

Roesink Microbiology Laboratories, LLC
4150 South Main St.
Akron, Ohio 44319
330-526-1914
www.rmicrobiolabs.com
info@rmicrobiolabs.com

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 Cattle Slaughter Establishments

The following information satisfies the requirements under the Pathogen Reduction/HACCP Regulation for Surface Sponge Sampling of cattle 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 cattle are: flank, brisket, and rump. For hide-on calves swab the inside of the flank, brisket, and rump. Swab sites in the order stated above. (i.e.: flank 1st, brisket 2nd, and rump 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 cattle: Locate the flank, brisket, and rump sampling sites using illustrations and directions in Figure 2 (cattle 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. Locate the flank sampling area (Figure 2). Place the template over this location.

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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 brisket area using the same side of the sponge that was used for the flank. Then repeat steps 7-8 for the rump area, using the side of the sponge opposite that was used for the flank and brisket.
10. After swabbing the rump, 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)

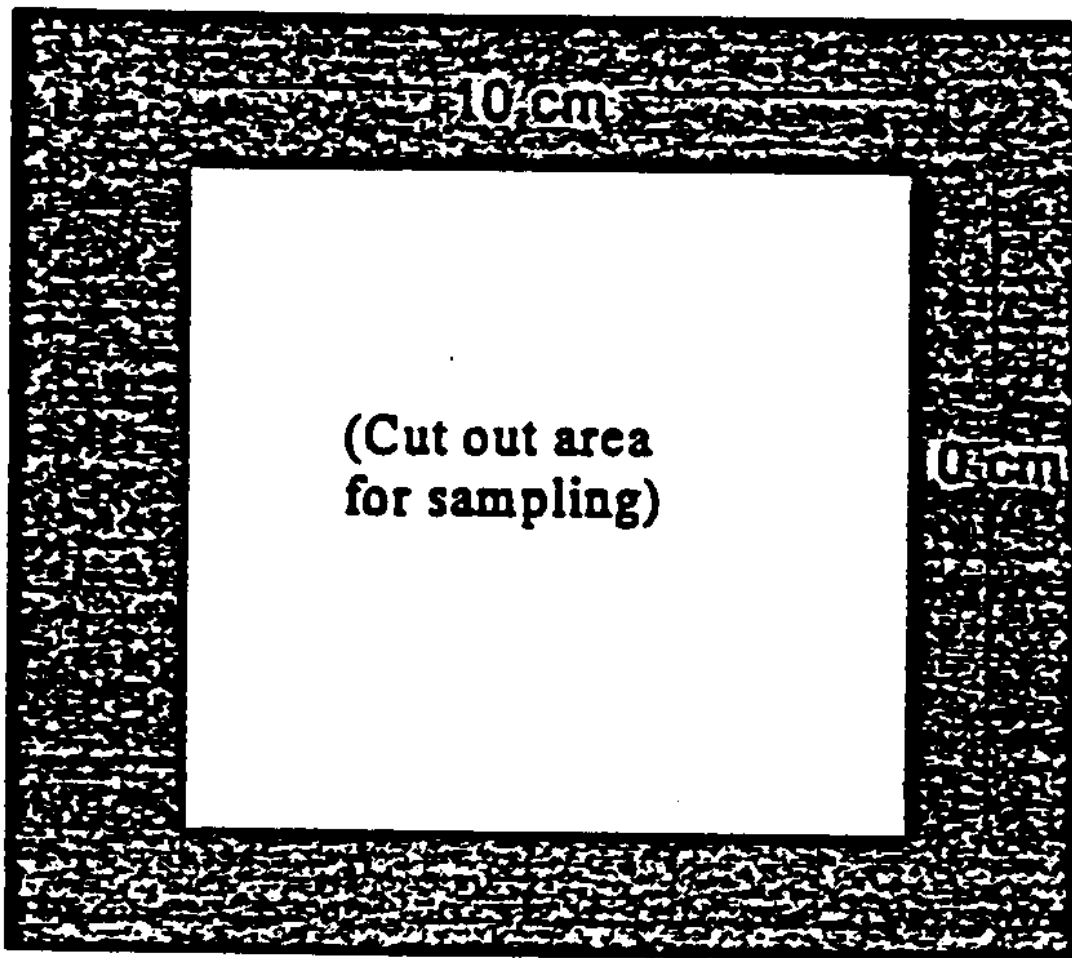
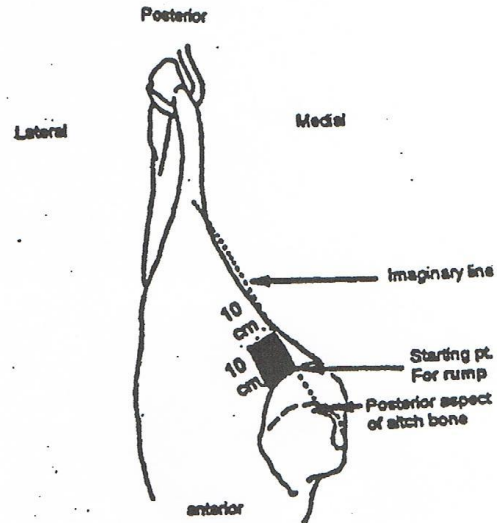


Figure 2. Sampling locations for *E. coli* testing of cattle carcasses

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.

