Guidelines for *Escherichia coli* Testing for Process Control Verification in Cattle and Swine Slaughter Establishments

INTRODUCTION

Under the Pathogen Reduction/HACCP Regulation, slaughter establishments are required to test carcasses for generic *E. coli* as a means of verifying process control. This document outlines sampling and microbial testing procedures that would meet this requirement. These guidelines may be helpful to your company microbiologist or analytic laboratory. This document is a supplement to the Regulation but not a substitute; in-depth details of microbial sampling and testing may be found in the Regulation.

Background

These guidelines describe a nondestructive sponge technique for sample collection from raw cattle and swine carcasses. The Pathogen Reduction/HACCP Regulation anticipated that this nondestructive technique would be used with samples taken from the same point in the slaughter process as were the samples in the FSIS Nationwide Microbiological Baseline Data Collection Programs. It was envisioned that a conversion factor would correlate sample results from the sponge method to results from the excised-tissue method used in the baseline studies. Performance criteria derived from the baseline studies - with acceptable, marginal, and unacceptable ranges - would then be applied to establishment sample results obtained by the sponging method. Analysis of recent data, however, has not been able to determine such a conversion factor for the two methods. For this reason, the regulatory criteria described in the Regulation remain relevant only to excised-sample results, upon which they were based.

Consequently, the Agency is requiring that establishments test for generic *E. coli* and employ standard statistical process control measures to demonstrate that their operations are in control, until such time as new baselines are established with the sponging method. Also provided here in an Addendum are the sampling and analytical techniques, using excised tissues, that were employed in the FSIS baseline studies; establishments may use the excised-tissue technique and apply the performance criteria originally defined for their specific operations.

Statistical Process Control - An Overview

The statistical process control approach required by the Agency is based on the principle that every product is produced by a process. All processes are subject to variation, which 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 is thus "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), and 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.

It is important to recognize that an in-control process may not necessarily result in product of the desired quality improvements may be needed or the entire process may require reconsideration. Problems in a process may stem from many sources, for example: inadequate knowledge of how a process should work or how a specific process is performing; errors or deficiencies in executing procedures; failure to recognize the need for preventive measures; unnecessary complexity in the process; and uncontrolled variation among inputs.

Specific techniques of statistical process control include the time plot, which charts measurements over time; this is the first technique to use with data collected over time and analyzed for patterns. A further development is the control chart, which plots data over time but also displays an upper control limit for specific measurements, and a centerline, above and below which there is an equal number of sample results (the centerline is in effect a median average). 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. Control charts have two essential uses: after-the-fact analysis of process performance and gaining and maintaining control of a process. In most situations more than one type of control chart would be applicable; detailed information can be found in texts on statistical quality control, under the topic "control charts."

In general, statistical process control techniques help to provide experience in "process thinking" (a central tenet of HACCP), develop an historical record of performance, evaluate the long-term stability of a process and determine process capability (i.e., how it is actually working), and judge the effectiveness of process improvement actions.

Generic *E.* coli testing conducted as part of statistical process control will not be directly useful for attaining and maintaining control of a process, as test results will come from the end of the process and in any case would not be timely enough; observations made earlier in the process would be more useful for attaining and maintaining control. Rather, *E.* coli testing would serve to verify process control. Process control techniques, applied and verified in this manner, would accomplish the essential intent

of the Regulation by integrating process control and microbial testing into slaughter operations.

GUIDELINES FOR SAMPLE COLLECTORS/MICROBIOLOGISTS

Pre-sampling Preparation

Sample collection shall be conducted by the individual(s) designated in the establishment's written procedures for microbiological sampling, as required by 9 Code of Federal Regulations (CFR) Part 310.25(a)(2)(i). These procedures shall also specify the location of sampling, the random sample selection method chosen by the establishment, and sample handling procedures that will ensure sample integrity.

Before beginning sample collection, assemble sampling supplies, such as sterile gloves, sterile sampling solutions, hand soap, and sanitizing solution, as well as specific materials needed for sampling different carcass types (i.e., specimen sponges in bags and template for sampling cattle or swine carcasses).

For cattle and swine carcass sampling, a template is recommended to mark off the area to sample. The template can be made of any suitable material, such as metal or aluminum foil, brown paper, or flexible plastic. Some disposable templates may be sterilized and individually prepackaged. To make a reusable template, cut out a 10 centimeters (cm) x 10 cm (3.94 inches x 3.94 inches) square from a sheet larger than the area to be sampled. (See Figure 1). If a reusable template is used, it will need to be sanitized with an approved sanitizing solution [e.g., hypochlorite (bleach) solution or alcohol]; however, the template needs to be dry before it is placed on the carcass. Aluminum foil or paper templates can be used once and discarded; foil or paper used for the template should be stored so as to prevent contamination. Since the area enclosed by the template will be sampled, take care not to touch this area with anything other than the sampling sponge. Using dirty or contaminated material may lead to non-representative results. If an autoclave is available, paper or aluminum foil templates can be wrapped in autoclavable paper and sterilized.

Sterile sampling solutions, such as Butterfield's phosphate diluent (BPD) or buffered peptone water (BPW), can be stored at room temperature; however, at least the day before sample collection, check such solutions for cloudiness and do not use solutions that are cloudy or turbid or that contain particulate matter. Place the number of containers of sampling solution that will be needed for the next day's sampling in the refrigerator.

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 service to the laboratory. A sample should arrive at the laboratory and be analyzed no later than the day after it is collected.

If sample collection, pick-up or shipment, and laboratory analysis cannot be carried out within this timeframe, the carcass selected for sampling

should be held until the process can be accomplished in the appropriate span of time. The same principle applies for samples that are analyzed in-plant: If a carcass cannot be sampled and the sample analyzed by the day after it is taken, the carcass should be held until this is possible. A collected sample should not be held; it should be either analyzed in-plant by the next day or immediately shipped for overnight delivery to the laboratory that will conduct the analysis.

The **Sample Shipment** section below gives information on shipping containers and transporting samples to off-site facilities.

Sampling frequency

Sampling frequency for *E. coli* testing is determined by production volume. The required minimum testing frequencies for all but very low production volume establishments are shown in Table 1 by type of livestock. An establishment need sample only the predominant species when two or more species are slaughtered.

Table 1. *E. coli* Testing Frequencies

Cattle	1 test per 300 carcasses, or at least 1 test per week
Swine	1 test per 1,000 carcasses, or at least 1 test per week

NOTE: These testing frequencies do not apply to very low volume establishments. See Table 2.

Very low volume establishments

Some establishments may be classified as very low volume establishments. The maximum yearly slaughter volumes for very low volume establishments are described in Table 2. An establishment need sample only the predominant species when two or more species are slaughtered.

Table 2. Maximum Yearly Livestock Slaughter Volumes for Very Low Volume Establishments

Type of Livestock	**Criteria (Yearly Slaughter Volume)**
Cattle	not more than 6,000 head
Swine	not more than 20,000 head
Cattle and Swine	not more than 20,000 total, with not more than 6,000 cattle

A very low volume establishment will sample the predominant species once per week beginning the first full week of operation after June 1, until at least 13 test results have been obtained or the following June 1, whichever

is first. The establishment will repeat the same sampling regime once per year, beginning the first full week of operation after June 1.

If a very low volume establishment is using the excised-sample technique and is slaughtering a type of livestock for which performance criteria have been determined, the establishment must continue sampling once per week until results show that it has met the m/M criteria outlined in the Pathogen Reduction/HACCP Regulation. See Addendum below.

Random selection of carcasses

Samples are to be taken randomly at the required frequency. For example, given that the frequency of testing for cattle is one test per 300 cattle slaughtered, if a plant slaughters 150 head of cattle an hour, one sample will be taken every two hours. Note: If more than one shift is operating at the plant, the sample can be taken on any shift.

Cattle and swine carcass selection

Different methods of selecting the specific carcass for sampling could be used, but all require the use of random numbers. Examples of methods include random number tables, calculator- or computer-generated random numbers, or drawing cards.

The carcass for sampling should be selected at random from all eligible carcasses of the predominant species. 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. Both the "leading" and "trailing" sides of a carcass should have an equal chance of being selected within the relevant time frame (based on the sampling frequency for the plant).

Cattle and swine carcasses may be designated for sampling at any time, but samples should be collected at the end of the slaughter process after chilling for at least 12 hours; hide-on or hotboned swine or cattle samples should be collected after the final wash.

Aseptic techniques/sampling

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 (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:

a) Peel open the package of sterile gloves from the top without contaminating (touching, breathing on, contacting) the exterior of the gloves.

b) Remove a glove by holding it by the inner surface of the wrist-side opening. Avoid any contact with the outer surface of the glove. Insert the washed and sanitized hand into the glove, taking care not to puncture the glove.

c) Taking care not to contaminate the exterior surface of the glove, repeat the above step for the hand you will use to physically handle the sample.

d) If at any time you are concerned that a glove may be contaminated, begin again with step a) above.

Preparation for Sample Collection

On the day of sampling, gather all sample collection bags, sterile gloves, sanitizer, hand soap, sterile solutions for sampling, and specific materials needed. Ensure that all sampling supplies are on hand and readily available before beginning sample collection.

Label the sample bags before starting the sampling procedure. Use permanent ink. If you are using paper labels, it is important that the label be applied to the bag at normal room temperature; it will not stick to the sample bag if applied in the cooler.

Outer clothing such as frocks, gloves, or head gear worn in other areas of the plant should be removed before entering the sampling area or preparing to collect samples. Replace outer clothing with clean garments, such as a laboratory coat, that have not been directly exposed to areas of the plant outside of the sampling area.

Sanitize the sample work area surfaces by wiping with a clean disposable cloth or paper towel dipped in a freshly prepared 500 ppm (parts per-million) sodium hypochlorite solution (0.05% sodium hypochlorite) or other approved sanitizer that provides an equivalent available chlorine concentration. The sample work area surfaces must be free of standing liquid before sample supplies or product containers are placed on them.

Before sampling, thoroughly wash and scrub hands to the midforearm. Use antibacterial hand soap. This procedure should include a sanitizer at 50 ppm equivalence available chlorine, if available. Dry the hands using disposable paper towels.

Cattle and Swine Surface Sponge Sample Collection Procedure

Materials:

1. Sterile specimen sponge in sterile Whirl-Pak®-type bag or equivalent[approximately 1 1/2" x 3" x 5/8" after hydration)

2. 25 ml sterile sampling solution (e.g., Butterfield's phosphate diluent [BPD] or sterile buffered peptone water [BPW])

3. Sterile ziplock-type or stomacher bag

4. Template for 100 CM^2 sampling area

5. Sterile gloves

6. Sanitizing solution

7. Small tote or caddy for carrying supplies

8. Wheeled ladder, platform, or step ladder

Collection

A sterile (non-latex, non-bactericidal) sampling sponge, which is usually dehydrated and prepackaged in a sterile bag, will be used to sample all sites on the carcass. The sites are for cattle: flank, brisket, and rump - see Figure 2 (for hide-on calves: inside the flank, inside the brisket, and inside the rump); for swine: belly, ham, and jowl - see Figure 3. It is important to sponge the areas in the order of least to most heavily contaminated in order to avoid spreading any contamination. Therefore, sponge the areas in the sequence indicated in this sampling protocol. Nondestructive surface sampling should be conducted as follows:

1. Ensure that all supplies are on hand, including the sampling template, and that all bags have been pre-labeled. The information needed for each sample includes the type of livestock sampled, the date and time of sample collection, and, if there is more than one slaughter line, the slaughter line from which the sample was collected. (An assistant may be helpful during the sampling process.)

2. If a reusable template is used, immerse the sampling template in an approved sanitizing solution for at least 1-2 minutes. Just before sponging the first sample site on the carcass, retrieve the sampling template from the sanitizing solution (step 12). Shake excess solution from the template and let dry, then protect the portion of the template that will contact the carcass from contamination.

3. For cattle: Locate the flank, brisket, and rump sampling sites using illustrations and directions in Figure 2 (cattle carcass sampling locations).

For swine: Locate the belly, ham, and jowl sampling sites using illustrations and directions in Figure 3 (swine carcass sampling locations).

4. While holding the sponge bag at the top corner by the wire closure, tear off the clear, perforated strip at the top of the bag. Open the bag.

5. Remove the cap from the sterile BPD/BPW bottle, being careful not to touch the bottle opening. Do not contaminate the lid.

6. Carefully pour the entire contents of the sterile BPD/BPW bottle (25ml) into the sponge bag to moisten the sponge.

7. Close the top of the bag by pressing the wire closures together. Use hand pressure from the outside of the bag and carefully massage the sponge until it is fully hydrated (moistened).

8. With the bag still closed, carefully push the moistened sponge to the upper portion of the bag, orienting one narrow end of the sponge up toward the opening of the bag. Do not open the bag or touch the sponge with your fingers. Hold the bag and gently squeeze excess fluid from the sponge using hand pressure from the outside. The whole-moistened sponge should still be in the upper portion of the bag, with the excess BPD/BPW in the lower portion.

9. Open the bag containing the sponge, being careful not to touch the inner surface of the bag with your fingers. The wire closure at the top of the bag should keep the bag open. Set bag aside.

10. Put on a pair of sterile gloves (use procedure on pp. 6-7).

11. Carefully remove the moistened sponge from the bag with the thumb and fingers (index and middle) of your sampling hand.

12. With the other hand, retrieve the template by the outer edge, taking care not to contaminate the inner edges of the sampling area of the template.

13. For cattle: Locate the flank sampling area (Figure 2). Place the template over this location.

    For swine: Locate the belly sampling area (Figure 3). Place the template over this location.

14. Hold the template in place with one gloved hand (Remember, only the sponge should touch the sampling area. Take care not to contaminate this area with your hands.)

15. With the other hand, wipe the sponge over the enclosed sampling area (10 cm x 10 cm) for a total of approximately 10 times in the vertical and 10 times in the horizontal directions. The pressure for sponging would be as if you were removing dried blood from the carcass;

however, the pressure should not be so great as to crumble or destroy the sponge. (Note: The template may need to be "rolled" from side to side during sponging since the surface of the carcass is not flat. This ensures that the 100 cm^2 area is enclosed while sponging.)

16. For cattle: Repeat steps 14-15 for the brisket area, using the same side of the sponge used for the flank.

    Then, repeat steps 14-15 for the rump area, using the side of the sponge opposite that used for the flank and brisket.

    For swine: Repeat steps 14-15 for the ham area, using the same side of the sponge used for the belly.

    Then, repeat steps 14-15 for the jowl, using the side of the sponge opposite that used to sponge the belly and ham.

17. For cattle: After sponging the brisket and rump areas, carefully place the sponge back in the sponge sample bag, taking care not to touch the sponge to the outside of the sample bag.

    For swine: After sponging the ham and jowl areas, carefully place the sponge back into the sponge bag. Do not touch the surface of the sponge to the outside of the sponge bag.

18. Press wire closures of the sponge bag together, expel excess air, then fold down the top edge of the bag 3 or 4 times. Secure the bag by folding the attached wire tie back against the bag. Place the closed sponge bag into the second bag and close the second bag securely.

19. a) If samples are to be analyzed at an on-site laboratory, begin sample preparation **(Analytical Methods** section)
    b) If samples are to be analyzed at an outside (off-site) laboratory, follow procedure in the **Sample Shipment** section.

Sample Shipment
If on-site facilities are not available, samples should be shipped to an outside laboratory the same day they are collected.

Samples should be analyzed no later than the day after collection.

Shipping containers and coolant packs

It is important that samples fit easily into the shipping containers so that the sample bags do not break. Correct use of coolant gel-ice packs and proper packing of the shipping container are necessary so that samples arrive at the laboratory at an acceptable temperature (0-10°C). Maintaining samples at improper temperatures may cause inaccurate results.

The sample should be kept refrigerated, not frozen, in the shipping container before it is picked up. The shipping container itself should not be used as a refrigerator; however, multiple samples (if needed) for that

day may be stored in the open shipping container in the cooler or refrigerator.

Recommended Procedure

1. Prechill shipping container by placing the open shipping container in the refrigerator at least the day before sampling.

2. Place the appropriately-labeled, double-bagged sample(s) in the prechilled shipping container in an upright position to prevent spillage. Newspaper may be used for cushioning the sample and holding it in the upright position. If more than one sample is collected during the day, take steps to ensure that samples are maintained at refrigeration temperature, as this helps limit multiplication of microorganisms.

3. Place a corrugated cardboard pad on top of sample(s). This corrugated cardboard pad prevents direct contact of frozen gel packs with the samples. Next place the frozen gel pack(s) on top of the corrugated pad. Use sufficient frozen coolant to keep the sample refrigerated (0-10°C) during shipment to the designated laboratory. Insert foam plug and press it down to minimize shipper head space.

4. Ship samples to the laboratory via overnight delivery or courier.

Suggested Criteria for Microbiological Laboratories

These suggestions are not meant to be exhaustive. Specific needs will vary from one processor to another.

Personnel

Both laboratory analysts and supervisors must have education, training, and experience in food microbiology. Specific familiarity with meat slaughter operations would be desirable. Personnel should be well versed in methods of analysis for meat samples and the organisms associated with meat products.

Facilities

Laboratory facilities should be suitable for conducting routine and specialized microbiological analyses and should provide adequate bio-safety precautions. It is crucial that the laboratory maintain separate, defined areas for sample receipt, preparation, and analytic work.

Equipment

The laboratory should have suitable equipment, appropriate preventive maintenance programs, readily available equipment manuals, and log books for documentation. Specialized equipment may be necessary for some applications.

Operations

The laboratory should have in place a written quality assurance (QA) program that is available to all employees. The QA program should include bio-safety equipment, media preparation, microbiological methods and procedures, control programs, equipment control, culture maintenance, sample receipt, handling, result reporting, and record keeping.

Records

Records should contain a complete sample description, including condition, source, lot code, date, quantity, etc. Results should be reported promptly and all data and summaries permanently recorded with the results.

Analytical Methods

All sample analyses must begin the day after collection. Samples must be analyzed using one of the *E.* coli (Biotype I) quantitation methods found in the Official Methods of AOAC International, 1611 edition, 3rd revision, 1997. Or, by any method that is validated by a scientific body in collaborative trials against the 3-tube Most Probable Number (MPN) method and that agrees with the 95% upper and lower confidence limits of the appropriate MPN index.

The following methods for generic *E.* coli quantitation in foods have been AOAC-approved:

1) 3-tube MPN method - AOAC 17.2.01-17.2.02

2) Modified 3-tube MPN method - AOAC 17.3.07 - Substrate Supporting Disc Method (ColiComplete®). ColiComplete® Substrate Supporting Discs are available from BioControl Systems, Inc., 19805 North Creek Parkway, Bothell WA 98011.

3) Modified 3-tube MPN method - AOAC 17.4-01 - Fluorogenic Assay for Glucuronidase. Lauryl sulfate tryptose broth with added 4-methylumbelliferyl-B-D-glucuronide (MUG) is used in a 3-tube MPN method.

4) Plating Method - AOAC 17.3.04 - Dry Rehydratable Film (Petrifilm *E.* coli Count Plate) Method. Medical-Surgical Division/3M, 275-5W 3M Center, St. Paul MN 55144.

5) Filtration/Plating Method - AOAC 17.3.09 - Hydrophobic Grid Membrane Filter/MUG (ISO-GRID) Method. QA Life Sciences, Inc., 6645 Nancy Ridge Dr., San Diego CA 92121.

Note: For most quantitative assays, weekend laboratory work can be kept to a minimum by refrigeration of incubated plates/tubes until Monday. A programmable refrigerated incubator is useful in such cases. For commercially available methods follow manufacturer's recommendations.

Suggested quantitation schemes

If a generic 1 milliliter (ml) plating technique is used for *E. coli* quantitation for cattle or swine carcass sponge sample analysis, the average plate count (if 2 plates are used) or the single plate count (if 1 plate is used) would be divided by 12 to equal the count of colony forming units per cm^2. **Record this value even if it is less than 1 cfu/cm.** For cattle samples the undiluted sample extract (10°) and a 1:10 dilution should be plated, preferably in duplicate. For swine samples the undiluted sample extract (10°), a 1:10, a 1:100, and a 1:1, 000 dilution should be plated, preferably in duplicate. Higher or lower dilutions may need to be plated based on the specific product.

If a hydrophobic grid membrane filtration method is used, the only difference would be filtration of 1 ml of the undiluted sample extract (10°) and 1:10 dilution for cattle samples and 1 ml of the 10° - 10^3 dilutions for swine samples. Additional dilutions of the original extract may need to be used if a 3-tube MPN protocol is used. The 3 highest dilutions that were positive for E. coli are used to calculate the MPN. MPN values from the appropriate MPN Table represent the count per ml of original extract and therefore must be divided by 12 to obtain the count per cm^2 of carcass surface area. **Record this value even if it is less than 1 cfu/cm^2.**

Recordkeeping

Each test result must be recorded in terms of colony forming units per square centimeter (cfu/cm2). **Record this value even if it is less than 1 cfu/cm^2.** A process control table or chart can be used to record the results and facilitate evaluation. Results should be recorded in the order of sample collection and include information useful for determining appropriate corrective actions when problems occur. The information needed for each sample includes date and time of sample collection, and, if more than one slaughter line exists, the slaughter line from which the sample was collected. These records are to be maintained at the establishment for 12 months and must be made available to Inspection Program employees on request.

For E. coli testing to be most useful for verifying process control, timeliness is important and the record should be updated with the receipt of each new result. Records should also be kept of any corrective actions taken if process control deviations are detected through microbiological testing.

Note: Occasionally, samples shipped to off-site laboratories may be lost during shipping or may arrive at the laboratory late or outside the acceptable temperature parameters for sample analysis (0-10@C). Any reasons for missing data should be documented.

Figure 1. Example of sampling template (not drawn to scale)15

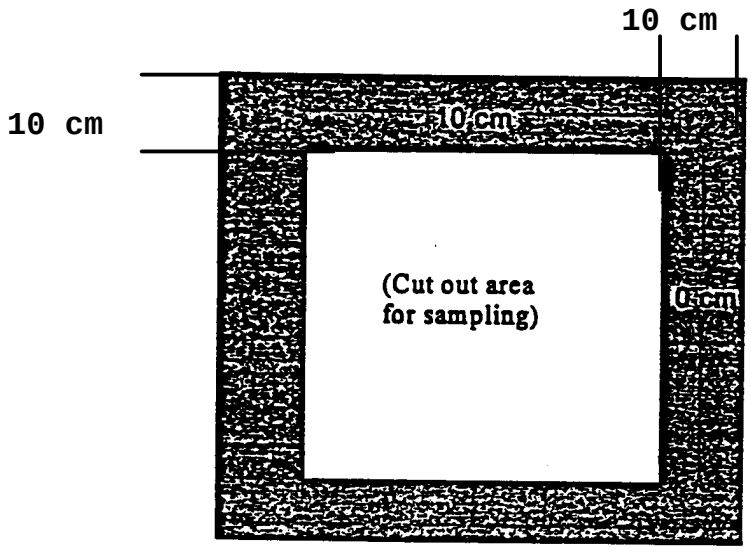
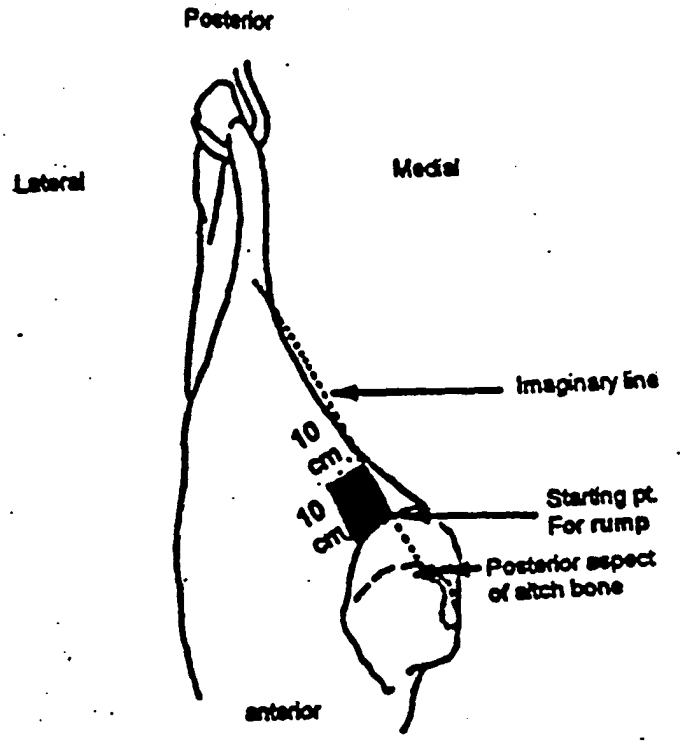


Figure 2. Sampling locations for *E. coli* testing of cattle carcasses

Rump Locate the posterior aspect of the aitch bone. Draw an imaginary line toward the achilles tendon. At the point where the line intersects the cut surface of the round is the starting point for the rump sample. Measure 10 cm up the line leading to the achilles tendon, then 10 cm over (laterally), then 10 cm back to the cut surface of the round, then 10 cm along the cut surface to form the 10 cm by 10 cm square area.

Note: The upper illustration has been purposely altered somewhat. A true lateral view of the carcass would not show the aitch bone. From a medial view, the whole 10 cm x 10 cm sampling area could not be seen. Therefore, a lateral view with a portion of the round removed so the location of the aitch bone is shown is illustrated.



Flank Locate the cutaneous flank muscle (external abdominal oblique) and follow the medial border of the muscle anteriorly until it comes within approximately 3" of the midline. This will be the starting point. Measure up (posteriorly) 10 cm (approximately 4 inches) along a line approximately 3" from the midline (measure up or parallel to the midline), then over (laterally) 10 cm (approx. 4 inches) to form a 10 cm wide by 10 cm long square sample.

Brisket Locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut. This will be the starting point. Measure up along the midline 10 cm (approximately 4 inches), then over 10 cm (approximately 4 inches) to form a 10 cm wide by 10 cm long square sample.

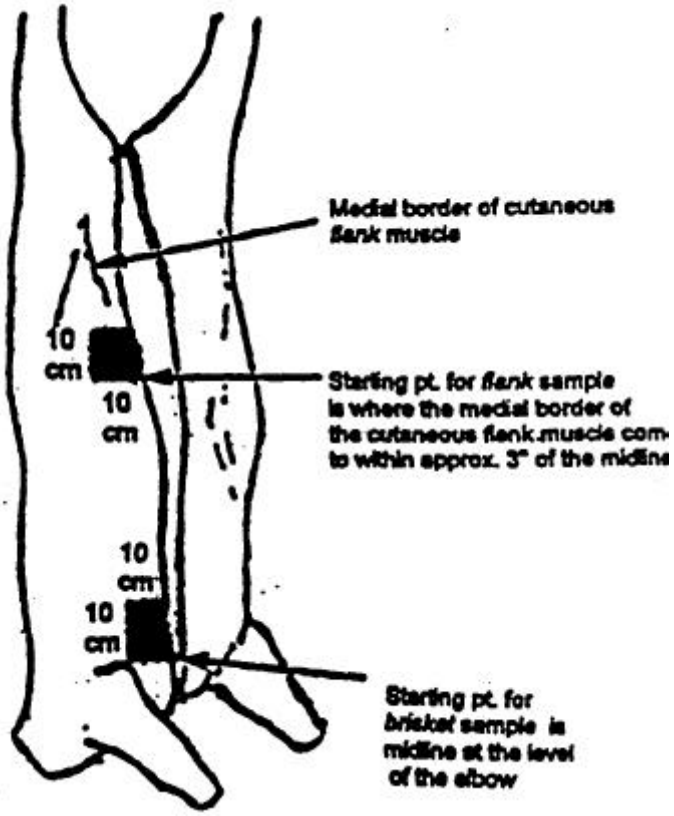
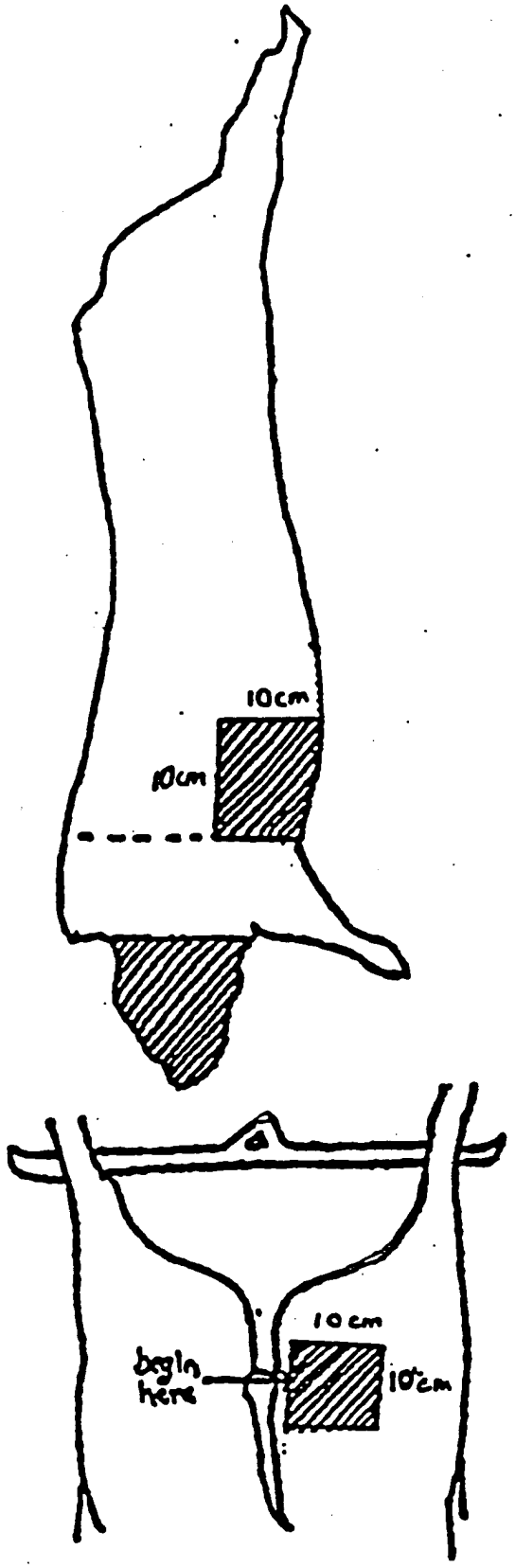


Figure 3. Sampling location for E. coli testing on swine, carcasses



belly Locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut. This will be the starting point. Measure up along the midline 10 cm(approximately 4 inches), then over 10 cm(approximately 4 inches) to complete the 10 cm long by 10 cm wide square sample. This square area will be the 100 cm2 area to swab for the belly sample.

jowls Draw an imaginary line from the atlas/axis joint to the ventral midline; all skin below that point will be considered the jowl.

ham From the dorsal position, locate the lateral surface of the base of the tail and measure up caudal) 5 cm along the lateral edge of the exposed fat margin, then 10 cm laterally. Now measure 10 cm down cranial), then 10 cm medially, then 5 cm up (posteriorly) to complete a 10 cm long by 6 cm wide rectangular sampling area.

ADDENDUM

EXCISION SAMPLE COLLECTION AND ANALYSIS TECHNIQUES USED IN THE FSIS NATIONAL BASELINE STUDIES

PREPARATION

1. Label 3 sample bags with the labels provided: for cattle brisket, flank, and rump; for swine - jowls, belly, and ham). It is important that the labels be applied to the bags at normal room temperature; they will not stick if applied in the cooler.

2. Measure the length of the blade on the knife you will be using for sample collection. You can use this measurement to estimate the size of the 10 inch long by 5 inch wide ham and belly samples for swine, and the 8 inch long by 6 inch wide samples for cattle.

3. Assemble the following:
   labeled bags
   knife (cleaned and sanitized)
   hook (or hemostat-type forceps), cleaned
   several packages containing sterile surgical gloves
   clean container (volume of 1 gallon or greater)
   FSIS-approved household bleach product
   potable water

   Also, locate a wheeled ladder, sampling platform, or step ladder that can be used to help you safely reach the ham or rump of the carcass.

   You will need at least one assistant to hold the sample bag and help you in other ways.

4. Prepare the chemical *sanitizing solution* by adding 2 to 4 oz. bleach to 1 gallon (128 oz.) potable water in the container (this will give a strength of 1000-2000 ppm hypochlorite, which is strong enough to sanitize properly even in the presence of some organic matter). Note: prepare fresh bleach solution immediately before going into the cooler for sampling; its strength diminishes upon standing.

SAMPLE COLLECTION

1. Have your assistant immerse the entire sampling tool (including the handle) in the bleach solution for 1-2 minutes. Have your assistant put on a pair of sterile gloves (taking care not to touch the sterile outer surface of the glove with fingers) before carefully retrieving the sampling tools from the bleach solution. Shake excess solution from utensils, and then protect the sampling ends of the tools from contamination. *Do* not put the sanitized sampling implements into your scabbard (it has not been sanitized).

2. Put on one sterile glove at a time, taking care not to touch the sterile outer surface of the glove with your fingers. Having both hands gloved will remind you that you need to follow aseptic

procedures. Remember: Touch nothing with your gloved hands except the sanitized instruments or the carcass if necessary.

3. Locate the 3 carcass sampling sites. (Note: Remember you can use the knife blade to estimate the length of samples.)

4. For cattle: Use the knife to mark out the sample borders of the brisket sample from one side of the carcass. You do not need to cut very deep since only the skin surface is needed; a ½ inch thick sample is sufficient. It is very important that the sample be 8 inches long by 6 inches wide (not 611 long and 8" wide) and that it be in one piece. Carefully excise the sample using the knife and the sanitized hook or forceps. Roll or fold the sample so that it fits easily into the sample bag (do not cut the sample into strips). Have your assistant hold open the labeled sample bag, and place the brisket sample inside the bag without touching the outside of the sample bag or the assistant. Have assistant close the sample bag.

   For swine: You will be collecting the surface (skin) of both jowls. A ½ inch thickness is sufficient; you do not need to cut very deep since only the skin is being sampled. It is very important that you collect all the skin from both jowls; the laboratory analysis requires that each of the two jowl samples provide a surface area equivalent to a 5 inch by 5 inch square. Surface area in excess of this is acceptable, as the laboratory will trim it down. The skin on each jowl should be carefully removed in one piece using the knife and the sanitized hook or forceps. Roll or fold the samples so that they fit easily into the sample bag (do not cut the samples into strips). Have your assistant hold open the labeled sample bag, and place the two jowl samples inside the bag without touching the outside of the sample bag or the assistant. Have assistant close the sample bag.

5. Do not put the sampling implements back into your scabbard. You may wish to "sheathe" your knife in the carcass where you will be able to reach it from the ladder or platform (insert it perpendicular to the floor, perhaps into the teat line or the medial flank).

6. For cattle: Remove the knife from the carcass and mark out the sample borders for the flank sample. Collect the 8 inch long by 6 inch wide by 4 inch thick skin sample and place it in the appropriately labeled bag.

   For swine: Use the knife to mark out the sample borders of the belly sample from one side of the carcass. You will be excising only the skin from an 10 inch long by 5 inch wide sample. You do not need to cut very deep since only the skin surface is needed; a ½ inch thick sample is sufficient. 'It is very important that the sample be 10 inches long by 5 inches wide (not 5" long and 10" wide) and that it be in one piece. Carefully excise the sample using the knife and the sanitized hook or forceps. Roll or fold the sample so that it fits

easily into the sample bag (do not cut the sample into strips), and place the sample in the bag.

7. Do not put the sampling implements back into your scabbard. You may wish to "sheathe" your knife in the carcass where you will be able to reach it from the ladder or platform (insert it perpendicular to the floor, perhaps into the teat line or the medial flank).

8. Remove your gloves and climb the ladder or platform, holding onto the hand-rail. Once at a convenient and safe height for sampling the ham or rump, carefully put on a new pair of sterile gloves.

9. For cattle: Remove the knife from the carcass and mark out the sample borders for the rump sample. Collect the 8 inch long by 6 inch wide by 4 inch thick skin sample and place it in the appropriately labeled bag. Knives and sampling implements may now be put into your scabbard as sampling has been completed.

   For swine: Remove the knife from the carcass and mark out the sample borders for the ham sample. Collect the 10 inch long by 5 inch wide by 1/2 inch thick skin sample and place it in the appropriately labeled bag. Knives and sampling implements may now be put into your scabbard as sampling has been completed.

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

SAMPLE SHIPMENT

Shipping containers (temperature-controlled container or similar container), gel-ice packs (specifically designed for shipment of refrigerated samples), and cardboard spacers should be specified by the designated receiving laboratory.

If samples are too large to fit into a single shipping container, use 2 shipping containers (make a duplicate of the data sheet and include a notation that you have used 2 shippers). Pre-chill shipping container properly. Address shipping container to the designated laboratory. Samples are to be shipped refrigerated.

1. Freeze gel-ice packs according to label instructions (0°F for 24 hours). Shipping box may be pre-chilled in the cooler if space permits.

2. Place samples into the shipping container as indicated in its instructions. If more space is needed, use another shipping container; do not force too much material into too small a space.

3. Check the sample data sheet for the designated receiving laboratory, and select the appropriate delivery service label. Be sure to enclose

the sample data sheet in a separate plastic bag and put it into the shipper with the samples.

4. Close and seal shipping container according to printed instructions on the carton. Apply the preprinted address label to the shipping box and ship immediately via the designated overnight-express delivery service so that the samples arrive chilled at the laboratory.

FSIS PROCEDURES FOR THE ENUMERATION OF GENERIC *E. COLI*

1. Sample Preparation

A. In order for the samples to be valid and to maintain consistency, analyses must begin the day following sample collection. Samples must meet temperature criteria (0-10°C) in order to be considered for analysis.

B. Three refrigerated subsamples, representing one sampled carcass, will be received by the laboratory. Sample condition and temperature upon receipt should be documented on each sample, together with the date of receipt. Only those samples received at = 0.0°C and 10.0°C will be analyzed.

1. Using a sterile trier (e.g. a circular trier with a 3.6 cm diameter, yielding approximately 10 cm^2 surface area), randomly cut 2 intact tissue discs from each subsample (flank, rump, and brisket for cattle; belly, ham, and jowls for swine). (Optional: Remaining subsample tissue may be retained under refrigeration in the event the analysis needs to be repeated-]

2. If the thickness of a disc greatly exceeds ½ inch, take a sterile scalpel and forceps and aseptically remove fat from the bottom of the plug so disc is approximately ½ inch thick. (Bacterial contamination should be limited to the outer, skin, surface of the disc, so excess tissue must be trimmed from the bottom surface.)

3. Label a sterile Stomacher 3500 bag so that it corresponds to the label on the original subsample bag. Aseptically place the 2 tissue discs from each single subsample into the sterile Stomacher 3500 bag.

C. Begin quantitative analyses for *E. coli* (biotype I) on the day of sample receipt. Using aseptic techniques, remove 2 tissue discs from each of the three subsample stomacher bags and place these 6 discs into the labelled Stomacher 3500 bag.

II. Analytical Methods

A. Preparation of sample homogenate for generic *E. coli*

1. Select the labelled Stomacher 3500 bag containing a composite of 6 discs that was prepared as described in Section IC (above).

2. Add 600 ml Butterfield's Phosphate Diluent (BPD) or buffered peptone water (BPW) to the core samples (this should approximate a 1:10 s.a./v. (surface area/volume] ratio).

3. Stomach for 2 minutes, prepare serial dilutions of 10^{-2} to 10^{-6}, and then proceed according to Instructions IIB (below).

B. Quantitative Test for generic *E. coli*

1. Samples must be analyzed using one of the generic *E. coli* quantitation methods found in the Official Methods of AOAC International, 16" edition, 3rd revision, 1997, or by any method that is validated by a scientific body in collaborative trials against the 3-tube Most Probable Number (MPN) method and that agrees with the 95% upper and lower confidence limits of the appropriate MPN index.

2. The excised sample is considered negative for generic *E. coli* when no *E. coli* colonies are present on plates of the lowest dilution (10-1). If *E. coli* colonies are present, multiply the average plate count by the appropriate dilution factor and record *E.* coli results as cfu/cm^2.

APPLYING PERFOP14ANCE CRITERIA TO EXCISED-SAMPLE TEST RESULTS

E. coli excised-sample results for cattle and swine tested in the FSIS baseline studies have been separated into three categories for the purpose of process control verification: acceptable, marginal, and unacceptable. In the Pathogen Reduction/HACCP Regulation, m and M, representing respectively the 80th and 98th percentile of sample results, leaving 18 percent of results in the marginal range denoted the upper limits for the acceptable and marginal ranges.

Table 3. Values for Marginal and Unacceptable Results for *E. coli* performance criteria

Slaughter Class	Acceptable Range	Marginal Range	Unacceptable Range
Cattle	*negative	positive but not above 100 cfu/cm^2	above 100 cfu/cm^2
Swine	10 cfu/cm^2	above 10 cfu/cm^2 but not above 10,000 cfu/cm^2	above 10,000 cfu/cm^2

*** An excised sample is considered negative when no *E. coli* colonies are present on plate(s) Of the lowest dilution (10- 1 1). If *E. coli* colonies are present, multiply the average plate count by the appropriate dilution factor and record the result as cfu/cm^2.**

To illustrate the use of Table 3, consider a cattle slaughter establishment. *E. coli* test results for this establishment will be acceptable if negative, marginal if positive but not above 100 cfu/cm^2 (>m but =M), and unacceptable if above 100 cfu/cm^2 (>M).

Verification Criteria

The verification criteria should be applied to test results in the order that samples are collected. The criteria consist of limits on occurrences of marginal and unacceptable results.

As each new test result is obtained, the verification criteria are applied anew to evaluate the status of process control with respect to fecal contamination. This way of looking at the number of marginal and unacceptable results is described as a "moving window" approach in the regulation. With this approach, results are accumulated until 13 have been accrued. After this, only the most recent 13 results - those in the "moving window" are considered.

1. An unacceptable result should trigger action to review process controls, discover the cause if possible, and prevent recurrence.

2. A total of more than 3 marginal results in the last 13 consecutive results also signals a need to reviewprocess controls. Having 3 marginal results out of 13 samples approximates the 18 percent found as marginal in the baseline studies.

An example of a record of results for cattle testing is shown below for an establishment performing 2 tests a day.

Test #	Date	Time Collected	Test Result (cfu/cm²)	Result Unacceptable?	Result Marginal?	Number Marginal or Unacceptable in last 13	Pass/ Fail?
1	10-07	08:50	10	No	Yes	1	Pass
2		14:00	negative	No	No	1	Pass
3	10-08	07:10	50	No	Yes	2	Pass
4		13:00	negative	No	No	2	Pass
5	10-09	10:00	negative	No	No	2	Pass
6		12:20	negative	No	No	2	Pass
7	10-10	09:20	80	No	Yes	3	Pass
8		13:30	negative	No	No	3	Pass
9	10-11	10:50	negative	No	No	3	Pass
10		14:50	negative	No	No	3	Pass
11	10-14	08:40	50	No	Yes	4	**Fail**
12		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		11:40	negative	No	No	2	Pass
17	10-17	10:20	**120**	Yes	No	3	**Fail**
18		14:40	negative	No	No	3	**Pass**