



Short communication

Development and testing of a prototype automatic trim sampler



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ABSTRACT

The N60 method currently mandated by regulatory bodies in North America for routine testing of beef trim for *Escherichia coli* O157:H7 requires that 60 slices of beef, up to 375 g, be removed from combo bins containing up to 10,000 lb of beef trim. The objective of this study was to design and test a prototype automatic trim sampler that would reduce the resource demands of, and result in more representative sampling than, N60. Ten commercial combo bins were each sampled at five locations of each of four levels, by swabbing an area of 1000 cm² and by excision of up to five meat pieces with a total area of 100 cm². The samples were enriched in modified Tryptone Soy Broth supplemented with novobiocin at 20 mg/L, and tested for generic *E. coli* using real-time PCR. A prototype trim sampler was constructed and tested for recovery of aerobes, coliforms and *E. coli* and was compared to manual swabbing, using beef trim artificially contaminated with *E. coli*. Overall, 21.5% of excision samples and 38.0% of swab samples from combo bins were positive for *E. coli*. Of the 40 levels that were sampled, 50.0% were positive by excision, 70.0% were positive by swabbing. The sampler, designed based on swab sampling, could process trim of ≤1 kg. Of the 12 pairs of manual sampling and automatic sampling compared, ≥ 8 were not significantly different for recovering each of the three groups of indicator organisms ($P > 0.05$). The findings of this study show that swab sampling can be more sensitive and representative than excision sampling for recovering small numbers of *E. coli* from beef trim and the prototype sampler can have efficacy comparable to manual swabbing for recovering all three groups of indicator organisms from beef.

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1. Introduction

Verotoxigenic *Escherichia coli* (VTEC) were first recognized as pathogens of great public concern in 1982, following outbreaks associated with the serotype O157:H7 in the US (Riley et al., 1983). Cattle are natural reservoirs of VTEC, but they are asymptomatic carriers (Gill & Gill, 2010). It is then not surprising that *E. coli* O157:H7 was detected in up to 94% of samples from hides of cattle after hide opening but before hide removal in a commercial beef packing plant in the US (Arthur et al., 2004). Most bacteria on carcasses are deposited during the skinning operations where the transfer of bacteria from the hide to the previously bacteria free meat is inevitable (Bacon et al., 2000; Bell, 1997; Nottingham, 1982, pp. 13–65). Thus, skinned carcasses can be contaminated with *E. coli* O157:H7. Despite the implementation of effective antimicrobial

interventions at beef plants, complete elimination of *E. coli* O157:H7 from beef is not yet attainable.

Due to the severity of illness caused by *E. coli* O157:H7 and its association with ground beef, the US Department of Agriculture–Food Safety and Inspection Service (USDA–FSIS) declared the organism a food adulterant in 1994 (Taylor, 1994). Shortly after the declaration, FSIS initiated a program to test for its presence in ground beef and subsequently in 2007, and expanded the testing to include beef trim intended for use in raw ground beef or beef patty products at the slaughter establishments that produced that trim (USDA–FSIS, 2013). Beef trim (trimmings) consists of small portions of beef that are trimmed away during the fabrication of primal and subprimal cuts from large portions of carcass sides (Arthur, Bono, & Kalchayanand, 2014). They are sorted, often according to visual observation of the fat content, and are collected into combo bins, which, when filled, can weigh up to 2000 lb each. The current sample collection method mandated by FSIS and the Canadian Food Inspection Agency (CFIA) for testing of beef for *E. coli* O157 is called

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N60 in which 60 slices of meat (6.25 g/slice) from a production lot are obtained by manual excision (CFIA, 2015; USDA/FSIS, 2014). A production lot contains up to 5 combo bins. At present the process at large beef packing plants in Canada requires 6–8 individuals per shift directly involved with sampling and another 2 individuals per shift involved with the paperwork, lot tracking, delivery of samples to the lab, etc. At current wage rates in Canada the cost of sampling exceeds \$1 million per year when both shifts at large facilities are considered. Thus, the N60 method is very resource intensive. In addition, it is difficult to obtain a truly representative sample by the N60 method for a number of reasons. Notable among these are the difficulty inherent to accessing meat for sampling anywhere other than in the top of the bin, 75 g of meat being a very small proportion of the 2000 lb in a bin, and inhomogeneous distribution of the pathogen in the bin. In addition, despite the severity of illness caused by *E. coli* O157:H7, it is found on beef at very low prevalence, and at very low numbers when it is present. The largest beef recall in Canadian history was triggered by detection of *E. coli* O157:H7 by the USDA in trim that had tested negative for the organism by routine testing at the plant where the trim was produced (Lewis, Corriveau & Osborne, 2013). The recall led to the closure of the beef plant which represented 35% of Canadian beef processing capacity. Therefore, automated approaches with improved representation of samples could save resources and reduce the amount of contaminated meat that would otherwise enter the market.

For automated sampling equipment to be devised, knowledge of whether sampling by swabbing can be used instead of technically more difficult excision sampling is required. The efficiency of swab sampling can be comparable with that of excision for recovering bacteria from carcasses of large animals (Gallina et al., 2015; Gill & Jones, 2000; Gill, Badoni, & McGinnis, 2001). Information on using swab sampling to recover small numbers of bacteria from manufactured beef, however, is largely lacking. Thus, this study was staged in two phases: 1. to determine whether automated sampling equipment would require excision sampling, in order to test beef trim at the level of contamination routinely attained at Canadian beef processing facilities; and 2. to construct and test the automated sampling equipment developed based on the findings from phase 1.

2. Materials and methods

2.1. Recovery of generic *E. coli* from commercial trim by swab and excision sampling

2.1.1. Sample collection

Ten combo bins of beef trim were obtained from a Canadian beef packing plant which processes feedlot cattle at rates up to 280 head per hour, through a process described in previous studies (Yang, Badoni, Tran, & Gill, 2015; Yang, Badoni, Youssef, & Gill, 2012). Each combo bin contained approximately 2000 lb of trim with a fat to lean ratio of 50:50. The combo bins were collected from the plant on 6 days at approximately weekly intervals, with one bin during each of the first two weeks and two bins during each of the following 4 weeks being collected. Upon collection from the plant, the combo bin(s) was transported in a refrigerated truck to a laboratory for further processing. The combo bins were designated as B1–10 in the order they were sampled.

Samples were collected from the four corners as well as the centre of the uppermost surface of each bin, by both excision and swabbing (Fig. 1). For excision, five thin strips measuring approximately 10 cm × 2 cm each were excised from trim in each of the five areas. The total weight of meat obtained from each area was ≤50 g. An area of approximately 1000 cm² of trim in each area from which strips of meat were excised was also swabbed using a sponge

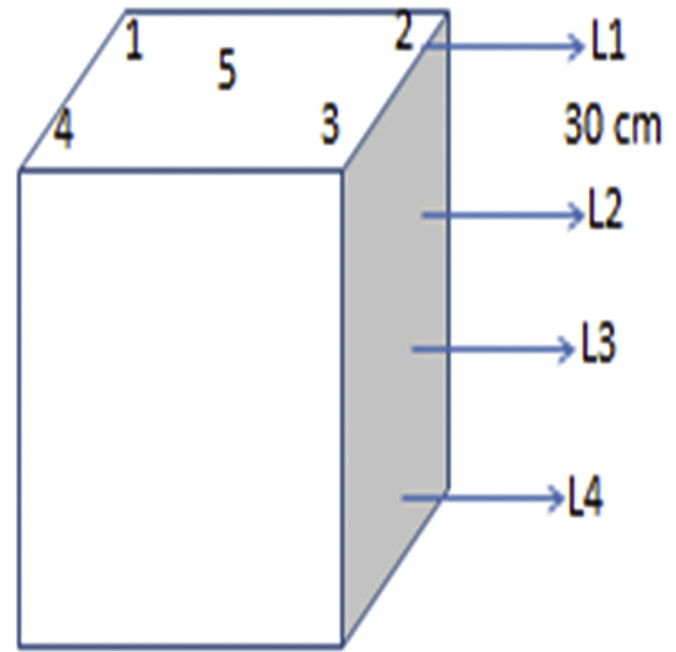


Fig. 1. Schematic diagram of sites of sample collection from combo bins. Samples were collected from four corners (1–4) and the centre of each of four levels (L1–4) of each combo bin of trim.

(Speci-sponge; VWR Canlab, Edmonton, Alberta, Canada) pre-moistened with 7 ml of 0.1% peptone water (Difco, Becton Dickinson, Sparks, MD). The meat sample or swab from each sampling site was placed in a sterile stomacher bag (Gill et al., 2001). Trim was removed from the bin until a fresh surface approximately 30 cm below the previous top surface was exposed. The bin edges were cut to make the new surface readily accessible. Five samples from the newly exposed surface were collected by excision or swabbing as before. The removal of meat and collection of samples were repeated for surfaces approximately 60 cm and 90 cm below the previous top surface. All stomacher bags containing samples were placed on ice and were transported to the laboratory for further analysis. A total of 200 samples were collected from excision or swabbing.

2.1.2. Quantification of *E. coli* in enrichment cultures

Ten ml of 0.1% peptone water was added to each swab sample which was then pummeled in a stomacher for 2 min. Five ml of the swab fluid, approximately half of the total volume, was withdrawn from the stomacher bag and was filtered through a hydrophobic membrane grid filter (HGME; Neogen, Lansing, MI). The filter was placed on a Lactose Monensin Glucuronate (LMG; Neogen) and incubated at 35 °C for 24 h. Subsequently, the filter was transferred to a plate of buffered MUG (BMA; Neogen). After incubation at 35 °C for 3 h, the filter was examined under long-wavelength UV light and the squares containing blue-white fluorescent colonies were counted as *E. coli* (Liu, Youssef, & Yang, 2016). This method is referred as direct filtration in this study.

One hundred or 250 ml of modified Tryptone Soy Broth (mTSB; Oxoid, Nepean, Ontario, Canada) supplemented with 20 mg/L novobiocin (N; Oxoid) was added to each stomacher bag containing a pummeled swab or meat pieces, respectively. The stomacher bags were placed into a plastic bag rack and incubated at 42 °C for 18–24 h, to enrich for *E. coli* (Yang, Badoni, Wang, & Gill, 2014). A 1.5 ml portion of each enrichment culture was withdrawn and centrifuged at 1500×g for 1 min to pellet large debris. One ml

portion of the resulting supernatant was transferred into a new 2 ml micro-centrifuge tube and centrifuged at $10,000\times g$ for 5 min to pellet cells, from which DNA was extracted and subsequently tested for *E. coli* using real-time PCR (Yang, Badoni, et al., 2015). The detection limit of the real-time PCR assay is 26 pg *E. coli* DNA per reaction, which yields a cycle threshold (Ct) value of 35 (Yang, Badoni, & Gill, 2011). Thus, samples yielding mean Ct values ≤ 35 were regarded as positive for *E. coli*. A standard curve using DNA extracted from cells with known concentrations of *E. coli* was constructed and used for estimation of the cell numbers in the enrichment cultures.

2.2. Development and construction of a prototype trim sampler

A prototype automatic sampler, employing swabbing mechanism, was conceptualized and designed and constructed with the assistance of engineers (Stanfos, Edmonton, Alberta, Canada). The sampler consists of four essential parts: a divided conveyor with a 4 cm gap, a sampling disc, a motor fitted for the conveyor, and a motor fitted for the sampling disc (Fig. 2A). The width and diameter of the sampling disc are 3 and 20 cm, respectively. Upon activation by pressurized air, the sampling disc will rise into the gap dividing the conveyor on which meat passes and run in contrary motion to the conveyor (Fig. 2B). When the sampling disc is activated, the top edge of the disc is about 1.5 cm above the conveyor. The speed of the conveyor and the sampling disc can be adjusted through their respective controlling motors, each with dials ranging from 1 to 10. The actual speed was determined by measuring the timed revolution of the belt or sampling disc, using a tachometer (Touchless Digital Tachometer; VWR). Beef bench trim or steaks of various shapes and sizes ranging from 0.11 to 1.3 kg were used to determine the carrying capacity of the conveyor belt and sampling disc.

2.3. Comparison of manual swabbing and automatic sampling for recovering bacteria from beef

2.3.1. Preparation and inoculation of beef

To minimize the impact of variation in meat background microflora, meat pieces freshly cut from primal cuts, instead of bench trim, were used for comparing the recovery of bacteria from beef by manual swabbing and automatic sampling. Inside round primal cuts collected from the cutting room of a federally inspected research abattoir in Alberta were vacuum-packaged. The vacuum packs were stored at $-20\text{ }^{\circ}\text{C}$ and used within 3 months of collection. The packs were partially thawed for 48 h at $7 \pm 2\text{ }^{\circ}\text{C}$ and then kept overnight in a walk-in cooler operated at $1 \pm 1\text{ }^{\circ}\text{C}$. Upon opening, the primal cut was placed on a sterile plastic tray and

made into pieces, to simulate trim. The meat pieces were approximately 1 cm thick and weighed up to 0.5 kg each. The dimensions of each meat piece were recorded and used for calculation of surface area.

A strain of wild type *E. coli* that was originally isolated from meat was used for inoculum preparation (Jones, Gill, & McMullen, 2002). *E. coli* was grown overnight at $35\text{ }^{\circ}\text{C}$ in tubes containing 10 ml of half strength brain heart infusion (BHI). The overnight cultures (ca. 10^9 CFU/ml) were 10-fold serially diluted in 0.1% peptone water up to 10^{-5} . The 10^{-5} dilution was held on ice and used as inoculum. An appropriate volume of the 10^{-5} dilution, calculated based on a ratio of inoculum volume (ml) to meat surface area (cm^2) of 1:100, was pipetted onto the top surface of each piece of meat. The inoculum was spread evenly over the entire top surface, using a sterile glass rod. The estimated counts of *E. coli* on the top surface of meat would be approximately $1\text{--}2\text{ log CFU/cm}^2$.

2.3.2. Sampling of inoculated beef trim

Gauze made from cheese cloth was used as it was not practical to wrap a sponge over the sampling disc and the recovery of bacteria from beef surface by swabbing with sponge and cheese cloth is comparable (Dorsa, Cutter, & Siragusa, 1996). Two strips of cheese cloth ($10\text{ cm} \times 60\text{ cm}$) were folded to make one gauze strip ($3\text{ cm} \times 60\text{ cm}$). Each such gauze strip was individually wrapped in aluminum foil and sterilized by autoclaving. The sampling disc of the sampler was first wrapped with a sterile gauze strip and then another sterile gauze strip that had been pre-moistened with 7 ml of 0.1% peptone water. The gauze strip was held in place by tape. Five pieces of inoculated meat were longitudinally placed in single file directly above the gap of the divided conveyor, with the inoculated side facing down. The dials of conveyor belt and sampling disc were adjusted to predetermined speeds and the sampler was turned on. Upon the first piece of meat passing the sampling disc, the sampler was stopped and the outer strip of gauze was removed and placed in a stomacher bag. Sterile gloves were worn when removing the gauze strip. A new pre-moistened sterile gauze strip was wrapped around the sampling disc as before. The sampling was repeated for the remaining four pieces of meat. Upon completion of sampling for all five pieces, the inner strip of gauze wrapped around the sampling disc was removed and replaced by a fresh one if more than one sampler setting was tested on that day. The sampler was tested at sampling disc dial settings of 4, 5, 7, and 9 with belt dial settings of 3, 6 and 9 for each disc dial setting. So in total, 12 automatic sampler settings were tested. On each day the sampler was tested, five pieces of inoculated meat were also sampled manually using sterilized gauze strips.

A total of 10 vacuum packaged primal cuts were used for the inoculation study. One piece of uninoculated meat selected at

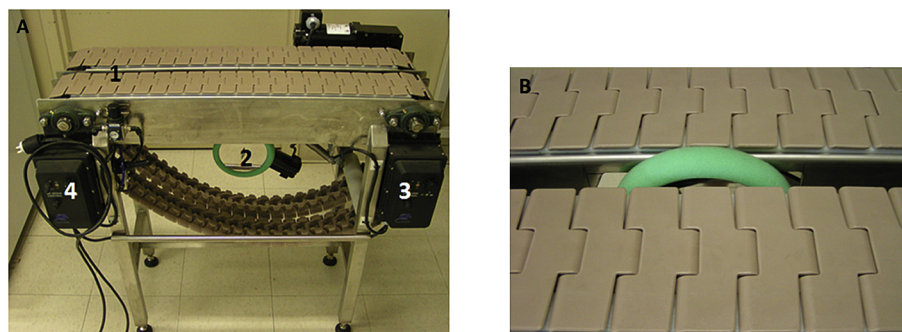


Fig. 2. Photograph of the automatic trim sampler (A) and the position of the sampling disc when activated in relation to the divided conveyor belt (B). The essential parts of the sampler are divided conveyor, 1; sampling disc, 2; motor fitted for the conveyor, 3; and motor fitted for the sampling disc, 4.

random from the meat pieces prepared from each primal was swabbed manually, for determination of meat background microflora.

2.3.3. Microbiological analysis of the samples

Eighteen ml of 0.1% peptone water was added into each stomacher bag containing a gauze strip. Each bag with accompanying diluent was stomached for 2 min. The gauze was squeezed to expel as much liquid as possible. The resulting stomacher fluid was used to determine the numbers of total aerobes, coliforms and *E. coli* as described before (Yang et al., 2012). All bacterial counts were transformed to log CFU/100 cm², with the surface area of each piece of meat sampled being calculated from the length of the meat and the width of the sampling disc. Counts of the same type obtained from each sampler setting or from the manual swabbing on days the sampler was tested were regarded as a set. Mean log values and standard deviations (SD) for each set were calculated. The difference in mean values for each automatic sampler setting and the accompanying manual swabbing was tested using the paired *t*-test function of Minitab version 16 (Minitab Inc., State College, PA), with a significance level of *P* < 0.05.

3. Results

By direct filtration, *E. coli* was recovered from 16 of the 200 (8.0%) swab samples from all the combo bins, with most positive samples yielding 1 *E. coli* and the maximum number of *E. coli* being 2 CFU. These positive samples were from 13 of the 40 levels examined (33%). For enrichment cultures, the number of *E. coli* positive samples per combo bin ranged from 0 to 13 for excision samples and from 0 to 20 for swab samples (Table 1). Overall, 21.5% of excision samples and 38.0% of swab samples were positive for *E. coli*. The location from which a positive sample was obtained was randomly distributed throughout the combo bin. Of the 40 levels that were sampled, 50% were positive by excision, 70% were positive by swabbing. The numbers of samples were 5 (2.5%), 18 (9.0%) and 20 (10.0%) for enrichment cultures from excision containing approximately 4, 3, and 2 log CFU/ml *E. coli*, respectively. The corresponding numbers for enrichment cultures from swabbing were 39 (19.5%), 8 (4.0%), and 29 (14.5%), respectively. The findings clearly show that swab sampling can be more sensitive than excision sampling for recovering small numbers of *E. coli* from beef

trim.

The dial settings 1–2 for the belt and 1–3 for the sampling disc were found to be unsuitable for a smooth operation of the belt or sampling disc, respectively. The sampling disc revolved at 43, 84, 120, 150 and 190 rpm at dial settings of 4, 5, 6 and 7, and 8 to 10, respectively. The belt revolved at 0.26, 2.1, 4.4, 6.4, 7.9, and 9.8, 9.9, and 9.9 rpm at dial settings of 3–10, respectively. The speed of the belt was then calculated to be 0.011, 0.09, 0.18, 0.26, 0.32 m/s and 0.40 m/s when the dial was set at 3 to 7 and at 8, 9 or 10, respectively. All bench trim and steaks weighing up to 1 kg went smoothly over the sampling disc if the meat was relatively even in thickness. However, when the meat was > 1 kg, the meat had a tendency to become stuck or to roll backward on the belt.

The background microflora on meat used for comparison of the sampler and manual swabbing was determined. Aerobes were recovered from all samples from the 10 pieces of meat, at numbers ranging from 1.64 to 3.52 log CFU/100 cm² and with a mean value of 2.65 ± 0.81 log CFU/100 cm². Coliforms and *E. coli* were recovered from 4 and 3 samples, respectively, with numbers ranging from 0.22 to 0.62 log CFU/100 cm².

All three groups of bacteria were recovered from all trim sampled by either manual or automatic sampling (Table 2). The total aerobes recovered by manual swabbing from different batches of trim ranged from 3.06 to 4.64 log CFU/100 cm², with standard deviations of five samples in each group varying from 0.08 to 0.96 log CFU/100 cm². The number of aerobes recovered by manual swabbing was always higher than the corresponding automatic sampling except one pair where the number of aerobes recovered by automatic sampling was 0.14 log CFU/100 cm² more than that by manual swabbing. Even so, the difference between 8 pairs of counts of aerobes recovered by the sampler and manual sampling was not significant (*P* > 0.05). The numbers of coliforms and *E. coli* recovered by manual swabbing differed by only 0.01 to 0.04 log CFU/100 cm² for all but one group, which had a difference of 0.21 log CFU/100 cm², suggesting that coliforms from those samples were exclusively *E. coli* and that the coliforms and *E. coli* counts were likely from the inoculated *E. coli*. The mean numbers of *E. coli* recovered by manual swabbing ranged from 2.28 to 2.63 log CFU/100 cm², with most groups yielding counts between 2.4 and 2.6 log CFU/100 cm². For each pair of automatic and manual sampling, the *E. coli* count from automatic sampling was always lower than that from manual sampling, with the difference ranging from 0.04 to

Table 1
Distribution of *Escherichia coli* in combo bins (B1–10) determined by excision (E) or swabbing (S).

Level	Location	B1		B2		B3		B4		B5		B6		B7		B8		B9		B10	
		E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S
1	1–1	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	+	–	+	–
	1–2	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	+
	1–3	–	–	–	+	–	+	–	+	–	+	–	–	+	–	–	+	–	+	+	+
	1–4	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	+	–	+	–	+
	1–5	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	–	+	+
2	2–1	–	–	–	+	–	–	–	+	–	–	+	+	–	–	–	+	–	–	–	+
	2–2	–	–	–	+	–	+	–	+	–	–	+	–	–	–	+	+	–	+	–	+
	2–3	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	+	–	+	–	+
	2–4	–	–	–	+	–	+	–	–	–	–	+	–	–	–	–	+	–	–	–	+
	2–5	–	–	–	–	+	–	–	–	–	–	+	–	–	–	+	+	–	–	–	+
3	3–1	–	–	–	–	–	+	–	+	–	–	+	–	–	–	+	+	–	–	+	+
	3–2	–	–	–	–	–	–	–	–	–	+	+	–	+	–	–	+	–	–	–	+
	3–3	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	+
	3–4	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	+
	3–5	–	–	–	+	–	–	–	+	–	–	–	–	–	–	+	+	–	–	–	+
4	4–1	–	+	–	+	–	–	–	+	–	–	–	–	+	–	+	+	+	+	–	+
	4–2	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	+	–	+	–	+
	4–3	–	–	+	–	–	–	–	–	–	–	–	+	–	–	+	+	–	+	–	+
	4–4	–	–	–	–	–	–	+	–	–	–	–	–	+	–	+	+	–	+	–	+
	4–5	–	–	–	–	+	+	–	–	–	–	–	–	+	–	–	+	–	+	–	+

Table 2Numbers of total aerobes, coliforms and *Escherichia coli* (log CFU/100 cm²) recovered from beef by manual swabbing (M) and the automatic sampler (A).

Sampler setting		Aerobes		Coliforms		<i>E. coli</i>	
Disc	Belt	M	A	M	A	M	A
4	3	3.09 ± 0.14 ^a	2.93 ± 0.55	2.42 ± 0.19	2.26 ± 0.19	2.38 ± 0.17	2.24 ± 0.18
	6	3.09 ± 0.14	2.68 ± 0.14*	2.42 ± 0.19	2.23 ± 0.08	2.38 ± 0.17	2.21 ± 0.08
	9	4.03 ± 0.32	3.19 ± 0.75	2.65 ± 0.14	2.31 ± 0.24	2.63 ± 0.13	2.30 ± 0.24
5	3	4.03 ± 0.32	3.40 ± 0.38*	2.65 ± 0.14	2.37 ± 0.34	2.63 ± 0.13	2.38 ± 0.38
	6	4.64 ± 0.60	3.99 ± 0.84	2.56 ± 0.21	2.06 ± 0.37*	2.54 ± 0.25	2.06 ± 0.36*
	9	4.64 ± 0.60	2.76 ± 0.71*	2.56 ± 0.21	1.86 ± 0.36*	2.54 ± 0.25	1.85 ± 0.35*
7	3	3.06 ± 0.08	2.82 ± 0.20	2.29 ± 0.17	2.18 ± 0.29	2.28 ± 0.17	2.17 ± 0.30
	6	3.16 ± 0.21	2.65 ± 0.21*	2.42 ± 0.25	2.16 ± 0.10	2.41 ± 0.25	2.15 ± 0.11
	9	3.16 ± 0.21	2.91 ± 0.26	2.42 ± 0.25	2.38 ± 0.12	2.41 ± 0.25	2.37 ± 0.12
9	3	3.38 ± 0.96	3.52 ± 0.34	2.75 ± 0.06	2.17 ± 0.21*	2.54 ± 0.46	2.10 ± 0.28
	6	3.38 ± 0.96	3.22 ± 0.26	2.75 ± 0.06	2.31 ± 0.19*	2.54 ± 0.46	2.30 ± 0.09
	9	4.38 ± 0.42	4.27 ± 0.39	2.54 ± 0.34	2.28 ± 0.26	2.51 ± 0.33	2.13 ± 0.23

^aThe pair of counts of each type from manual swabbing and automatic sampling were significantly different ($P < 0.05$).^a Mean ± SD.

0.69 log CFU/100 cm². Of the 12 pairs of *E. coli* counts from manual and automatic sampling, 10 were not significantly different ($P > 0.05$). The standard deviations for all three groups of bacteria resulting from both sampling methods were in similar ranges.

4. Discussion

The first phase of this study, detection of *E. coli* in commercial combo bins using swab and excision sampling methods, was originally envisioned to use combo bins that had tested positive for *E. coli* O157:H7. However, improvement of carcass decontaminating treatments and of operations of carcass dressing and breaking has resulted in greatly enhanced control over the microbiological contamination of beef produced at the plant (Yang et al., 2012; Yang, Badoni, et al., 2015). Consequently, combo bins found to be positive for *E. coli* O157:H7 in routine testing were extremely rare, i.e., one positive bin in a four-month period. Therefore, the work was carried out with combo bins of *E. coli* O157 negative product and testing for total *E. coli*, which has long been regarded as an indicator organism for the possible contamination of meat with enteric pathogen (Brown et al., 2000). Less than 40% of the enrichment samples tested positive for *E. coli* by either swabbing or excision and the numbers, calculated from Ct values, of *E. coli* in the *E. coli*-positive enrichment cultures were less than 5 log CFU/ml. These findings indicate that numbers of *E. coli* recovered from the samples before enrichment were likely less than 10 per sample. These numbers were in agreement with those from direct filtration, i.e. 8% of the filters yielded *E. coli* at ≤ 2 CFU per sample. The level of *E. coli* on trim in this study was comparable with those in recent studies conducted at federally inspected Canadian beef packing plants which reported that *E. coli* was recovered from a minority of samples and at levels ranging from 0.3 to 1.76 log CFU/2500 cm² (Yang, Tran, Youssef, & Gill, 2015; Yang et al., 2012; Youssef, Badoni, Yang, & Gill, 2013).

In this study, the numbers of overall positive samples and positive levels from swabbing were 17 and 20% more than those by excision, respectively. This suggests that swab sampling can be more sensitive than excision for recovering small numbers of *E. coli* from beef trim. The recovery of bacteria by swabbing can easily be improved further simply by increasing the surface area sampled (Gill & Jones, 2000). Published accounts on comparison of the destructive sampling method excision with non-destructive methods, primarily involving swabbing with various materials, for recovery of very small numbers of *E. coli* are largely lacking. However, comparison of excision and swabbing of meat surfaces with bacteria at log unit levels or higher has been the subject of

investigation of a number of studies (Dorsa et al., 1996; Gallina et al., 2015; Gill & Jones, 2000; Gill, Badoni, Moza, Barbut, & Griffiths, 2005; Gill et al., 2001; Pearce & Bolton, 2005). Most of these studies evaluated carcass surfaces. As would be expected, the recovery of bacteria by different methods may vary with animal species, swabbing materials, bacterial species, conditions and types of meat surfaces. Even so, the numbers of bacteria recovered by swabbing were, in general, comparable with or consistently a good portion of those by excision.

The findings of this study, in particular, that the swabbing method can yield a more representative and sensitive sampling scheme than excision for recovering very small numbers of *E. coli* from trim, rendered it possible to design a swabbing-based prototype of an automatic trim sampler. Automatic sampling, under most combinations of the sampler parameters, resulted in counts similar to manual swabbing for *E. coli*, coliforms and aerobes. In-plant validation studies for *E. coli* O157 including minimum sample size required for the automatic sampling to be equivalent of N60, more automation, such as timed sampling and automatic change of swab, would be required before the sampler can be used in commercial settings. Nevertheless, the findings of this study suggest that automatic sample collection from trim for detection of *E. coli* is possible and that, for quality control purposes, the application could be extended to allow for monitoring of indicator organisms or spoilage bacteria.

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