

Carcass Sampling Protocol

U.S. Meat Animal Research Center

*P.O. BOX 166, Spur 18D
Clay Center, Nebraska 68933*

USMARC Carcass Sampling Protocol

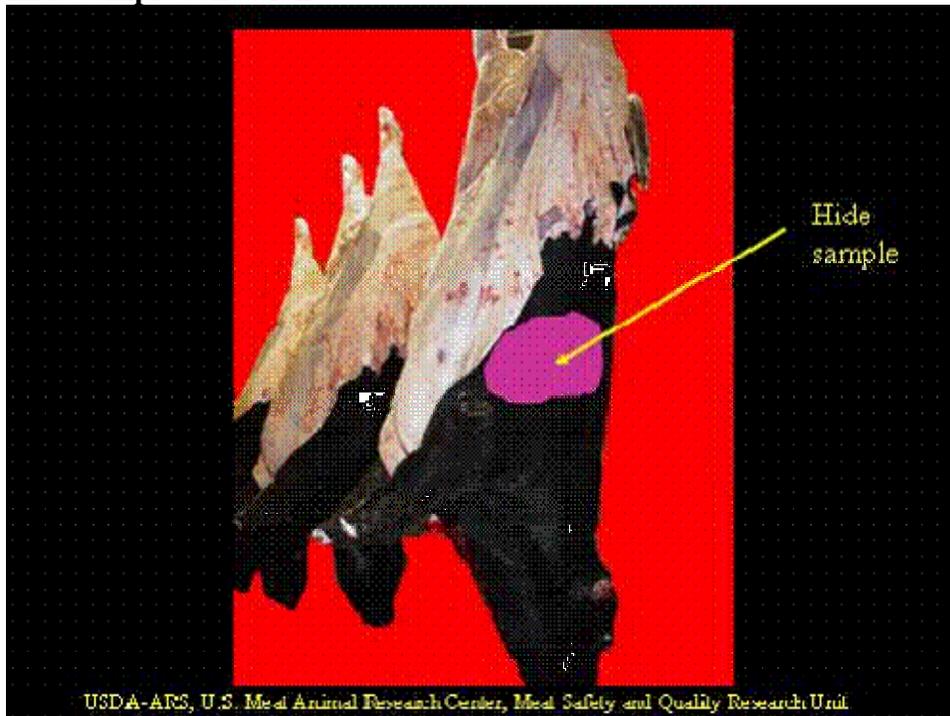
Sampling. Sample using wetted sponges at three separate points on the process line: (1) the plate area of the hide, after hide opening but before hide removal; (2) pre-evisceration, immediately following hide removal before any antimicrobial applications; (3) post-intervention, in the hot box cooler after all antimicrobial interventions. Tag individual carcasses when hide sampled to track through the entire process so the same carcass is sampled for (1) hide, (2) pre-evisceration, and (3) post-intervention.

Obtain hide and carcass samples using Speci-Sponges (Nasco, Fort Atkinson, WI) moistened with 20 and 10 ml, respectively, of buffered peptone water (BPW, Difco Laboratories). Use a new glove on the sampling hand for each sample. While the gloves do not need to be sterile, the gloves should not contact anything but the sponge and the sampling area of the carcass. Wring out the BPW from the sponge inside the bag, then remove the sponge from the bag, and use to swab the hide or carcass.

The hide sample should be taken from a 1000-cm² area over the plate (see picture below). Approximately 5 vertical and 5 horizontal passes (up-and-down or side-to-side are considered one pass) should be made. The carcass sample consists of an 8,000 cm² area obtained by sampling two 4,000 cm² areas (see pictures below) from one carcass side for pre-evisceration and from the other side if you also are sampling for post-intervention. Sampling of carcasses is facilitated by using two sponges (one for the inside and outside round area and one for the navel-plate-brisket-foreshank area), each moistened with 10 ml of BPW. Later in the laboratory, the two sponge samples are combined into one sample bag. If samples are not processed immediately, they should be cooled as quickly as possible in a refrigerator or by packing in coolers with refreezable ice packs (use a layer of cardboard to avoid direct contact between samples and ice packs). The samples in coolers should be transported back to the laboratory or shipped overnight in order to be processed the next day.

The inside and outside round and the navel-plate-brisket-foreshank areas were selected for sampling because those areas include or lie adjacent to points where the hide is opened (hide pattern line), and, therefore these points are thought to be "hotspots" for hide-to-carcass cross contamination. Because contamination is not evenly distributed, large areas are sampled to insure that the sample is representative at each sampling point.

Hide sample collection



Tag the carcass (leading side of plate) at this point to track through processing and obtain 1,000 cm² sample.

Pre-evisceration carcass sample collection

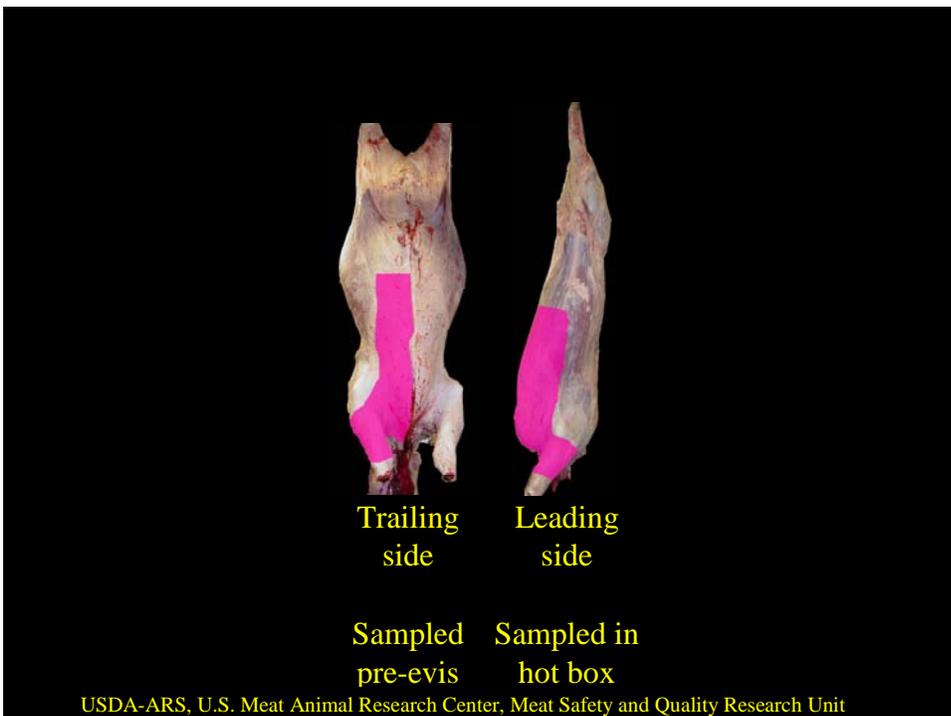


Collect ~4000cm² from the inside and outside round and 4,000 cm² the navel-plate-brisket-foreshank areas as shown each with a separate sponge. Combine sponges later in laboratory for processing giving an 8,000 cm² sample.

Post-intervention (hot-box cooler) sample collection.



Collect ~8000cm² from the inside and outside round and the navel-plate-brisket-foreshank areas as shown from the carcass side not sampled for pre-evisceration. Combine sponges later in laboratory for processing.



During carcass sampling alternate between leading and lagging sides for pre-evisceration and post-intervention carcass samples to prevent bias.



Dual bag system used to facilitate collection of carcass samples. The above photo shows the two bags used for carcass sample collection. The sponge in the bag labeled Pre-Evis Bottom is used for the navel-plate-brisket-foreshank sampling and the sponge in the bag labeled Pre-Evis Top is used for the inside and outside round area. After the samples arrived at the laboratory, the Pre-Evis Bottom is allowed to absorb all of the buffer (10 ml) and matter collected by the sponge during sampling and is carefully transferred to the Pre-Evis Top bag. The contents of the Pre-Evis Top bag (two sponges, 20 ml of buffer, and matter absorbed by the sponges during sampling) is then massaged to achieve homogeneity before removing aliquots for testing. Subsequently, 80 ml of enrichment buffer (tryptic soy broth) is added for detection of *E. coli* 0157:H7.

Sample Processing. Sponge bags are massaged thoroughly and aliquots of up to 2.5 ml are removed prior to the addition of enrichment media. The removed sample aliquots are used for enumerations of indicator bacteria (total aerobic bacteria, *Enterobacteriaceae*, *E. coli* O157:H7 and *Salmonella*).

Total aerobic counts and *Enterobacteriaceae* counts are processed on a Bactometer (BioMerieux, Hazelwood, MO) or for those samples with bacterial levels too low to count on the Bactometer (Post-intervention), Petrifilm Aerobic Count Plates or *Enterobacteriaceae* Count Plates (3M Health Care, St. Paul, MN.).

Enumeration of *E. coli* O157:H7 and/or *Salmonella* is performed according to established USMARC protocols using direct spiral plating or hydrophobic grid membrane filtration.

O157:H7 Culture and Detection. Eighty milliliters of tryptic soy broth (TSB) is added to the sample bags. All sample bags are homogenized by hand massaging then incubated for 2 h at 25°C followed by 6 h at 42°C then held at 4°C overnight. After incubation, 1 ml of each enrichment is subjected to immuno-magnetic separation and plated on sorbitol MacConkey agar (SMAC) supplemented with 0.05 mg/L cefixime and 2.5 mg/L potassium tellurite and a chromogenic media such as Rainbow agar (BioLog) supplemented with 20 mg/L novobiocin and 0.8 mg/L potassium tellurite or ChromeAgar O157 (DRG International) supplemented with 5 mg/L novobiocin and 2.5 mg/L potassium tellurite. After the plates are incubated for 16-24h at 37°C, up to five suspect colonies should be picked and tested by latex agglutination (DrySpot *E. coli* O157; Oxoid, Basingstoke, England). To overcome occasional instances of excessive background organism growth, a second ChromeAgar plate may be used. This plate receives 50uL of a 1:50 dilution of the recovered IMS beads, is incubated at 42°C for 16-24 hours, and interpreted as above.

Salmonella may also be cultured and detected by IMS from the same samples. In this case anti-*Salmonella* beads are used and the recovered beads are enriched in Rappaport-Vassiliadis-Soya (RVS) by incubating at 42°C overnight. *Salmonella* present in the samples is detected by swabbing the RVS enrichment onto (i) Hektoen Enteric agar containing novobiocin (5mg/liter) (HEn) and (ii) Brilliant Green medium with Sulfadiazine. Following incubation, 37°C for 18 to 20 h, suspect colonies are picked for confirmation by PCR for the *Salmonella* specific gene *invA*.

Peer reviewed and published scientific paper based on this procedure:

Arthur, T. M., J. M. Bosilevac, M. P. Kent, D. Jaroni, X. Nou, S. D. Shackelford, T.L. Wheeler, B. Pauling, D. M. Allen, and M. Koohmaraie. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *E.coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67:658-665. 2004.

For additional information or questions contact:

Terry Arthur
402-762-4227
Terry.Arthur@ARS.USDA.GOV