) of this section; and

(iii) Maintain records of such analytic results in accordance with paragraph (a)(4) of this section.

(2) Sampling requirements.

(i) Written procedures. Each establishment shall prepare written specimen collection procedures which shall identify employees designated to collect samples, and shall address location(s) of sampling, how sampling randomness is achieved, and handling of the sample to ensure sample integrity. The written procedure shall be made available to FSIS upon request.

(ii) Sample collection. The establishment must collect samples from all chilled livestock carcasses, except those boned before chilling (hot-boned), which must be sampled after the final wash. Samples must be collected in the following manner;

(A) For cattle, establishments must sponge or excise tissue from the flank, brisket and rump, except for hide-on calves, in which case establishments must take samples by sponging from inside the flank, inside the brisket, and inside the rump.

(B) For sheep, goat, horse, mule, or other equine carcasses, establishments must sponge from the flank, brisket and rump, except for hide-on carcasses, in which case establishments must take samples by sponging from inside the flank, inside the brisket, and inside the rump.

(C) For swine carcasses, establishments must sponge or excise tissue from the ham, belly and jowl areas.

(iii) Sampling frequency. Slaughter establishments, except very low volume establishments as defined in paragraph (a)(2)(v) of this section, must take samples at a frequency proportional to the volume of production at the following rates:

(A) Cattle, sheep, goats, horses, mules, and other equines: 1 test per 300 carcasses, but, a minimum of one sample during each week of operation.

Swine: 1 test per 1,000 carcasses, but a minimum of one sample during each week of operation.

(iv) Sampling frequency alternatives. An establishment operating under a validated HACCP plan in accordance with Sec. 417.2(b) of this chapter may substitute an alternative frequency for the frequency of sampling required under paragraph (a)(2)(iii) of this section if,

(A) The alternative is an integral part of the establishment's verification procedures for its HACCP plan and,

(B) FSIS does not determine, and notify the establishment in writing, that the alternative frequency is inadequate to verify the effectiveness of the establishment's processing controls.

(v) Sampling in very low volume establishments.

(A) Very low volume establishments annually slaughter no more than 6,000 cattle, 6,000 sheep, 6,000 goats, 6,000 horses, mules or other equines, 20,000 swine, or a combination of livestock not exceeding 6,000 cattle and 20,000 total of all livestock.

Very low volume establishments

that collect samples by sponging shall collect at least one sample per week, starting the first full week of operation after June 1 of each year, and continue sampling at a minimum of once each week the establishment operates until June 1 of the following year or until 13 samples have been collected, whichever comes first. Very low volume establishments collecting samples by excising tissue from carcasses shall collect one sample per week, starting the first full week of operation after June 1 of each year, and continue sampling at a minimum of once each week the establishment operates until one series of 13 tests meets the criteria set forth in paragraph (a)(5)(i) of this section.

(B) Upon the establishment's meeting requirements of paragraph (a)(2)(v)(A) of this section, weekly sampling and testing is optional, unless changes are made in establishment facilities, equipment, personnel or procedures that may affect the adequacy of existing process control measures, as determined by the establishment or FSIS. FSIS determinations that changes have been made requiring resumption of weekly testing shall be provided to the establishment in writing.

(3) Analysis of samples. Laboratories may use any quantitative method for analysis of *E. coli* that is approved as an AOAC Official Method of the AOAC International (formerly the Association of Official Analytical Chemists) ² or approved and published by a scientific body and based on the results of a collaborative trial conducted in accordance with an internationally recognized protocol on collaborative trials and compared against the three tube Most Probable Number (MPN) method and agreeing with the 95 percent upper and lower confidence limit of the appropriate MPN index.

² A copy of the current edition/revision of the "Official Methods of AOAC International," 16th edition, 3rd revision, 1997, is on file with the Director, Office of the Federal Register, and may be purchased from the Association of Official Analytical Chemists International, Inc., 481 North Frederick Ave., Suite 500, Gaithersburg, MD 20877-2417.

(4) Recording of test results. The establishment shall maintain accurate records of all test results, in terms of CFU/cm² of surface area sponged or excised. Results shall be recorded onto a process control chart or table showing at least the most recent 13 test results, by type of livestock slaughtered. Records shall be retained at the establishment for a period of 12 months and shall be made available to FSIS upon request.

(5) Criteria for evaluation of test results.

(i) An establishment excising samples from carcasses is operating within the criteria when the most recent *E. coli* test result does not exceed the upper limit (M), and the number of samples, if any, testing positive at levels above (m) is three or fewer out of the most recent 13 samples (n) taken, as follows:

Table 1--Evaluation of *E. coli* Test Results

Type of livestock	Lower limit of marginal range (m)	Upper limit of marginal range (M)	Number of sample tested (n)	Maximum number permitted in marginal range (c)
Cattle.....	Negative ^a	100 CFU/ cm ²	13	3
Swine.....	10 CFU/ cm ²	10,000 CFU/ cm ²	13	3

^a Negative is defined by the sensitivity of the method used in the baseline study with a limit of sensitivity of at least 5 cfu/cm² carcass surface area.

(ii) Establishments sponging carcasses shall evaluate *E. coli* test results using statistical process control techniques.

(6) Failure to meet criteria. Test results that do not meet the criteria described in paragraph (a)(5) of this section are an indication that the establishment may not be maintaining process controls sufficient to prevent fecal contamination. FSIS shall take further action as appropriate to ensure that all applicable provisions of the law are being met.

(7) Failure to test and record. Inspection shall be suspended in accordance with rules of practice that will be adopted for such proceedings upon a finding by FSIS that one or more provisions of paragraphs (a) (1)-(4) of this section have not been complied with and written notice of same has been provided to the establishment.

Ratites

Sec. 381.94 Contamination with Microorganisms; process control verification criteria and testing; pathogen reduction standards

Sec. 381.94 Contamination with microorganisms; process control verification criteria and testing; pathogen reduction standards for establishments that slaughter ratites.

(a) Criteria for verifying process control; *E. coli* testing. (1) Each official establishment that slaughters ratites shall test for *Escherichia coli* Biotype I (*E. coli*). Establishments that slaughter ratites and livestock, shall test the type of ratites or livestock slaughtered in the greatest number. The establishment shall: (i) Collect samples in accordance with the sampling techniques, methodology, and frequency requirements in paragraph (a)(2) of this section;

(ii) Obtain analytic results in accordance with paragraph (a)(3) of this section; and
(iii) Maintain records of such analytic results in accordance with paragraph (a)(4) of this section.

(2) Sampling requirements. (i) Written procedures. Each establishment that slaughters ratites shall prepare written specimen collection procedures which shall identify employees designated to collect samples, and shall address location(s) of sampling, how sampling randomness is achieved, and handling of the sample to ensure sample integrity. The written procedure shall be made available to FSIS upon request.

(ii) Sample collection. The establishment must collect samples from whole ratites at the end of the chilling process. Samples from ratites may be collected by sponging the

carcass on the back and thigh or samples can be collected by rinsing the whole carcass in an amount of buffer appropriate for that type of bird.

(iii) Sampling frequency. Establishments that slaughter ratites, except very low volume ratite establishments as defined in paragraph (a)(2)(v) of this section, must take samples at a frequency proportional to the establishment's volume of production at the following rate: 1 sample per 3,000 carcasses, but at a minimum one sample each week of operation.

(iv) Sampling frequency alternatives. An establishment operating under a validated HACCP plan in accordance with Sec. 417.2(b) of this chapter may substitute an alternative frequency for the frequency of sampling required under paragraph (a)(2)(iii) of this section if,

(A) The alternative is an integral part of the establishment's verification procedures for its HACCP plan and,

(B) FSIS does not determine, and notify the establishment in writing, that the alternative frequency is inadequate to verify the effectiveness of the establishment's processing controls.

(v) Sampling in very low volume ratite establishments. (A) Very low volume ratite establishments annually slaughter no more than 6,000 ratites. Very low volume ratite establishments that slaughter ratites in the largest number must collect at least one sample during each week of operation after June 1 of each year, and continue sampling at a minimum of once each week the establishment operates until June of the following year or until 13 samples have been collected, whichever comes first.

(B) Upon the establishment's meeting the requirements of paragraph (a)(2)(v)(A) of this section, weekly sampling and testing is optional, unless changes are made in establishment facilities, equipment, personnel or procedures that may affect the adequacy of existing process control measures, as determined by the establishment or by FSIS. FSIS determinations that changes have been made requiring resumption of weekly testing shall be provided to the establishment in writing.

(3) Analysis of samples. Laboratories may use any quantitative method for analysis of *E. coli* that is approved as an AOAC Official Method of the AOAC International (formerly the Association of Official Analytical Chemists) or approved and published by a scientific body and based on the results of a collaborative trial conducted in accordance with an internationally recognized protocol on collaborative trials and compared against the three tube Most Probable Number (MPN) method and agreeing with the 95 percent upper and lower confidence limit of the appropriate MPN index.

(4) Recording of test results. The establishment shall maintain accurate records of all test results, in terms of colony forming units (CFU)/ml of rinse fluid. Results shall be recorded onto a process control chart or table showing at least the most recent 13 test results. Records shall be retained at the establishment for a period of 12 months and shall be made available to FSIS upon request.

(5) Establishments shall evaluate *E. coli* test results using statistical process control techniques.

(6) Failure to meet criteria. Test results that do not meet the criteria described in paragraph (a)(5) of this section are an indication that the establishment may not be maintaining process controls sufficient to prevent fecal contamination. FSIS shall take

further action as appropriate to ensure that all applicable provisions of the law are being met.

(7) Failure to test and record. Inspection will be suspended in accordance with rules of practice that will be adopted for such proceeding, upon a finding by FSIS that one or more provisions of paragraphs (a) (1) through (4) of this section have not been complied with and written notice of same has been provided to the establishment.

Poultry

Sec. §381.65 Operations and procedures, generally

(g) *Procedures for controlling contamination throughout the slaughter and dressing operation.* Official poultry slaughter establishments must develop, implement, and maintain written procedures to prevent contamination of carcasses and parts by enteric pathogens and fecal contamination throughout the entire slaughter and dressing operation. Establishments must incorporate these procedures into their HACCP plans, or sanitation SOPs, or other prerequisite programs. At a minimum, these procedures must include sampling and analysis for microbial organisms in accordance with the sampling location and frequency requirements in paragraphs (g)(1) and (2) of this section to monitor their ability to maintain process control.

(1) *Sampling locations.* Establishments, except for very small establishments operating under Traditional Inspection or very low volume establishments operating under Traditional Inspection must collect and analyze samples for microbial organisms at the pre-chill and post-chill points in the process. Very small establishments operating under Traditional Inspection and very low volume establishments operating under Traditional Inspection must collect and analyze samples for microbial organisms at the post-chill point in the process.

(i) Very small establishments are establishments with fewer than 10 employees or annual sales of less than \$2.5 million.

(ii) Very low volume establishments annually slaughter no more than 440,000 chickens, 60,000 turkeys, 60,000 ducks, 60,000 geese, 60,000 guineas, or 60,000 squabs.

(2) *Sampling frequency.* (i) Establishments, except for very low volume establishments as defined in paragraph (g)(1)(ii) of this section, must, at a minimum, collect and analyze samples at a frequency proportional to the establishment's volume of production at the following rates:

(A) *Chickens.* Once per 22,000 carcasses, but a minimum of once during each week of operation.

(B) *Turkeys, ducks, geese, guineas, and squabs.* Once per 3,000 carcasses, but at a minimum once each week of operation.

(ii) Very low volume establishments as defined in paragraph (g)(1)(ii) of this section must collect and analyze samples at least once during each week of operation starting June 1 of every year. If, after consecutively collecting 13 weekly samples, a very low volume establishment can demonstrate that it is effectively maintaining process control, it may modify its sampling plan.

(iii) Establishments must sample at a frequency that is adequate to monitor their ability to maintain process control for enteric pathogens. Establishments must maintain accurate records of all test results and retain these records as provided in paragraph (h) of this section.

(h) *Recordkeeping requirements.* Official poultry slaughter establishments must maintain daily records sufficient to document the implementation and monitoring of the procedures required under paragraph (g) of this section. Records required by this section may be maintained on computers if the establishment implements appropriate controls to ensure the integrity of the electronic data. Records required by this section must be maintained for at least one year and must be accessible to FSIS.