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Providing Quality, Time Sensitive Microbiological Testing

IMPORTANT! Roesink Microbiology Laboratories does not accept Category A Infectious Substances (Biosafety Level 3 or 4 materials or any pathogen that causes highly contagious or otherwise serious disease).

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

The following information satisfies the requirements under the Pathogen Reduction/HACCP Regulation for Surface Sponge Sampling of swine slaughter establishments

Materials:

Sterile specimen sponge with 10 ml buffer.
Zip-lock bag
Template for 100 cm₂
Sterile gloves
Sanitizer
Small tote or caddy for carrying sample supplies
Ladder or platform
Marker

Collection:

Overview: Thoroughly wash hands prior to collecting samples and wear gloves. Using the sample supplies provided to you, carefully push the moistened sponge, from the outside of the bag, to the upper portion of the bag and orient one narrow end of the sponge up toward the opening of the bag. 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 buffer in the lower portion. Open the bag and set aside on a sanitized surface or have a co-worker hold it. (Note: Make sure the co-worker has cleaned and sanitized their hands and is wearing gloves.) Using aseptic technique, remove the sterile gloves from their package and place on hands. Being careful not to touch the outside of the bag or any surrounding objects, remove the sponge from the bag with the thumb and forefingers. The sites for swine are: belly, ham, and jowl. Swab sites in the order stated above. (i.e.: belly 1st, ham 2nd, and jowl 3rd) The sponge is sterile and is to be used to sample all sites on the carcass. It has been pre-moistened with 10 ml of buffer. The remaining 15 ml are added upon receipt by Roesink Laboratories.

When taking samples the following information needs to be included.

- a. Establishment Name
 - b. The type of livestock sampled and sample number
 - c. Sample Description or animal I.D.
 - d. The date and time of sample collection
 - e. The slaughter date and line from which the sample was collected if there is more than one slaughter line.
1. 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. Shake excess solution from the template and let dry, then protect the portion of the template that will contact the carcass from contamination
 2. For swine: Locate the belly, ham, and jowl sampling sites using illustrations and directions in Figure 3 (swine carcass sampling locations).
 3. 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. Do not open the bag yet. 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. 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 buffer in the lower portion. Open the bag and set aside on a sanitized surface or have a co-worker hold it. (Note: Make sure the co-worker has cleaned and sanitized their hands and is wearing gloves.)
 4. Using aseptic technique, remove the sterile gloves from their package and place on hands. Being careful not to touch the outside of the bag or any surrounding objects, remove the sponge from the bag with the thumb and forefingers.
 5. 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.
 6. For Swine: Locate the belly sampling area (Figure 3). Place the template over this location.

7. 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.)
8. 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.
9. Repeat steps 7-8 for the ham area using the same side of the sponge that was used for the belly. Then repeat steps 7-8 for the jowl area, using the side of the sponge opposite that was used to sponge the belly and ham.
10. After sponging the ham and jowl areas, carefully return the sponge to the sample bag being careful to not touch the sponge to the outside of the bag. Expel the excess air, fold down the top edge of the bag 3-4 times and secure the bag by folding the wire tie back against the bag. Place the closed sponge bag in a zip-lock bag and seal.

Sample shipment:

Samples should be analyzed no later than the day after collection. Samples should arrive to Roesink Laboratories at an acceptable temperature (0-10°C). Maintaining samples at improper temperatures may cause inaccurate results. Samples should be kept refrigerated and not frozen. Samples may be placed in the shipping cooler with the lid off and stored in a cooler until they are picked up or shipped.

Recommended Procedure

1. Pre-chill 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 in the pre-chilled 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 Roesink Laboratories. Place lid on cooler.
4. Ship samples to Roesink Laboratories via overnight delivery or courier service.

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

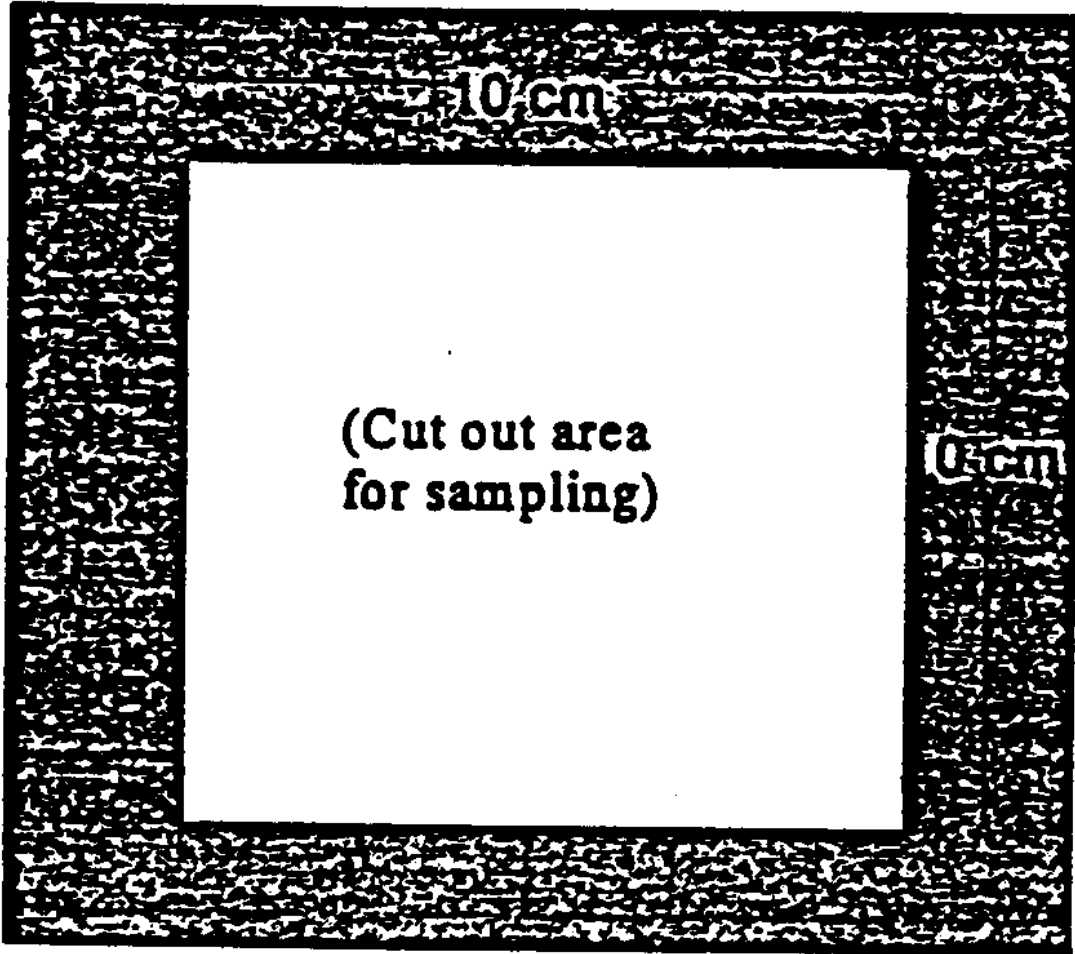
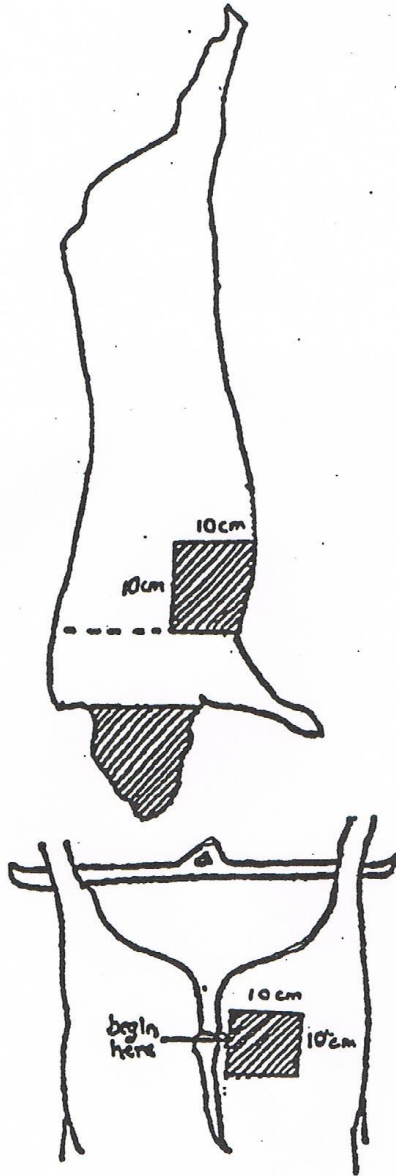


Figure 3. Sampling location for *E. coli* testing on swine, carcasses



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.