

Incident Investigation Team Methodology for *Escherichia coli* (*E. coli*) O157:H7 in Beef Slaughter Establishments

I. Introduction

This guidance material provides the methodology Incident Investigation Teams (IITs) will use for determining the effectiveness of a beef slaughter establishment's food safety system in controlling and preventing *Escherichia coli* (*E. coli*) O157:H7 contamination. IIT members should consult FSIS Directive 6500.2, Incident Investigation Team Reviews for general information concerning IIT reviews.

Appendix I, "Notes on *Escherichia coli* (*E. coli*) O157:H7 Ecology in Beef Slaughter Establishments," contains guidance and exploratory questions for the team to consider in the investigation. Appendix II, "Review of Microbiological Controls: Beef Carcass Swab Sampling for *E. coli* O157:H7," provides carcass sampling methodology.

The IIT's findings will be used by OFO to determine whether any enforcement action should be taken against the establishment, and if so, what enforcement action is supportable. If OFO determines that the establishment is preparing product under insanitary conditions that may render the product injurious to health; that is, the establishment is failing to address this pathogen successfully, this finding will be submitted to the District Office for consideration of enforcement action against the establishment.

II. First Meeting

When the IIT meets or teleconferences offsite to begin planning their investigation, the IIT member with knowledge of microbiological sampling will estimate the amounts of supplies that will be needed and arrange for them to be sent to the inspection program personnel at the establishment. This activity will occur in close consultation with the supporting Office of Public Health Science laboratory to ensure that laboratory personnel and resources are available for the required testing.

III. Entrance Conference

At the entrance conference, the Team Leader (TL) will advise the establishment that sampling of product, contact surfaces, and the environment may occur. If product is tested, the establishment will be notified, so that the product represented by the sample can be placed on hold pending test results.

IV. Onsite Investigation

A. Initial Review

The IIT's initial review is an in-depth observation and examination of the steps in an establishment's operations

The IIT will integrate findings from reviews of:

1. Establishment documents,
2. Inspection findings,
3. Conditions in the establishment during operations, and
4. Microbiological testing

The IIT will specifically examine locations in the slaughter establishment where *E. coli* O157:H7 may survive and multiply and conduct routine predetermined microbiological sampling¹ (Appendix II, "Review of Microbiological Controls: Beef Carcass Swab Sampling for *E. coli* O157:H7"). The IIT will conduct hide and carcass testing to assess the effectiveness of sanitary dressing procedures and other interventions.

B. Further Investigation

After integrating the findings of the reviews of the establishment documents, inspection findings, conditions in the establishment during operations, and microbiological testing, the IIT will focus on those areas where problems exist. Regulatory actions are deferred until all evidence is obtained and analyzed. However, if immediate actions are necessary, the IIT should bring them to the attention of in-plant inspection personnel for appropriate action, e.g., documenting on a Non-Compliance Record.

If the IIT determines that further microbiological testing of product or the environment is needed, a plant-specific sampling plan should be developed in consultation with the supporting laboratory supervisor to ensure that necessary laboratory resources are available to support the sampling.

C. Synthesis and Analysis of Data

Since each review is unique, broad discretion is needed to facilitate synthesis and analysis of the findings, including:

- HACCP, Sanitation SOPs, and prerequisite program records and document review

¹ The establishment is advised of *E. coli* O157:H7 testing and given an opportunity to hold product until results are obtained.

- Interventions and supporting documentation
- Observations made by the IIT during Pre-op and operations (e.g., sanitary dressing)
- Microbiological analyses, including the establishment's microbiological data and the team's sampling results

V. The Team Report

After the data has been synthesized and analyzed, it will be used to develop the report. The general outline of the report should include the following sections:

Section 1: Scientific Findings – any data and findings, including the microbiological sampling and observation of operations.

Section 2: Public Health Impact – any public health implications from the findings.

Section 3: Regulatory Analysis – an evaluation of the potential regulatory non-compliances.

Appendix I: Notes on *Escherichia coli* (*E. coli*) O157:H7 Ecology in Beef Slaughter Establishments

Within a slaughter and processing operation, the following steps provide risk of contamination:

- Arrival of live animals
- De-hiding
- Decontamination
- Evisceration
- Splitting
- Final washing
- Chilling
- Carcass fabrication
- Grinding
- Storage and
- Transportation.

Within the slaughter establishment, rafters and other surfaces can harbor *E. coli* O157:H7 for weeks after contamination. Airborne dispersion is a concern, particularly at the de-hiding station, since contaminated hides are the major source of carcass contamination. Best slaughter practice recommendations include a mud score program to identify problem cattle.

Cross contamination can be reduced by adequate spacing between carcasses on conveyers. Knives and clothes can become contaminated, and disinfection procedures for these items may not be adequate. Rinsing of worker's hands between carcasses is an effective means of removing hide derived microflora. Contact between carcass and un-rinsed worker's hands introduce contamination.

Questions for Consideration:

These exploratory questions are not intended to be comprehensive. They point to locations where *E. coli* O157:H7 product contamination could occur.

General:

Has the establishment taken measures to prevent the spread of potential pathogens through the facility and to product? For example:

1. Does the plant control air flow and aerosolization? Has the establishment assessed the air flow and taken measures to minimize contamination?
2. Has attention been given to "clean" vs. "dirty" in traffic and product flow from the hide-on to the hide-off side of production?

3. Is the establishment maintaining equipment/facilities to prevent harborage of pathogens (e.g., belts, flooring)? Are there any obvious cleaning/sanitizing improvements that could be made?
4. Are the sanitary dressing/processing procedures likely to lead to carcass contamination with *E. coli* O157:H7?
5. Do employee hygiene practices increase the likelihood of product contamination?

Receiving Facility:

Has the establishment taken measures to prevent the introduction of potential pathogens into the facility from the live receiving area? For example:

1. Are there foot baths for workers walking inside plant?
2. Are animals in lairage overcrowded?
3. Are lairage, knock boxes, restraining chutes and holding pens maintained in a sanitary condition?

Hide Removal/Evisceration:

Is the establishment maintaining proper sanitary dressing and process control? For example:

1. Are there frequent and prolonged stoppages of the slaughter line?
2. Are the rail-out loops overcrowded?
3. Are the sticking knives adequately sanitized (e.g., after use for each animal)?
4. Do employees touch carcasses with contaminated hands during process?
5. Does the establishment prevent loosened hide from contaminating carcasses (e.g., through the use of shields between the hide and the carcass)?
6. Do employees prevent the contamination of carcasses with the loosened hide (e.g., by preventing the hide from flapping)?
7. Is the legging process designed to prevent contamination of the carcass?

Carcass Wash:

Has the establishment adequately designed its carcass washing procedures to prevent contamination of product? For example:

1. Could high pressure be driving contaminants into the carcass?
2. Do employees wash carcasses from top to bottom when using hoses?

Interventions:

1. What are the conditions of use of antimicrobial interventions?
 - a. Concentration
 - b. Time
 - c. Temperature
 - d. Pressure
 - e. Surfaces application; any surfaces missed?
2. What does establishment data show for effectiveness in the reduction of *E. coli* O157:H7? Has the establishment established and documented that a log kill is obtained with the intervention and quantified the incoming load of bacteria on the cattle?
3. What interventions are present? Are there multiple interventions, and is the order of interventions conducive to reducing the pathogen?

Appendix II: Review of Microbiological Controls: Beef Carcass Swab Sampling for *E. coli* O157:H7

Thirty beef carcasses will be swabbed for microbiological testing. Each carcass will be tagged and sampled at three successive locations in the slaughter establishment (Table 1):

- Prior to hide removal,
- Pre-evisceration, and
- Post wash

The IIT will notify the establishment that samples will be collected from post-wash carcasses and advise them to segregate and hold any carcasses that are sampled.

Carcasses contaminated with *E. coli* O157:H7 contain parts which may be processed into non-intact products (ground beef; beef that has been mechanically tenderized by needling, cubing, Frenching, or pounding devices; and beef that has been reconstructed into formed entrees). Any carcass testing positive for *E. coli* O157:H7 is adulterated unless it is further processed to destroy the pathogen. See Directive 10,010.1, Revision 1, "Microbiological Testing Program and Other Verification Activities for *Escherichia coli* O157:H7 in Raw Ground Beef Products and Raw Ground Beef Components and Beef Patty Components."

Prior to Hide Removal Location

The hide-on carcass swab will be collected from a 100 cm² area of the hide approximately 15 cm from the midline near the diaphragm (See Figure 1). A template may be placed over the area of the hide to facilitate sampling. A standard procedure will be used, with 10- two direction strokes per specimen. The sponge will be turned over after the anterior area has been swabbed.

Pre-evisceration and Post Wash Location

The pre-evisceration and post wash carcass swabs will be collected over an area of 8000 cm² that includes two approximately equal sized parts: 1) the posterior area, from the lateral hock over the round and to the rump, and 2) the anterior area, laterally from the midline over the short plate and brisket (See Figure 2). A single swab will be collected by taking 10-two direction strokes in both the posterior and anterior sampling areas of the carcass. Sampling will proceed from anterior (brisket) to the posterior (hock). The sponge will be turned over after the anterior area has been swabbed. Pre-evisceration and post wash swabs are collected from opposite sides of the midline.

Note: In some instances, it will be necessary to use two swabs for the pre-evisceration and post-wash samples, respectively because of factors such as the

speed of the line or the distance between the anterior and posterior sampling areas.

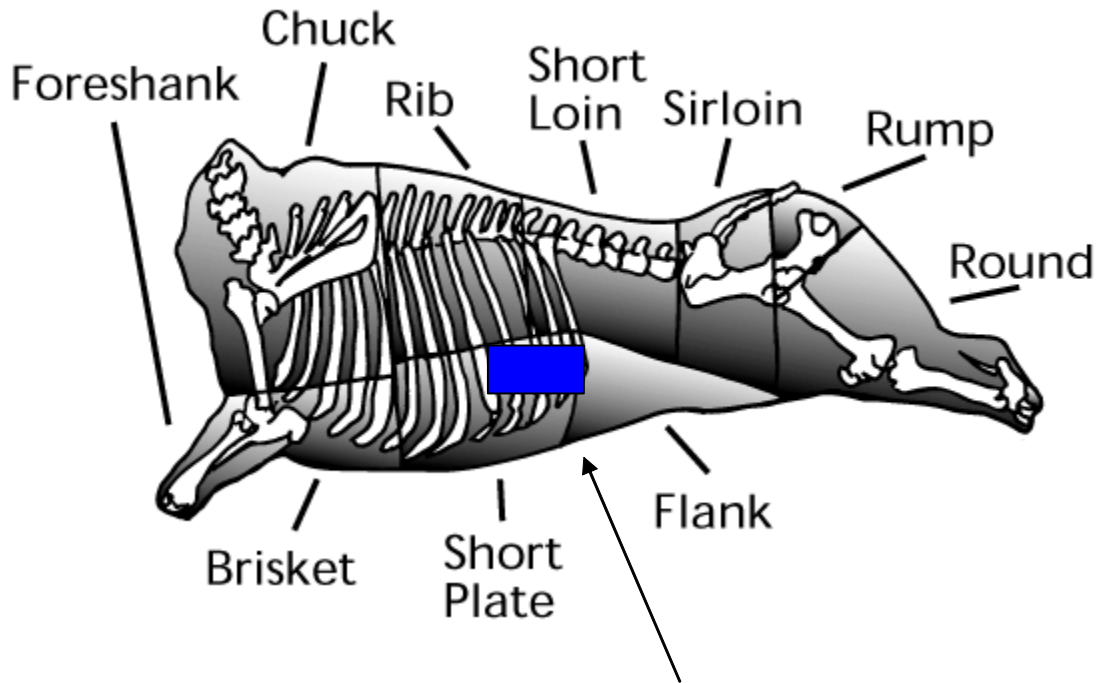
After each swab is collected, it will be placed in a Whirl-Pak bag containing 25 milliliters (mL) of Buffered Peptone Water (BPW). The swab bags will be hand mixed until a uniform suspension is visible (approximately five manipulations). Swabs will be immediately placed on icepacks and shipped by overnight mail to the designated FSIS laboratory for the selected microbiological analyses.

Table 1. Locations, Sites, Materials, and Methods for Sampling.

Location	Site(s) swabbed	Materials	Method*
Prior to hide removal	Swab 100 cm ² area of hide Lateral to midline in mid flank area (See Figure 1)	BPW pre-moistened Speci-Sponge Whirl-Pak bag with 25 mL BPW	- Template on hide - 10-two direction strokes - Turn sponge midway through sampling
Pre-evisceration	Swab 8000 cm ² area: 4000 cm ² anal-hock area and 4000 cm ² brisket-plate area (one side of midline) (See Figure 2)	BPW pre-moistened Speci-Sponge Whirl-Pak bag with 25 mL BPW	- 10-two direction strokes - Turn sponge midway through sampling
Post wash	Swab 8000 cm ² area: 4000 cm ² anal-hock area and 4000 cm ² brisket-plate area (opposite side as pre-evisceration swab) (See Figure 2)	BPW pre-moistened Speci-Sponge Whirl-Pak bag with 25 mL BPW	- 10-two direction strokes - Turn sponge midway through sampling

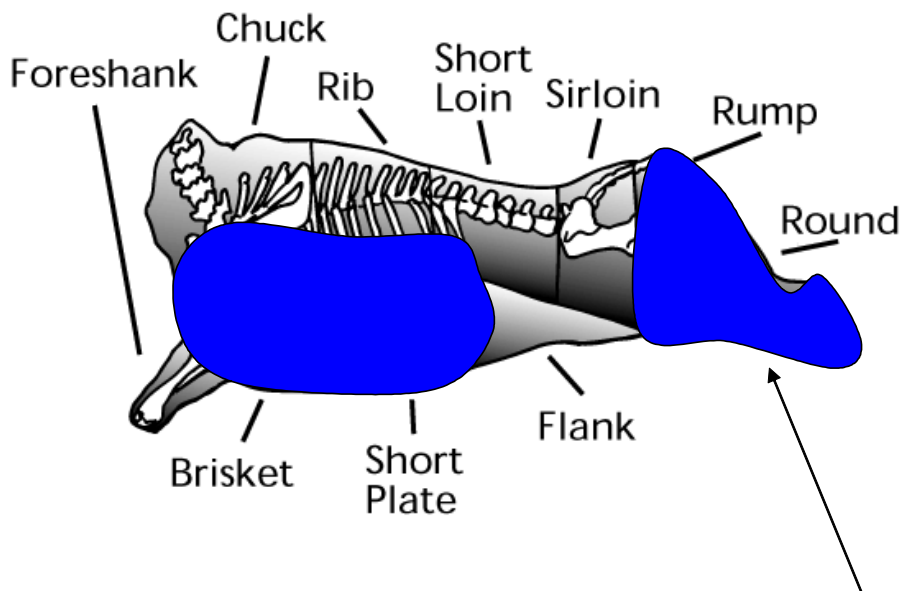
*Wash hands and use sterile latex gloves for each swab. A change of protective clothing and boot wash are required before movement from hide removal to pre-evisceration station and from pre-evisceration station to hot scale, pre-wash, and chilled carcass station.

Figure 1. The 100 cm² sampling site for prior to hide removal swab, approximately 15 cm from the midline near the diaphragm



Collection site for hide sample

Figure 2. The 8000 cm² sampling site for pre-evisceration and post wash carcass swabs (4000 cm² anterior region: lateral brisket and short plate and 4000 cm² posterior region: lateral hock, round, and rump)



Anterior sampling area

Posterior sampling area

Microbiological Testing

Aliquots (1.5 mL) will be taken for serial 10-fold dilutions. Aerobic Plate Counts (APC) will be estimated for all swabs from each of the three locations described above. Counts will be measured over three serial dilutions to bracket the midpoint of the quantitative range as described by Koohmaraie and colleagues². Enumeration will be performed in duplicate. AC Petrifilm will be used to measure APC. An undiluted aliquot from hide, pre-evisceration, and post wash swabs will be cultured for the presence of *E. coli* O157:H7.

Judgment is needed to evaluate microbial counts and *E. coli* O157:H7 test results. For example, it is not uncommon to isolate *E. coli* O157:H7 on 80% of hides. In contrast, 10% prevalence on pre-evisceration carcasses is considered acceptable, while a 60% level would be viewed as marginal. No *E. coli* O157:H7 contamination should occur on post-wash carcasses. Arthur and colleagues reported mean aerobic plate counts on hides of 7.8 CFU/100 cm², respectively, and 1.4 CFU/100 cm² on chilled carcasses. When counts on pre-evisceration carcasses were related to counts on corresponding hides, the carcasses with highest counts of indicator bacteria were from those animals whose hides carried the highest numbers of indicator bacteria.

Table 2. Microbiological Testing Performed

Location	Site(s) swabbed	Aerobic Plate Count	<i>E. coli</i> O157:H7 Positivity
Prior to hide removal	A 100 cm ² area of hide 15 cm from midline, mid flank	YES	YES
Pre-evisceration	Swab 8000 cm ² area: anal-hock, brisket, plate	YES	YES
Post wash	Swab 8000 cm ² area: anal-hock, brisket, plate	YES	YES

² Arthur TM, Bosilevac JM, Nou X, Shackelford SD, Wheeler TL, Kent MP, Jaroni D, Pauling B, Allen DM, Koohmaraie M. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. J Food Prot. 2004; 67:658-65.