

# SPONGING AND EXCISING AS SAMPLING PROCEDURES FOR FRESH BEEF CARCASS TISSUE

L.M. Ware, M.L. Kain, J.N. Sofos, K.E. Belk and G.C. Smith

## Summary

Sponging and excising were evaluated as sampling procedures for microbiological analysis of beef carcass tissue. Brisket tissue portions (10 x 10 cm) were inoculated with 2 ml of an *Escherichia coli* ATCC 25922 cell suspension. After 30 min, the portions were sampled by excising (EX) or swabbing (SP) with a sterile sponge (Whirl-Pak™, Nasco, Modesto, CA), and analyzed for aerobic plate counts (APC) on tryptic soy agar (Difco Laboratories, Detroit, MI), and for total coliform counts (TCC) and *E. coli* counts (ECC) on 3M Petrifilm™ *E. coli* count plates (3M Healthcare Products, St. Paul, MN). Another set of inoculated samples was analyzed after being spray-washed with water (6 sec, 35°C, 3.4 bar), acetic acid (2%, 6 sec, 35°C, 2.1 bar), water (20 sec, 42°C, 20.7 bar) and acetic acid (2%, 6 sec, 35°C, 2.1 bar). Additional samples were analyzed after chilling at 7°C for 24 h. Bacterial counts recovered were influenced ( $P \leq 0.05$ ) by procedure of sampling (EX vs. SP), time of sampling (0.5 h vs. 24 h), and their interactions. Counts recovered 0.5 h after inoculation, from unwashed or spray washed samples, were similar between the two sampling procedures (EX, SP). However, counts recovered after 24 h of sample storage, were significantly ( $P \leq 0.05$ ) lower for the SP compared to the EX sampling procedure. The results indicated that as the carcass tissue was stored, recovery of bacteria by SP was less efficient compared to EX.

## Introduction

Implementation of the 1996 Pathogen Reduction: Hazard Analysis and Critical Control Point Systems; Final Rule (HACCP) systems regulation for use in United States Meat and Poultry Inspection (2) requires food animal slaughtering facilities to sample and analyze carcasses for levels of *Escherichia coli* as a part of their process control verification of performance criteria, while the Food Safety and Inspection Service (FSIS) samples and tests meat products for incidence of *Salmonella* as a part of its verification of pathogen reduction performance standards.

Following implementation of the U.S. Meat and Poultry Inspection regulation questions have arisen concerning selection of the most efficient method of carcass tissue sampling for maximum recovery of bacteria. Currently, the regulation (2) allows companies to take samples for microbial monitoring by either the sponging or excising sampling methods. Recent research indicated that exposure of *E. coli* cell suspensions to sampling sponges may reduce numbers of bacteria by as much as 99% (5). This lack of recoverability was potentially attributed to the diluent used, possible antimicrobial properties of antioxidants in the sponge, or the possible presence of chemical residues in the sampling bag (3). These findings generated concern and prompted discussion among scientists of FSIS, academia, and industry on the most appropriate procedure for carcass tissue sampling (3,4).

This study evaluated the effectiveness of two sampling methods, sponging (SP) and excising (EX), for recovering bacteria from beef carcass tissue. In addition, the effects of different diluents (Butterfield's phosphate—BP and peptone water—PW), of a tissue spray-

washing process and of sample storage, simulating carcass storage for chilling before sampling for analysis, were evaluated.

## Materials and Methods

### *Sample Preparation*

Brisket fat samples were collected on each day of experimentation at a commercial beef slaughtering plant. The samples were collected, immediately following the hide side pullers, and transported to the laboratory within one hour in insulated coolers. Approximately 1 to 2 cm of the top surface of each sample was removed with a sterile blade to expose a fresh surface for testing. The cutting boards and forceps used for sample preparation were treated to reduce contamination by spraying with ethyl alcohol and flaming before and after each use. Each sample was cut to fit (10 x 10 cm) on a clean plastic sample holder board for a custom-made spray-washing apparatus (1).

A 24 h *E. coli* (ATCC 25922) culture was used as the inoculum. The culture was prepared from a stock inoculated into tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI) and incubated at 37°C for 24 h. The inoculum was then prepared by diluting in Butterfield's phosphate buffer (Difco) to a McFarland 1.0 standard (approximately  $3 \times 10^8$  cells/ml). Each brisket sample was inoculated with a 2-ml portion of this suspension that was spread over a 100-cm<sup>2</sup> area with a sterile bent glass rod, yielding a target inoculum of approximately 6 log CFU/cm<sup>2</sup>.

### *Sampling of tissue*

Sampling by sponging followed the procedure described in the U.S. Meat and Poultry Inspection Regulation (2). Ten ml of buffer were used to rehydrate a sterile sponge (Whirl-Pak<sup>TM</sup>, Nasco, Modesto, CA). Another 15 ml were added to the sponge in the bag, to bring the total volume to 25 ml after swabbing the sample area with the sponge (9). Swabbing consisted of 10 strokes horizontally and 10 strokes vertically. One stroke consisted of a side-to-side motion up and down (vertically) or an up-and-down motion from left to right (horizontally). Sampling by excision involved cutting of a 10 x 10 cm sample using a sterile blade and forceps. The excised samples were placed in (18.5 X 30 cm) sterile sample bags (Whirl-Pak<sup>TM</sup>) and sterile buffer was added to the original weight to obtain a 10<sup>-1</sup> dilution.

### *Treatments*

In addition to the two types of tissue sampling procedures (sponging and excising), the study included evaluation of tissue storage for 0.5 or 24 h. at (7°C) before sampling; sampling and analysis before or following spray-washing of the inoculated tissue; and comparison of two types (evaluated in one of two experiments) of buffer (Butterfield's phosphate or peptone water) (Difco) used in sample preparation. One set of inoculated samples was held for 0.5 h. and sampled either by sponging or excising. A second set of inoculated samples was placed into 14 x 14 cm plastic storage containers (uncovered) and held 24 h (7°C) before sampling (excising or sponging) for analysis. Additional samples were inoculated and spray-washed 10 min after inoculation. Following spray washing, these samples were analyzed after 0.5 h at ambient temperature or after 24 h of storage at 7°C. Spray washing involved a series of consecutive treatments (1) which included, in sequence, water (6 sec, 35°C, 3.4 bar), acetic acid (2%, 6 sec, 35°C, 2.1 bar), water (20 sec, 42°C, 20.7 bar) and acetic acid (2%, 6 sec, 35°C, 2.1 bar).

### *Microbiological analysis*

The sponges and the excised samples were stomached (Stomacher 400, Tekmar, Inc., Cincinnati, OH) for 2 min, and then serially diluted in the corresponding buffer (Butterfield's phosphate or peptone water) and plated on tryptic soy agar (TSA) (Difco) for aerobic plate counts (APC) and on 3M E. coli Petrifilm™ count plates (3M, Health Care Products, St. Paul, MN) for total coliform counts (TCC) and *E. coli* counts (ECC). The APC and TCC were determined following incubation at 37°C for 48 h, while the determination for ECC was done after 24 h at 37°C. The TSA plates were inoculated with a spiral plating system (Model 500A, Spiral Biotech, Inc. Bethesda, MD) and counted using a CASBA data processor (Model 800, Spiral Biotech, Inc.). The colonies on 3M Petrifilm™ E. coli count plates (TCC and ECC) were counted manually, as described in the instructions provided by the supplier. All counts were converted to log CFU/cm<sup>2</sup>.

### *Data analysis*

A total of three replications (two samples each) were done on inoculated samples that were either sponged (n = 6) or excised (n = 6) after 0.5 h of storage and sponged (n = 6) or excised (n = 6) after 24 h (7°C) of storage, using only one buffer (Butterfield's phosphate). In addition, a total of four replications were done on inoculated samples that were unwashed or spray washed and sponged (n=8/treatment) or excised (n =8/treatment) after 0.5 h of storage, using two buffers (Butterfield's phosphate or peptone water). There were also four replications of inoculated samples that were unwashed or spray washed and sponged (n=8/treatment) or excised (n=8/treatment) after 24 h of storage at 7°C. The data were analyzed using the general linear model procedure of SAS and sample means were separated with the least square mean test (6).

## **Results and Discussion**

Overall, results indicated a significant ( $P \leq 0.05$ ) difference between the two sampling procedures (sponging and excising). In one experiment, using only Butterfield's phosphate buffer, sampling method had no significant ( $P > 0.05$ ) effect on APC, but had a major influence on TCC and ECC ( $P \leq 0.05$ ) (Table 1). The sampling method also had significant effects ( $P \leq 0.05$ ) on all counts (APC, TCC, ECC) recovered in the other trial (comparing buffers) of unwashed samples (Table 2) and of spray washed samples (Table 3), which also compared sampling buffers (Butterfield's phosphate or peptone water).

In addition to sampling procedure, counts recovered were also affected ( $P \leq 0.05$ ) by sampling time and the interaction of sampling method and time. Sampling buffer had no significant ( $P > 0.05$ ) effect on bacterial counts recovered (Tables 2 and 3). The two sampling methods (excising and sponging) did not result in major differences in bacterial counts recovered from freshly inoculated and sampled beef carcass tissue. However, sampling following a 24 h storage (7°C), to simulate carcass chilling, resulted in greater recovery of bacteria counts by excising compared to sponging.

This study confirmed the widely accepted conclusion that excising generally results in recovery of higher bacterial counts than swabbing, especially following product storage (which allows for increased bacterial cell attachment). Either method (sponging or excising) can be used in carcass sampling for process control verification, provided that the results are compared against data or baseline standards obtained following the same procedure of sampling.

## References

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## Tables

Table 1: Analysis of variance of aerobic plate counts (APC), total coliform counts (TCC), and *Escherichia coli* counts (ECC) recovered from inoculated unwashed beef brisket tissue sampled by sponging or excising (Butterfield's phosphate buffer).

Variable	DF	APC		TCC		ECC	
		Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Replicate (R)	2	11.3	25.9**	2.1	42.0**	2.6	19.8**
Sampling method (M)	1	0.01	0.02	0.9	18.7**	2.3	18.2**
Sampling time (T)	1	6.8	15.6**	8.3	170.3**	7.4	57.5**
R x M	2	0.04	0.1	0.3	5.4*	0.6	4.4*
R x T	2	3.5	7.9**	0.2	2.4	2.3	17.5**
M x T	1	0.01	0.01	0.4	7.1*	0.3	2.2
R x M x T	2	0.3	0.6	0.2	2.8	0.1	1.0

Each sample (10 x 10 cm) was inoculated with 2 ml of inoculum ( $3 \times 10^8$  cells/ml) of *Escherichia coli* ATCC 25922.

M= excising or sponging; T= sampled and analyzed 0.5 h or 24 h (7°C) following inoculation.

\* P ≤ 0.05; \*\* P ≤ 0.01.

Table 2: Analysis of variance of aerobic plate counts (APC), total coliform counts (TCC), and *Escherichia coli* counts (ECC) recovered from inoculated, unwashed beef brisket tissue sampled by sponging or excising (Butterfield's phosphate buffer or peptone water).

Variable	DF	APC		TCC		ECC	
		Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Replicate (R)	3	0.0	0.5	0.2	0.6	1.0	1.3
Sampling method (M)	1	2.0	51.6**	5.2	15.6**	6.8	8.6**
Sampling time (T)	1	8.0	203.5**	4.4	13.2**	6.1	7.7*
Sample buffer (B)	1	0.1	1.3	0.1	0.2	0.7	0.8
M x T	1	6.6	169.0**	3.3	9.8**	3.5	4.4*
M x B	1	0.0	0.6	0.5	1.5	0.0	0.0
T x B	1	0.0	0.8	0.1	0.2	0.0	0.0
M x T x B	1	0.0	0.3	0.4	1.2	0.2	0.3

Each sample (10 x 10 cm) was inoculated with 2 ml of inoculum ( $3 \times 10^8$  cells/ml) of *Escherichia coli* ATCC 25922.

M= excising or sponging; T= sampled and analyzed after 0.5 h or 24 h (7°C) following inoculation.

B= 0.1% peptone water or Butterfield's phosphate buffer.

\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ .

Table 3: Analysis of variance of aerobic plate counts (APC), total coliform counts (TCC), and *Escherichia coli* counts (ECC) recovered from inoculated spray washed, beef brisket tissue sampled by sponging or excising (Butterfield's phosphate buffer or peptone water).

Variable	DF	APC		TCC		ECC	
		Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Replicate (R)	3	0.1	1.4	1.3	5.2**	1.4	2.6
Sampling method (M)	1	2.1	35.3**	8.5	33.1**	8.2	14.6**
Sampling time (T)	1	12.5	206.9**	16.3	63.5**	12.9	22.8**
Sample buffer (B)	1	0.1	0.8	0.3	1.1	0.6	1.1
M x T	1	4.7	77.4**	3.6	14.0**	3.8	6.7*
M x B	1	0.0	0.2	0.0	0.1	0.2	0.3
T x B	1	0.1	1.7	0.5	1.9	0.0	0.0
M x T x B	1	0.1	1.7	0.8	3.3	0.0	0.0

Each sample (10 x 10 cm) was inoculated with 2 ml of inoculum ( $3 \times 10^8$  cells/ml) of *Escherichia coli* ATCC 25922.

M= excising or sponging; T= sampled and analyzed after 0.5 h or 24 h (7°C) following inoculation.

B= 0.1% peptone water or Butterfield's phosphate.

Spray washing involved the sequence of water (6 sec. 35°C, 3.4 bar), 2% acetic acid rinse (6 sec. 35°C, 2.1 bar), water (20 sec. 42°C, 20.7 bar) and followed by 2% acetic acid rinse (6 sec. 35°C, 2.1 bar).

\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ .

