

Module 4a: Microbiological Testing—*E. coli*

Goal To inform inspectors of the regulatory and operational requirements that plants must implement for *E. coli*.

Objectives After completing this module, participants will be able to

1. Explain why *E. coli* testing is used.
2. State who will conduct *E. coli* testing.
3. Explain the difference between process microbiological control guidelines and performance standards.
4. Explain how industry defines greater than very low volume and very low volume plants.
5. List dates for implementation of *E. coli* testing according to plant volume.
6. List testing frequency based on plant volume.
7. List the species that must be tested.
8. Describe how industry may decide which of the three methods of sample collection to use for testing.
9. Name the sites of sample collection for cattle, swine, and turkeys.
10. List the plant recordkeeping requirements for its *E. coli* testing program.
11. State the options that industry may select for demonstrating statistically based process control.
12. Discuss *E. coli* sampling techniques recommended in the FSIS guidelines.

Introduction

E. coli testing is done by slaughter plant employees. Establishments are required to maintain sanitary conditions and use good manufacturing practices to avoid contamination with visible feces, ingesta, and the associated bacteria. Fecal contamination is one of the principal sources of pathogenic organisms that contaminate carcasses. The single best indicator of fecal contamination is *Escherichia coli*, Biotype I, also called generic *E. coli*, because it is commonly found in the intestinal tract of food animals.

E. coli testing by slaughter establishments is a plant verification activity designed to supplement the FSIS organoleptic inspection of the physical removal of visible contamination. This microbial testing verifies that the slaughter process with respect to prevention and removal of fecal material, ingesta, and associated bacteria is controlled.

Performance Criteria

E. coli testing is not a performance standard. It is, instead, an indicator of process control for fecal contamination or sanitary dressing in slaughter establishments. However, some performance criteria by which results can be evaluated have been developed for *E. coli* testing. These performance criteria were developed from the Agency's Microbiological Baseline Studies and will continue to be updated with further microbiological surveys. In some cases, data collected from the industry might even be used to develop performance criteria. Establishments must use statistical process control to evaluate their test results when they slaughter species for which the Agency has not developed performance criteria or when they use sampling techniques for which values have not yet been developed.

The *E. coli* performance criteria are not enforceable regulatory standards. Test results that show an establishment is meeting or exceeding the criteria provide evidence that the establishment is maintaining adequate process control for fecal contamination.

Species Tested

The requirements for *E. coli* testing currently include only establishments that slaughter any market classes of cattle, swine, chickens and turkeys. According to the regulations, “cattle” includes calves, cows, bulls, steers, heifers, and hide-on calves. “Swine” includes sows, boars, gilts, market hogs, suckling pigs, and pigs. “Chicken” includes broilers, light and heavy fowl, Cornish hens, and capons. “Turkey” includes hens, toms, heavy and light breeders, and young turkeys.

Sheep, goats, horses, ratites, quail, rabbits, and ducks are not required to be tested for *E. coli* at this time. If an establishment slaughters a non-amenable species, such as ratites, in greatest number and also slaughters an amenable species required to be tested, such as cattle, then testing is required for the amenable species.

If a combination of types of livestock or poultry is slaughtered, the establishment will sample only from the type it slaughters in the largest number. It is only necessary to sample one type of livestock or poultry to provide information that establishments can use to verify their process controls over sanitary dressing. For example, if an establishment slaughters both cattle and swine, and its yearly average shows that it slaughters mostly cattle, then it will conduct *E. coli* testing only on cattle.

In some plants the major species slaughtered might be a type of livestock that does not yet require testing. In that case, that establishment will not be required to conduct *E. coli* testing **on that species, but would be required to test the species specified in the regulations. For example, an establishment that slaughters mostly sheep and some cattle will be required to conduct *E.coli* testing on the cattle.**

Plant Size

For *E. coli* testing purposes, slaughter establishments are divided into two categories: very low volume plants (VLV) and greater than very low volume plants. The two categories of plants are based on an average annual slaughter volume.

Very low volume plants are described as follows.

Cattle: Annually slaughter less than 6,000 cattle

Swine: Annually slaughter less than 20,000 swine.

Cattle/Swine Combination: Annually slaughter less than a combination of 6,000 cattle and 20,000 animals total.

Chickens: Annually slaughter less than 440,000 chickens.

Turkeys: Annually slaughter less than 60,000 turkeys.

Chicken/Turkey Combination: Annually slaughter less than 60,000 turkeys and less than 440,000 birds total.

Effective Dates

Based on plant size, there are two separate dates for beginning *E. coli* testing programs in plants. Greater than very low volume slaughter establishments started *E. coli* testing first on January 27, 1997. Very low volume establishments started *E. coli* testing during their first full week of operation after June 1, 1997.

Testing Frequency

The regulation stipulates testing frequencies to be used by plants.

For greater than very low volume establishments the following frequency of testing is required.

Cattle:	1 test per 300 carcasses
Swine:	1 test per 1,000 carcasses
Chickens:	1 test per 22,000 carcasses
Turkeys:	1 test per 3,000 carcasses

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

Note: In smaller plants slaughtering no more than 50 animals per year, not more than 25% of the carcasses will be sampled.

In some cases an establishment operating under a validated HACCP plan may substitute an alternative frequency for the frequency in the regulation. This is allowed if the alternative frequency is an integral part of the establishment's verification procedures for its HACCP plan. Establishments currently using an alternative *E. coli* sampling frequency for process control purposes, but not yet under a HACCP plan, will have to test at the frequencies specified in the regulation unless they are granted an exemption by the FSIS Office of Policy, Program Development, and Evaluation in Washington, D.C. Field employees who have questions about alternative frequencies should contact the Technical Service Center for assistance.

Sampling Methods

There are three sampling methods an establishment may use to collect *E. coli* samples: excision sampling, sponging, and whole bird rinsing.

Excision sampling involves aseptically cutting a surface section from the carcass (8 x 6 x ½ inch thick for beef and 10 x 5 x ½ inch thick for swine) and either sending the excision sample for laboratory analysis or running the analysis in-house. Excising tissue from a carcass is, of course, a destructive method of sampling.

Sponging involves aseptically swabbing a sterile sponge on a surface of the carcass (10 cm x 10 cm for beef and swine, and 10 cm x 5 cm for turkey) and either sending the

sponge to the laboratory for analysis or running the analysis in-house. Sponging is a nondestructive method of sampling.

Whole bird rinsing involves shaking the whole carcass in a bag with a sterile sampling solution, collecting the rinse fluid, and either sending it to the laboratory for analysis or running the analysis in-house. This is also a nondestructive technique.

Establishments slaughtering cattle and swine may choose either excision sampling or sponging. Establishments slaughtering chickens must use the whole bird rinse. Establishments slaughtering turkeys may use either the whole bird rinse or the sponging method.

Sampling Sites and Locations

The regulation requires that samples be taken from specific locations in the plant. Samples from cattle and swine will be collected from carcasses in the cooler 12 hours or more after slaughter. Both chicken and turkey carcasses must be sampled after the chill tank, at the end of the drip line, or at the last readily accessible point prior to packing or cut-up. Any carcasses to be hot-boned should be sampled after final wash.

Samples must also be taken from specific sites on cattle and swine carcasses. The three sites from which either excision or sponging samples must be taken on cattle carcasses are the flank, the brisket, and the rump. In the case of hide-on calves, the three sites from which either excision or sponging samples must be taken are inside the flank, inside the brisket, and inside the rump. In the case of swine, the three excision or sponging samples must be taken from the belly, the ham, and the jowl.

For chickens, the whole bird is rinsed in a sterile solution and the rinse is sampled. For turkeys, a whole bird rinse may be used, or the company might elect to use the sponging technique. The sponging technique requires that two sites, the back and the thigh of the turkey, be swabbed. The size of the sponged area is a 5 cm x 10 cm area, which is smaller than for livestock.

Cattle and swine establishments may choose either excision of three sites or nondestructive sponging of three sites. If excision is selected, the establishment must use the performance criteria of “m” (minimum value) and “M” (maximum value) published in the regulations for evaluating test results. These criteria were developed from the Agency’s Microbiological Baseline Studies. If the sponging method is selected, the establishment must use statistical process control for evaluating test results.

Chicken slaughter establishments must use the whole bird rinse method and are required to use FSIS “m” and “M” performance criteria. Baseline studies have not been established for turkeys, however, and all turkey slaughter establishments must use statistical process control methods to evaluate test results whether they use the whole bird rinse method or the sponging method of sampling.

FSIS is currently conducting additional baseline studies to cover the sponging method in cattle, swine, and both turkey sampling methods. When these studies are completed around the middle of 1998, “m” and “M” values will be established for these methods of sample collection and provided to slaughter establishments. Until the baseline data is complete, FSIS is publishing interim sponging “m” and “M” criteria which establishments slaughtering swine and cattle may use.

Carcass Selection

Regulations require that carcasses to be sampled be selected at random. Different methods of selecting the specific carcass may be used. For example, random number tables, computer-generated random numbers, or drawing cards may be used.

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 swine, each whole carcass represents one unit eligible for sampling. The half-carcass or carcass eligible for sampling should be selected from those in the cooler 12 or more hours after slaughter. This location may be at the transfer

chain, a rail, or a similar place that contains carcasses that have chilled 12 hours or more. In cases where the carcasses are inaccessible in the cooler, or employee safety is jeopardized, it is acceptable to select random samples before carcasses enter the cooler. Selected carcasses may be chilled in a more accessible area and sampled after 12 hours. Use a similar random sample selection method in those establishments conducting hot-boning operations, but select the samples after the final wash.

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

The half-carcass or carcass for sampling must be selected at random from all the eligible half-carcasses or carcasses, so if there are multiple lines or chillers, randomly select the line or chiller from which the sample will come during each collection interval.

Poultry carcasses will be selected at random after chilling, at the end of the drip line, or at the last readily accessible point prior to packing or cut-up. A whole, untrimmed carcass (with or without the neck) is required for sampling. The time of sampling is based on the appropriate sampling frequency. For example, based on the frequency of sampling, the company might identify a carcass at the predetermined collection point -- a carcass which was selected by the random number method -- then count back five carcasses and select the next carcass for sampling. If the sixth carcass is not a whole, untrimmed bird, count back another five carcasses for sample selection.

Use a similar sample selection method in poultry establishments conducting hot-boning operations on whole or split carcasses. Samples are selected at the end of the slaughter line prior to chilling.

Sampling Guidelines

FSIS assumes that meat plants following the "Guidelines for *E. coli* Testing for Process Control Verification in Cattle and Swine Slaughter Establishments" and poultry plants following the "Guidelines for *E. coli* Testing for Process Control Verification in Poultry Slaughter Establishments" 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 plant may choose to use a comparable sampling technique and not be out of compliance. With this in mind, let's discuss the excision, sponging, and whole bird rinse sampling techniques described in the guidelines for cattle, swine, and poultry.

Sampling Techniques

For all three types of sampling, aseptic techniques should be used. Extraneous organisms from the environment, hands, clothing, sample containers, sampling devices, etc., may contaminate samples and lead to non-representative analytical results. It is necessary to use aseptic sampling techniques and clean, sanitized equipment and supplies.

An area should be designated for preparing sampling supplies. A stainless steel, wheeled cart or table would be useful during sampling. A small tote or caddy could be moved to the location of sampling and used for carrying supplies. Sample bags could be placed on the tote or caddy when sterile solutions are added to the bags.

Sterile gloves should be used for collecting samples. Nothing should contact the external surface of the glove except the exposed sample being collected or the sterile sample utensil, like a specimen sponge. Keep in mind that the outside surfaces of the sample container are not sterile. The following procedure for putting on sterile gloves can be followed when collecting samples.

The package of sterile gloves is peeled open from the top without contaminating the exterior of the gloves by touching them, breathing on them, or contacting them.

A glove is removed by holding it by the inner surface of the wrist-side opening. Any contact with the outer surface of the glove must be avoided. The washed and sanitized hand is inserted into the glove. Care should be taken not to puncture the glove.

The exterior surface of the glove must not be contaminated. This step is repeated for the other hand.

If at any time there is concern that a glove might be contaminated, this entire process must be repeated with a sterile pair of gloves.

Let's begin by describing the excision method of sample collection used in cattle and swine slaughter.

In preparation for sampling, plant personnel should assemble sample bags, a cleaned and sanitized knife, a cleaned and sanitized hook or hemostat-type forceps, sterile surgical gloves, a clean one-gallon or larger container, and FSIS-approved bleach. Potable water should be readily accessible. A wheeled ladder, sampling platform, or stepladder will help plant employees safely reach the rump or ham of the carcass. At least one assistant to the sampler makes the job much easier because s/he can, at a minimum, hold the sample bag. Prior to the employee climbing the ladder to sample the rump or ham, it is acceptable, for safety reasons, to sheath the knife in the midline where it can be safely retrieved for sampling from the ladder.

First, the plant employee should label three sample bags. For cattle, label one brisket, one flank, and one rump. For swine, label one jowls, one belly and one ham. It is important to apply the labels to the bags at normal room temperature because they will not stick if applied in the cooler or to a cold surface.

Measure the length of the blade of the knife used for sample collection. Employees can use this measurement to estimate the size of the 8 inches long by 6 inches wide samples for cattle, and the 10 inches long by 5 inches wide samples for swine.

Next, the sample taker should prepare the chemical sanitizing solution by adding two to four ounces of bleach to one gallon of potable water in the clean container. This solution should give a strength of 1000-2000 ppm hypochlorite, which is strong enough to sanitize properly even in the presence of some organic matter. The bleach solution should be prepared immediately before going into the cooler for sampling because it will lose its strength if left standing for a while.

To collect the sample, the assistant should immerse the entire sampling tool, including the handle, into the bleach solution for one to two minutes. The assistant should put on sterile gloves before carefully retrieving the sampling tools from the bleach solution. S/he should then shake the excess solution from the utensils, being careful not to contaminate the sampling end of the tools.

The sample taker should put on sterile gloves before touching the sanitized instruments or the carcass.

The sample taker locates the three carcass sampling sites. In cattle, collect the brisket, flank, and then the rump samples in that order of least to most contaminated. In swine, the samples will be collected from the belly, the ham, and then the jowls.

For cattle, the knife is used to mark out the borders of the sample from one side of the carcass. It is not necessary to cut very deep since only the skin surface is needed for the sample. A 1/2 inch thickness is sufficient. The samples should be in one piece, so the samples should be carefully excised using the knife and sanitized hook or forceps. The employee rolls or folds the sample so that it fits easily into the sample bag. S/he must not cut the samples into strips. S/he places the samples inside the bag without touching the outside of the bag or the assistant holding the bag. It would be best if the assistance closes the sample bag.

The sample taker must not put the knife back into a scabbard between samples. It is not sanitized and there are still two more cattle samples to take.

For swine, the skin of both jowls, the belly, and the ham will be collected. A 1/2 inch thickness is sufficient. Each sample must provide a surface area equivalent to a 10 inch by 5 inch square. The skin of each sample should be carefully removed in one piece using the knife and the sanitized hook or forceps. The employee rolls or folds the sample so it fits easily into the sample bag. S/he must not cut the samples into strips. S/he places the samples inside the bag without touching the outside of the sample bag or the assistant holding the bag. It would be best if the assistant closes the sample bag.

The sample taker must not put the knife back into the scabbard. It has not been sanitized and there are still two more swine samples to take. The knife may be sheathed in the carcass where it can be reached from the ladder or platform. To do this, it is inserted perpendicular to the floor, perhaps into the teat line of the medial flank.

In the event a sample is dropped, that sample is discarded. Go to the companion carcass side and sample the area corresponding to the dropped sample. If gloves and/or instruments touch any surface other than the carcass or the sanitized instruments, gloves will need to be changed and/or instruments sanitized.

Next, let's review the sponging method of sample collection used for swine and cattle slaughter establishments. Let's look at swine first.

The sterile sampling supplies for swine are a pair of gloves, a sponge in a Whirl-pak bag, a template, and 10 milliliters of a sterile sampling solution.

Recall that prior to actually taking the sample, the plant employee had randomly determined where and at what time to take the sample, gathered the sampling supplies, labeled the sponge bag, and prepared supply-contact surfaces, as well as his or her hands.

At the sample location, the plant employee chooses the sample by counting back 5. S/he allows sufficient room to safely collect the sample beginning with the belly, continuing to the ham and, finally, the jowl. By wiping with the sponge in this order of "least to most" contaminated, spreading contamination on the carcass is avoided.

A ladder or similar equipment needs to be positioned near enough to the carcass to easily and safely sponge the ham.

It is important to avoid touching sterile surfaces from this point on during the sampling procedure.

The sponge bag is opened by holding a corner of the wire closure and tearing off the clear perforated strip at the top of the bag. The two white tabs should be pulled to open the mouth of the bag. It is important to **not touch the inner surface of the bag at any time!** The employee removes the cap from the sterile sampling solution container and pours all of the solution into the sponge bag. S/he should hold the bag closed and massage the sponge through it. This hastens the sponge's absorption of the solution.

When the sponge is fully moistened, the employee must carefully push it to the upper part of the bag and open the bag. The wire closure should keep the bag open, as well as maintain the sponge in place at the opening. This is set aside, with care **not** to contaminate the sponge.

Next, the template bag is carefully opened and set aside. Care is also taken to avoid contaminating the template.

The sterile gloves are put on as discussed earlier. The employee carefully removes the sponge without touching the bag. This is done with the hand used to sponge the carcass. We will call this the "sampling hand".

With the other hand, the employee removes the template from its bag. S/he must only handle it by the outer edges.

The employee lays the template over the section of the belly to sample. This is close to the underarm section. S/he must not touch the enclosed sampling area or the inner edge of the template with his or her hands. One of two sponging techniques may be used. Either of these may be used on the different sites, but only one is used per site. One way is to start at the top of the area in the template. The employee wipes down firmly but not so hard as to crumble the sponge. An even pressure is used that would be sufficient to remove dried blood. S/he must lift the sponge at the end of one wipe and then rotate it. If the sponge isn't lifted during the rotation, the sponge may contact other surfaces. It's important to always have the same side of the sponge in contact with the carcass. This is done for 10 vertical wipes, then s/he turns the sampling hand and does 10 horizontal wipes. Each pass of the sponge counts as one wipe. It may be necessary to roll the template when sponging since the carcass surface is not flat.

Next, the employee transfers the template to his or her "sampling hand". S/he carefully climbs the ladder or platform. Available hand holds or rails should be used with the free hand. Once s/he is at a convenient and safe height for sampling the ham, the template must be transferred back to the other hand and laid over the ham. The inner edges of the template or section of the ham sampled must not be touched. The same side of the sponge used for the belly is used on the ham.

For the second sponging technique, s/he may wipe downward. When s/he reaches the bottom, the sponge is lifted and started at the top again. This is done 10 times vertically and another 10 times horizontally. The sponge must not contact anything but the area inside the template and, of course, the sampling hand.

The template is transferred back to the sampling hand and the employee climbs down. The sponge is turned over and the **unused** side of the sponge is used to properly sponge the jowl area. The sponge is then put back into its bag. The excess air is expelled and the top edge of the bag is folded over 3 or 4 times. Then, the wire is folded back against the bag.

Let's move on to sampling beef half-carcasses. The procedures for randomly selecting the location and the sample, and then preparing the supplies, sampling area and the employee are the same as for swine. The supplies are also the same.

Sponging samples are taken at the flank, brisket, and rump, in that order from "least to most" contaminated. A ladder or such is needed to be able to easily and safely sponge the rump. Just as was done for swine, the sponge bag is opened; the sponge is moistened with the sterile sampling solution; the template bag is opened; the gloves aseptically put on; the template is laid over the sample area; and the sampling area is sponged 10 times each vertically and horizontally. The template may need to be rolled. This sponging sequence is repeated for the brisket area, using the same side of the sponge.

The employee then carefully transfers the template to the "sampling hand". S/he safely climbs the ladder. S/he puts the template back into the other hand. The sponge must be turned over and the rump area sampled with the **unused** side of the sponge.

The employee climbs down the ladder, again using the handrail. Care not to contaminate the sponge must be taken. The sponge is put back in its bag, then excess air is expelled and the bag sealed. S/he cleans up the supplies and stores the sample.

Now we shall turn our attention to poultry. Let's start with turkeys. Turkey carcasses may be sponged or rinsed. We shall cover the sponging method first.

Sponge samples for turkeys are taken similarly to livestock sponge samples. However, the supplies are two pair of sterile gloves, a sponge in a Whirl-pak bag, 10 milliliters of a sterile sampling solution, and a 5 cm by 10 cm sterile template. The sterile solution must be clear. It needs to be refrigerated and chilled before use.

The supplies, sampling area and employee are prepared the same as for sampling livestock. But to prevent the turkey carcass from slipping while sampling, the employee

may want to place clean paper towels, tray-pack absorbent pads, or a sanitized wire rack on the sanitized sampling work surface.

There are only two sites on the turkey carcass to sample. These are the back and the thigh.

The employee puts on a pair of sterile gloves using the method described earlier. S/he should go to the end of the drip line or chiller and randomly select an intact turkey carcass. It is important to obtain a **whole** carcass. This means one that has not been trimmed, whether or not it has the neck attached. If the sixth carcass is not whole, count back 5 more and choose *that* sixth one. This is repeated as often as necessary until s/he can collect a **whole** carcass. For safety, s/he waits for the bird to drop off the line before picking it up. The employee must grasp the turkey by its drumsticks and be sure not to touch the back or thigh area.

The carcass is taken to the sample area and carefully placed breast down on the towels. It is okay if the carcass leans on one side of the breast. But the back and the thigh to be sampled must not touch any supporting surfaces.

The sample taker removes and discards the gloves. They are now contaminated from handling the carcass.

S/he must be very conscious to avoid contaminating sterile surfaces from this point on during the sampling procedure.

Just as was described for swine and beef, the sponge bag is opened. The employee pours all of the sterile sampling solution into the sponge bag, and completely moistens the sponge. S/he pushes the sponge to the top of the bag, sets it aside, opens the template bag and sets it aside also. If the template is not in a sterile package, it must be sanitized before use. It must be completely dry before it is used on the turkey.

S/he then puts on the second pair of sterile gloves and carefully removes the sponge without touching the bag. This is done with the hand that will be used to sponge the carcass. Remember, we called this the “sampling hand”.

With the other hand, s/he removes the template from its bag and only handles it by the outer edges.

The template is laid over the section of the back to sample. This is over the vertebral column and just in front of the tail. The template should be evenly placed on either side of the vertebral column. The enclosed sampling area or the inner edge of the template must not be touched with the hands. Either of the two sponging techniques mentioned earlier with the swine may be used to make the 10 horizontal and 10 vertical wipes. The same side of the sponge must always be in contact with the carcass. The template may need to be rolled since the turkey surface is not flat.

Next, the template is placed over one of the thighs. The sample area starts at the hip joint and extends to cover the thigh. The sampler must turn the sponge over and use the “clean” side of the sponge, holding it by its edges only. The sponge cannot contact anything but the area inside the template and, of course, the sampling hand.

After sponging the thigh, the sampler sets the template aside and puts the sponge back into its bag. S/he expels the air and seals it just as was done for swine and beef.

The turkey is returned to the point from which it was collected. The supplies are cleaned up and the sample stored.

We shall move on now to sampling chicken carcasses. Turkeys may also be sampled using the whole bird rinse. No sponge is used here because the whole carcass is rinsed in a bag with sterile sampling solution. Random selection of the location, time, and carcass is similar to that of the other species discussed. Again, a **whole** carcass is needed (with or without the neck).

The supplies needed for a whole bird rinse are a pair of sterile gloves, one large and one small zip-lock bag, 400 milliliters of sampling solution (600 ml for turkeys), and a sealed container. The sampling supply contact surfaces and the sampler are prepared as discussed earlier.

The sample taker must carefully open the large zip-lock bag. **The inside must not be touched** since it is sterile. The opened bag can be laid on its side on a sanitized surface.

The sterile gloves must be put on using the aseptic technique. S/he will use only one hand to get the selected carcass by the legs and remove it from the line. For safety, the sampler should wait for the bird to fall from the shackles before picking it up. Excess fluid must be drained out.

With the other hand, the sampler picks up the open sample bag and places the bird in it so that the vent and legs are toward the bag opening. S/he must not touch the inside of the bag.

The bottom of the bag can rest on a sterile surface, with the bag opened. The employee uncaps the sterile sampling solution and pours all of it into the carcass cavity. Most of the air is expelled from the bag and it is zipped closed. S/he supports the bottom of the bag with one hand and the top with the other. The bird in the bag is inverted 30 times. This takes about one minute. This ensures that all interior and exterior surfaces are rinsed thoroughly.

The sampler sets this bag aside and opens the small zip-lock bag. S/he removes the cap from the sample container and places the cap in this bag to prevent it from getting contaminated. **Neither the inside of the cap nor its container can be touched.**

Now, s/he opens the bag containing the bird. With one hand, s/he holds the carcass through the bag by the leg. With the other, s/he holds the top corner of the bag to form a “V” at the bottom corner. Using this “V” as a pour spout, the sampler carefully pours the

rinse fluid into the open sample container. It is only filled to the 30 milliliter volume line. The bag is set aside again. The sampler takes the cap out of the bag and closes the sample container of rinse fluid. **S/he must not touch the inside of the cap.** The cap must be secure.

The sample container is then put into the small zip-lock bag, the excess air is expelled, and the bag is zipped closed. The remainder of the rinse fluid is poured into a drain and the carcass is put back at the point where it had been collected. S/he cleans up the supplies and stores the sample.

We have previously mentioned storing the sample. Samples need to be maintained at refrigeration temperatures until the analysis is run. They must not be frozen. The analysis must be run as soon as possible. If the establishment does not have an in-house laboratory, then the sample must be sent to a lab for analysis. Some samples may be hand-carried, but others might need to be mailed.

Samples to be mailed must be shipped by overnight delivery on the **same day that the sample** was taken. The sample has to be as fresh as possible for analysis.

To pack a sample, the plant employee will gather the sample, shipping container and frozen gel-packs. S/he will place the sample in the shipper, put a cardboard pad on top of the sample and the gel-pack on top of the cardboard. The gel-packs must not contact the sample container, because it could lower the sample temperature too much and possibly kill bacteria.

Next, s/he should place a foam plug on top and press it down to minimize headspace.

Sample Analysis

After samples are collected they must be analyzed. The establishment may analyze its samples in its own in-house laboratory or it may ship the samples to an outside laboratory. Regardless of who does the analysis, test results must come from one of the generic *E. coli* quantitation methods found in the Official Methods of Analysis of AOAC

International or from a method that is approved and published by a scientific body based on the results of a collaborative trial conducted in accordance with an internationally recognized protocol on collaborative trials.

Recordkeeping

Establishments must keep *E. coli* test results data. Each test result must be recorded in terms of colony forming units per square centimeter (cfu/cm²) for excision and sponging results and in colony forming units per milliliter (cfu/ml) for whole bird rinses.

Remember that “m” and “M” criteria and sample data applies to swine and cattle sampling if the excision sampling technique is used and to chickens using the whole bird rinse technique. If, however, the cattle or swine establishment is using the sponge technique, statistical process control must be used, not the “m” and “M” criteria. All turkey establishments must use statistical process control, since “m” and “M” criteria are still being developed.

Charts or tables of the sample results data must show at least the most recent 13 test results.

Let’s address the Agency performance criteria known as “m” and “M”. The *E. coli* performance criteria, or “m” and “M”, are not enforceable regulatory standards. Instead, guidelines were designed to assist establishments who are striving to ensure that fecal material, ingesta, and associated bacterial contamination is prevented and reduced on carcasses.

The “m” and “M” criteria are intended to provide each establishment with a benchmark for assessing its performance against a national norm.

E. coli test result levels are separated into three categories for the purpose of process control verification: acceptable, marginal (represented by “m”), and unacceptable (represented by “M”).

Marginal results (“m”) are those within the worst 20% of overall industry performance in terms of *E. coli* counts. More than three marginal results in the last 13 tests are deemed unacceptable. Results worse than the 98th percentile (“M”) are within the worst 2% of overall industry performance. Any single test result exceeding “M” is deemed unacceptable.

The “m” and “M” values are applied to a moving window of 13 test results. That means each day only the last 13 test results are evaluated to determine if the performance criteria are met. Any single test result exceeding “M” is unacceptable. More than three marginal results in the last 13 tests are also unacceptable.

This table shows the “m” and “M” values for *E. coli* performance criteria set forth by the Agency for the species that have had a baseline study completed.

Slaughter Class	Acceptable Range	Marginal Range (m)	Unacceptable Range (M)
Cattle.....	Negative	Positive value not more than 100 cfu/cm ²	Above 100 cfu/cm ²
Chicken.....	100 cfu/ml or less	Over 100 cfu/ml but not over 1,000 cfu/ml	Above 1,000 cfu/ml
Swine.....	10 cfu/cm ² or less	Over 10 cfu/cm ² but not over 10,000 cfu/cm ²	Above 10,000 cfu/cm ²

To illustrate the use of this table, consider a steer/heifer slaughter establishment. An *E. coli* test result for this establishment is acceptable if it comes back negative, marginal if the test result is positive but not above 100 cfu/cm², and unacceptable if it is above 100 cfu/cm².

The company may document its test results in table form, in chart form, or both.

The table on the following page is representative of tables that may be used by plants for recordkeeping.

Test #	Date	Time Collected	Test Result (cfu/cm ²)	Result unacceptable?	Result marginal?	Number marginal or unacceptable in last 13	Pass/Fail ?
1	10-07	08:50	10	No	Yes	1	Pass
2	10-07	14:00	Negative	No	No	1	Pass
3	10-08	07:10	50	No	Yes	2	Pass
4	10-08	13:00	Negative	No	No	2	Pass
5	10-09	10:00	Negative	No	No	2	Pass
6	10-09	12:20	Negative	No	No	2	Pass
7	10-10	09:20	80	No	Yes	3	Pass
8	10-10	13:30	Negative	No	No	3	Pass
9	10-11	10:50	Negative	No	No	3	Pass
10	10-11	14:50	Negative	No	No	3	Pass
11	10-14	08:40	50	No	Yes	4	Fail
12	10-14	12:00	Negative	No	No	4	Fail
13	10-15	09:30	Negative	No	No	4	Fail
14	10-15	15:20	Negative	No	No	3	Pass
15	10-16	07:30	Negative	No	No	3	Pass
16	10-16	11:40	Negative	No	No	2	Pass
17	10-17	10:20	120	Yes	No	3	Fail

Looking at this plant record you can make the following observations. First, test number eleven conducted on October 14, at 8:40 A.M., documents the fourth test result in the marginal (“m”) range. Therefore, the plant has entered 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.

Secondly, tests number twelve and thirteen are negative, therefore, in the acceptable range. However, if you consider the last 13 test results, or the 13-test moving window, there are still more than three results in the marginal range. The company has marked its record to show that it is still in a failing mode because of the 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. Do not treat the failure documented on the table for tests twelve and thirteen as evidence of a new problem. The log or documentation of corrective action taken for the first failure at test number eleven should be adequate to verify that the problem was addressed.

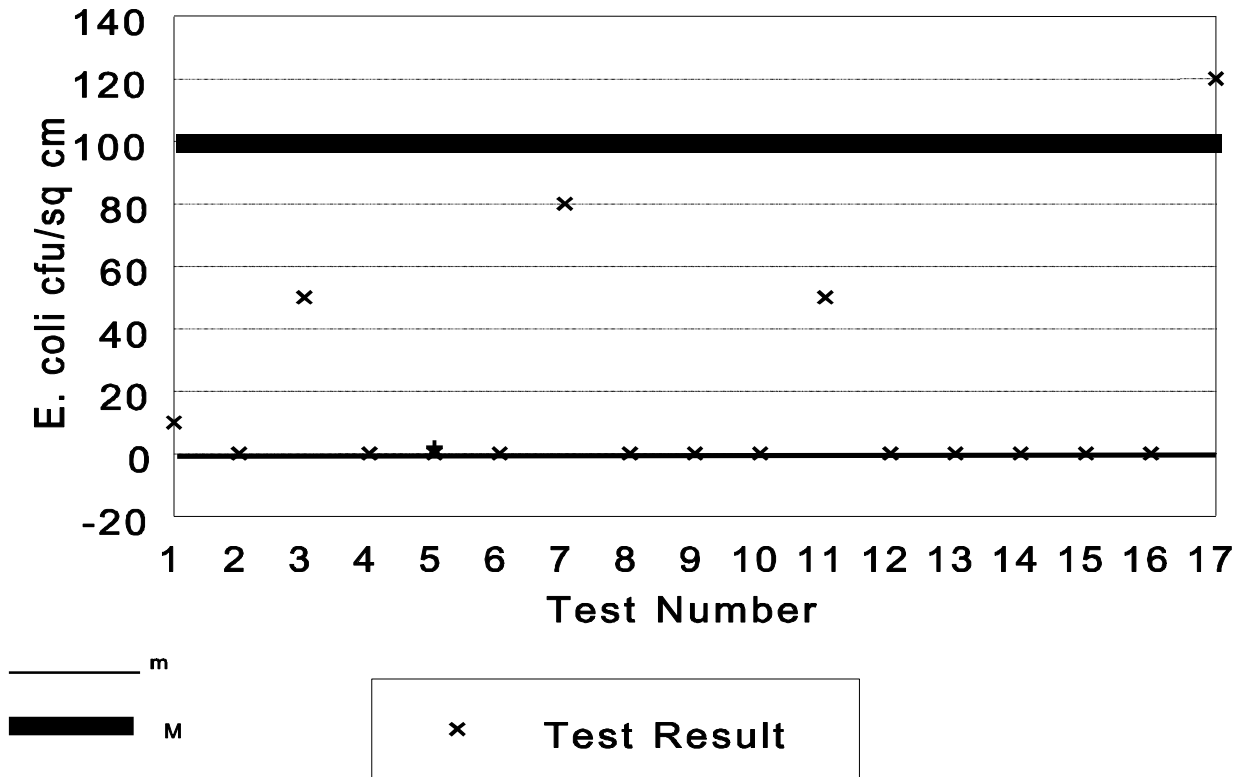
Third, notice that at test number fifteen (October 15 at 3:20 P.M.), the number of marginal results in the last thirteen tests window is reduced to three. The marginal result for test number one (October 7, at 8:50 A.M.) is dropped and replaced by an acceptable result as the 13-test window moves ahead one test.

The fourth observation you could make from this company record is that the test result for test number seventeen (October 17, at 10:20 A.M.) exceeds 100 cfu/cm², the “M” value for cattle. Any result over 100 cfu/cm² is automatically unacceptable. It only takes one test in the “M” range to indicate the establishment may not have adequate process control.

Another method the company can use to document its *E. coli* test results is a control chart. The seventeen test results written in the table you just looked at are plotted on the following control chart with an “X” marking each value. The vertical Y-axis shows how many colony forming units (cfu) of *E. coli* were found in a square centimeter (cm²) of media at the laboratory. The horizontal X-axis indicates the test number. Marking an “X” at the point the X and Y-axes cross plots each test value. For ease of reading, the company has drawn a line on its chart 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



Statistical Process Control

As we mentioned earlier, cattle and swine slaughter establishments that use the sponging technique for sampling and all turkey slaughter establishments may not use the “m” and “M” performance criteria we just discussed. The Agency has not completed its baseline studies that will set the “m” and “M” limits for sponge sampling or any turkey sampling method. In the meantime, these establishments are still required to document their test results on a process control chart and use an acceptable method of statistical process control to evaluate their results. A company will collect test results until it can set its own upper control limit based on its history of *E. coli* test results. If data points on

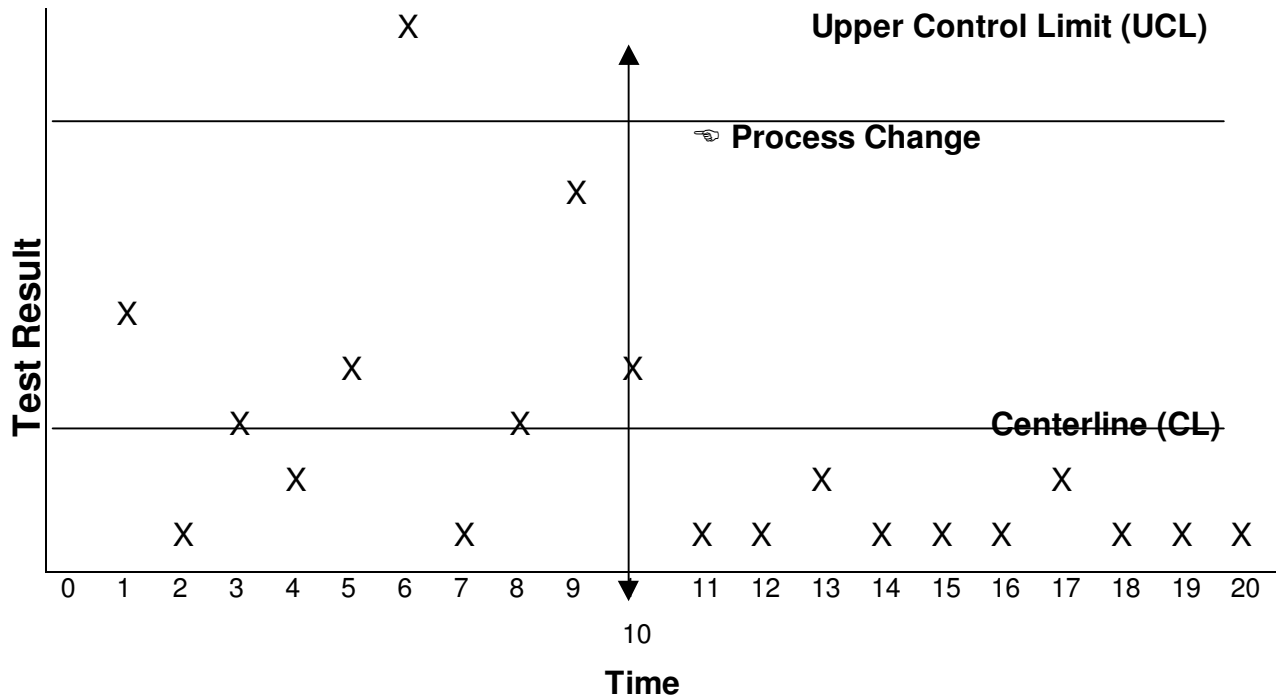
the company chart stay within the control limits set by the company, the process is considered in control.

The statistical process control approach required by FSIS is based on the principle that every product is produced by a process. All processes are subject to variation. Variation should be understood and controlled by statistical methods. A process that is in control is stable in terms of average level and degree of variation, i.e., it is predictable within limits and, thus, is “doing its best”. Processes that have not been subjected to analysis are not likely to be in control. Control is attained, often by degrees, by detecting and eliminating special causes of variation -- those not present all the time or not affecting all product output. This involves initially evaluating data to determine process capability (the typical process performance level), then checking subsequent data to see if they are consistent with this baseline level, i.e., the process is in control and variations are within normal and acceptable limits. This is accomplished by checking for unreasonably high results, trends, etc., and looking for and correcting problems in the process when these signals occur.

Look at the example of a statistical process control chart on the next page. Notice that the company has plotted the test result in terms of time along the horizontal X-axis against test results along the Y-axis. This establishment has set a centerline value for its process control. This line indicates the center point of the range considered acceptable by the company. The upper control limit line marks the highest test result value that is considered acceptable by the company. Notice that the test result shown at test number 6 is above the upper control limit. The company recognized this result as the likely presence of a special cause or causes of variation in its process that needed to be tracked down, eliminated, and prevented from recurring. The chart was marked with a vertical arrow to show the point at which a change was made in the process. According to the chart, the correction was effective because the following test results were back in the acceptable range.

Sample Control Chart

Following process Change



When FSIS completes its baseline studies for sponge sampling of cattle, swine, and turkeys, and its baseline studies for the whole bird rinse sampling method for turkeys, new “m” and “M” values will be implemented in plants currently using statistical process control.

Official establishments are not required under the new rule to maintain a file of laboratory reports received from either an in-house laboratory or an outside laboratory. They are only required to keep a table or a chart of the results.

Whenever a plant determines that its *E. coli* test results do not meet “m” and “M” performance criteria it must take corrective action to bring the process back into control. In the case of plants using statistical process control, when *E. coli* test results do not meet *E. coli* limits set by the plant, corrective action to regain process control must be taken. Although the plant is required to make corrections to its process to regain control of contamination, it is **not** required to document those corrective actions.

When establishments do not evaluate their test results (§318.94(a)(5) or §325.10), they might not be maintaining process controls sufficient to prevent fecal contamination. The District Office will be notified of these instances and will take further action as appropriate to ensure all applicable provisions of the law are being met.

FACT SHEET: E. COLI TESTING, VERY LOW VOLUME ESTABLISHMENTS

The following information is a summary of *E. coli* testing requirements that very low volume establishments must meet. For additional details copies of the regulations and the Technical Amendment, including sampling guidelines are enclosed.

Species Required to Sampled and Tested for *E. coli*

All market classes of cattle and swine (for example, including calves, sows, boars) and all market classes of chickens and turkeys (for example, including fowl and young toms) are required to be sampled and tested for *E. coli*.

In establishments slaughtering more than 1 type of poultry or livestock, for example cattle and swine, then the establishment must collect samples from the type it slaughters in the largest number.

Testing Requirements for Market Classes

For cattle and swine two sampling techniques, sponging or excision may be used. If sponging, a non-destructive swabbing, is used then test results are evaluated using Statistical Process Control. If excision sampling (slicing a 5 X 7 X .5 cm area) is used, then the m/M criteria contained in CFR 310.25, must be used to evaluate test results.

For chickens and turkeys, rinsing of whole birds with buffer is the sampling technique. It is expected that sponging of turkeys will also become available in the future. For chickens, the m/M criteria contained in CFR 381.94, must be used to evaluate test results and for turkeys, Statistical Process Control must be used to evaluate test results.

Statistical process control involves initially evaluating data to determine the typical process performance level, then checking subsequent data to see if these are consistent with the baseline level. This is accomplished by checking for unreasonable high results, trends, etc., and looking for and correcting problems in the process when these signals occur.

Sampling Frequencies

Very low volume establishments must begin sampling the first full week they operate after June 1 and continue collecting one sample per week in each week they operate until the sampling requirement of 13 samples are completed.

Sampling Sites

Three sampling sites on each swine and cattle carcass are required to be sponged or excised. For cattle the flank, rump and brisket are sampled. For swine the belly, ham and jowls are sampled.

Sampling Locations

Carcasses should be sampled after chilling for 12 hours (livestock) or after exiting the chilling tanks (poultry). In establishments that slaughter, dress or chill types of livestock or poultry by using nontraditional methods, such as hot boning of swine and poultry and chilling of split turkey carcasses, samples may be collected after the final wash.

Two guidelines to assist establishments, especially small businesses, in meeting the proposed sampling and testing requirements are enclosed. Guideline A is titled, "Guidelines for *Escherichia coli* Testing for Process Control in Cattle and Swine Slaughter establishments." Guideline B is titled, "Guidelines for *Escherichia coli* Testing for Process Control in Poultry Slaughter Establishments."