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Research Paper

Novel Continuous and Manual Sampling Methods for Beef Trim Microbiological Testing

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ABSTRACT

A sampling method that represents a greater proportion of the beef trimmings in a 900-kg combo bin should improve the current pathogen sampling and detection programs used by fresh beef processors. This study compared two novel, nondestructive sampling methodologies (a continuous sampling device [CSD] and a manual sampling device [MSD]) with the current industry methodologies, the N60 Excision (the "gold standard") and N60 Plus, for collection of trim samples. Depending on the experiment, samples were analyzed for naturally occurring *Escherichia coli* O157:H7 or *Salmonella,* inoculated surrogates, or indicator organisms in multiple plants, on multiple days, across multiple lean percentage mixtures. Experiments 1A and 1B with natural contamination found no *E. coli* O157:H7 but similar ($P > 0.05$) prevalence of *Salmonella* (CSD 9.2% versus N60 Excision 6.0%) and similar ($P > 0.05$) levels of indicator organisms for CSD compared with both N60 methodologies. In experiments 2 and 3, CSD cloth sampling had the same or higher prevalence of naturally occurring *E. coli* O157:H7 and *E. coli* O157:H7 surrogate organisms, as well as similar levels of indicator organisms compared with the N60 methodologies. In experiment 4, MSD cloth sampling detected similar ($P > 0.05$) prevalence of *E. coli* O157:H7 surrogate organisms, as well as slightly lower ($P < 0.05$) levels of indicator organisms compared with N60 Plus. In experiment 5, the MSD found similar ($P > 0.05$) prevalence of naturally occurring *E. coli* O157:H7 and the same or slightly higher ($P < 0.05$) levels of naturally occurring indicator organisms compared with N60 Plus. In experiment 6, the MSD detected the same ($P > 0.05$) prevalence of naturally occurring *Salmonella* as did N60 Excision. The results of these experiments collectively demonstrate that sampling beef trim using either the CSD or MSD provides organism recovery that is similar to or better than the N60 Excision or the N60 Plus methodologies.

Key words: Beef trimmings; *Escherichia coli* O157:H7; Indicator organisms; Pathogen testing; *Salmonella*; Sampling method

Foodborne illnesses caused by microorganisms are a food safety concern among consumers and regulatory agencies. Foodborne *Escherichia coli* O157:H7 and *Salmonella* are common human infectious agents throughout the world *(3, 13, 23)* and can cause severe debilitating symptoms and in some cases may result in death. These pathogens can contaminate beef carcasses during processing steps, especially during hide removal *(15, 16, 24)*. Carcass contamination is one of the biggest challenges to the meat industry; therefore, meat processors implement comprehensive, robust food safety systems to keep meat safe and wholesome for consumers *(5)*. Beef trim sampling for pathogen testing is one of the final steps in the food safety system *(4)*.

Traditional N60 Excision sampling consisting of 60 or more surface excision slices, resulting in a total sample of ~375 g per lot (a lot is usually five 900-kg combo bins of beef trimmings but can be a single-combo bin) is recognized as the "gold standard" sampling method *(4)* and is utilized in Food Safety Inspection Service (FSIS) verification sampling *(19)*. In addition, the N60 Plus method has been issued a letter of no objection by FSIS, providing a method for obtaining samples for pathogen testing from a single combo of beef trimmings. Both N60 methods of sampling beef trim for the purpose of conducting pathogen testing have been effective but leave room for improvement because neither method samples a large proportion of the trim in a 900-kg combo bin.

A new approach using continuous sampling of the trim as the combo is filled was developed that is nondestructive. This continuous sampling device (CSD) is positioned at the end of the conveyor so that the trim pieces rub against a sampling cloth as they fall into the combo bin. For situations in which the combo is not filled by a conveyor, a second method was developed that uses the CSD cloth to manually sample all of the trim on the top of the combo by hand (manual sampling device [MSD]). The objective of these experiments was to compare, in a commercial setting, organism recovery for these two new approaches (CSD and MSD) to traditional N60 Excision and N60 Plus methods for

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collecting a sample from a single-combo bin of beef trim for pathogen and microbiological testing.

MATERIALS AND METHODS

Experiments. Six experiments were conducted that include various combinations of CSD and MSD compared with N60 Excision or N60 Plus or both by using naturally occurring organisms or surrogate inoculations. All comparisons used paired sampling on the same combo bins within an experiment. All experiments were conducted in commercial beef processing facilities in collaboration with industry partners. A robust comparison was obtained from these multiple experiments by analyzing for naturally occurring *E. coli* O157:H7 or *Salmonella,* inoculated surrogates, as well as indicator organisms in five different processing plants, on multiple days, across multiple lean percentages (50, 80, 90, and 93% lean). The CSD method samples a vast majority of the meat pieces as they fall into the combo and rub against the cloth. The MSD samples all of the exposed meat at the top of the combo (~11,000 cm^2), while the current methods sample ~1,100 cm^2 (N60 Excision) or slightly less (N60 Plus).

Experiment 1. This experiment includes two preliminary proof-of-concept tests of the continuous sampling concept conducted in large commercial beef processing plants. At that point in development, the sampling material was a cellulose sponge held in place with a stainless steel clamping device positioned at the end of the trim conveyor belt at the combo bin fill station. After the combo bin was filled, the sponge was aseptically removed and placed into a sterile bag for transport to the laboratory for analysis. Experiment 1A compared the CSD to N60 Excision and N60 Plus using paired single-combo lot sampling for naturally occurring organisms from fifty 93% lean trim and fifty 50% lean trim combos from a large fed-beef processing plant. *E. coli* O157:H7 and indicator organisms (aerobic plate count [APC], *E. coli,* and coliforms) were measured. Experiment 1B compared the CSD to N60 Excision by using paired single-combo lot sampling for naturally occurring organisms from 214 combos of 90% lean trim from a cull cow processing plant. *Salmonella* and indicator organisms (APC and *Enterobacteriaceae*) were measured.

Experiment 2. Because of the amount of cellulose sponge that was needed for the CSD, it was deemed too expensive for 24- to 36-in.-wide (ca. 61- to 91.4-cm-wide) conveyor belts often used to convey beef trimmings to various collection points in the processing plants. An alternative material, spunbond olefin polymer cloth, was identified as a replacement that had acceptable characteristics for strength and absorbency, had food-grade approval, and was less expensive than cellulose sponge material. This initial in-plant test of the CSD concept by using a spunbond olefin polymer cloth for the sampling material was conducted in a large commercial fed-beef processing plant. This experiment used surrogates, green fluorescent protein–labeled (GFP) *E. coli* biotype 1 *(6, 14)*, per an FSIS letter of no objection to compare the CSD with N60 Excision and N60 Plus by using paired single-combo lot sampling on 20 combos each ($n = 20$) of 50 and 93% lean trim combos. Surrogates were prepared and used as described in the following. A bolus of surrogate-inoculated trim was introduced into the trim stream on the conveyor at a different randomly selected fill time for each combo bin. This inoculation strategy ensured inoculated trim had the opportunity to be localized to areas throughout the combo from top to bottom representing nonhomogeneous contamination. Samples were tested for the surrogate organism, as well as APC and *E. coli*.

Experiment 3. Additional CSD testing was conducted in three large commercial fed-beef processing plants. Each plant tested both 50 and 80% lean trim combos for naturally occurring *E. coli* O157:H7 comparing the CSD to N60 Plus by using paired single-combo lot sampling on a total of 578 combos for each method.

Experiment 4. This was the initial in-plant test of the MSD concept in a large commercial fed-beef processing plant using the same cloth material as used for the CSD for sampling in experiments 2 and 3. The same GFP *E. coli* biotype 1 surrogates from experiment 2 were used to compare the MSD to N60 Plus by using paired single-combo lot sampling of 25 combos each ($n = 25$) of 50 and 80% lean trim combos. Samples were tested for the surrogates, as well as APC and coliforms.

Experiment 5. Additional MSD testing was conducted in another large commercial fed-beef processing plant. One hundred seventy-five combos each ($n = 175$) of 50 and 80% lean trim combos were tested by using paired single-combo lot sampling for naturally occurring *E. coli* O157:H7 comparing the MSD to N60 Plus. In addition, a subset of 25 combos each ($n = 25$) of these 50 and 80% lean trim combos were tested for indicator organisms (APC, coliforms, and *E. coli*). One sample of 80% lean trim was lost for N60 Plus; thus, the corresponding MSD sample was discarded.

Experiment 6. MSD testing was conducted in a commercial cull cow processing plant. One hundred eighty-two 90% lean trim combos were tested by using paired single-combo lot sampling for naturally occurring *E. coli* O157:H7 and *Salmonella* comparing N60 Excision to MSD.

General sampling procedures. CSD sampling occurred as individual trim combos were filled to provide single-combo lot samples. N60 Excision sampling was conducted by trained plant personnel according to standard procedures *(4, 18, 21)* to provide five combo lot composite samples for experiment 2, but for experiments 1A, 1B, and 6, N60 Excision was conducted to provide single-combo lot samples. N60 Plus sampling was conducted by trained plant personnel according to IEH Laboratories and Consulting (Lake Forest, WA) procedures by using the IEH N60 PLUS SAMPLER to provide single-combo lot samples. MSD sampling was conducted on single combos after they were filled with trim. CSD sampling was always conducted as the combo was filled. N60 Plus was conducted before N60 Excision, and MSD sampling was conducted last. Processing lines involved in the experiments were running peracetic acid intervention sprays near the end of the trim conveyor lines as part of standard trim processing strategies. No neutralizers were added to the samples in this study other than the enrichment media.

CSD sampling procedure. The stainless steel cloth holder was installed at the end of the identified trim conveyor belts at the combo fill station. Prior to starting to fill a combo, chemical sanitizer was applied to the cloth holder to sanitize all surfaces of the holder and then wiped down and allowed to air dry. For experiments 1A and 1B using cellulose sponge as the sampling material, the sponge was installed into the holder to provide an exposed area (24 by 6 in. [144 in^2] or ca. 61 by 15.2 cm [927 cm^2] for experiment 1A and 36 by 6 in. [216 in^2] or ca. 91.4 by 15.2 cm [1,389 cm^2] for experiment 1B) for sampling. For the two CSD experiments using the cloth as the sampling material (experiments 2 and 3), the cloth (24 by 10 in. [240 in^2] or ca. 61 by 25.4 cm

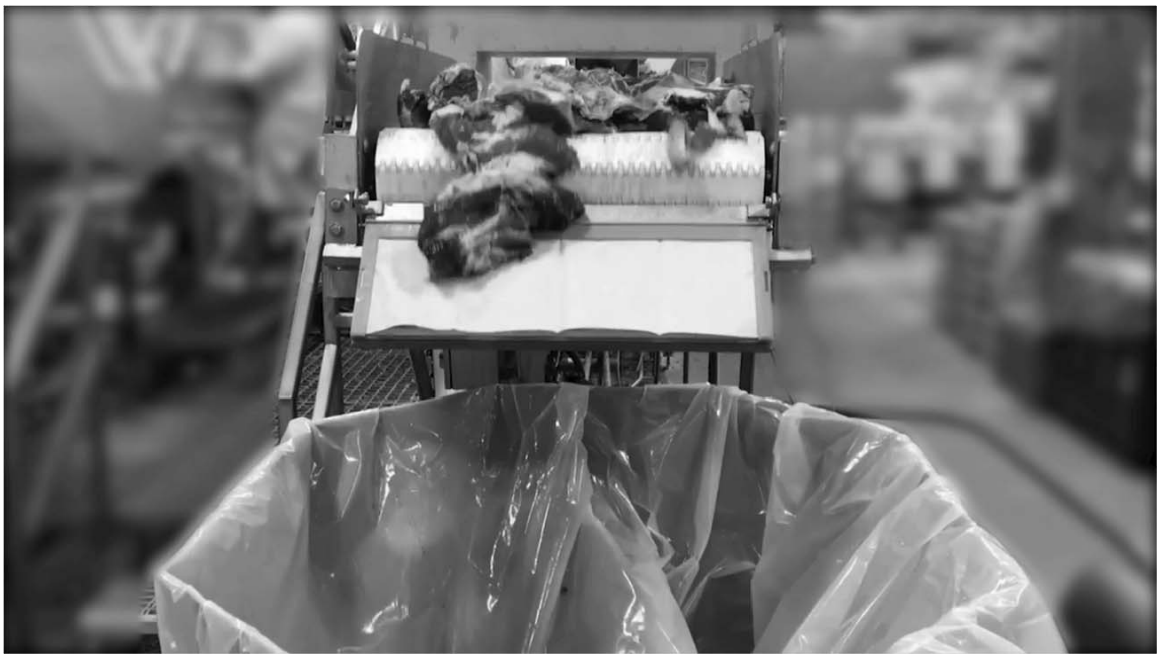

FIGURE 1. *Photograph of continuous sampling device (CSD).*

[1,549 cm^2]) was installed into the holding device such that cloth (24 by 6 in. [144 in^2] sampling area) was exposed for contact with the trim for sample collection (Fig. 1). The trim contacted the sponge or cloth per normal flow from the conveyor into the combo as the combo filled. Once the combo was full with trimmings and after the combo was pulled away, the sponge or cloth was aseptically removed, folded, and placed into a sterile bag (MicroTally swab, Fremonta, Fremont, CA). Between combos, the holder was sanitized and a new sponge or cloth was installed. When a new empty combo was in place, the belt was restarted as normal to fill the combo with trimmings and collect the next sample.

N60 Excision sampling procedure. Excision sampling equipment and plastic sleeves and gloves were sanitized by applying chemical sanitizer to all surfaces. For experiment 2, 60 surface slices were aseptically excised from five combos by obtaining 12 pieces (approximately 3 in. long by 1 in. wide and 1/8 in. thick [ca. 7.6 by 2.5 by 0.32 cm]) per combo *(4, 18, 21)*. Each sample slice was obtained from different, individual pieces of trim. The 12 slices per combo were placed into a separate sample bag. The five bags were placed into a mother bag. Samples were ~75 g per combo to provide an ~375-g composite sample. Five consecutive combos of the same lean type from the same fill site were placed in the mother bag. For experiments 1A, 1B, and 6, N60 Excision was conducted to collect 60 pieces from one combo to provide paired single-combo lot samples.

N60 Plus sampling procedure. Plastic sleeves and gloves were sanitized by applying chemical sanitizer to all plastic surfaces. A sterile sampling bag and hot water–sanitized IEH N60 PLUS SAMPLER with the sample removal tool were carried to the combo to be sampled. Samples were collected from five areas (four corners and center) of each combo by inserting the sampler to its maximum depth into the combo bin. If necessary, the sampler was inserted more than five times to ensure that the device was filled with surface material and that the collected sample was ~165 g. The entire head of the sampling device was placed into the sample bag, and the sanitized sample removal tool was used to push the collected sample out of the tip and into the bag.

MSD sampling procedure. Plastic sleeves and gloves were sanitized by applying chemical sanitizer to all plastic surfaces. After the combo was filled with trimmings and after the combo was pulled away, the MSD cloth (24 by 8 in. [61 by 20.3 cm]) was swabbed or rubbed vigorously over the entire top surface of the trim meat in the combo (Fig. 2). One side of the cloth was used to swab one-half of the meat exposed on top of the combo, and then the cloth was flipped over and the other side of the cloth was used to swab the other half of the meat exposed on top of the combo. Swabbing was conducted for approximately 1.5 min, ensuring that the top and sides as well as spaces between meat pieces were sampled. Sanitized gloves were worn during sample collection, and care was taken so that gloved hands did not contact anything but the cloth and the combo being tested. After sample collection, the cloth material was placed in an appropriately identified sterile bag for transport to the laboratory.

Surrogate inoculations. Two different *E. coli* biotype 1 strains (ATCC accession no. BAA 1429 and BAA 1431) transformed to include GFP markers, have been evaluated and identified as suitable surrogates for *E. coli* O157:H7 in various slaughter and processing applications *(6, 14, 20)*. Each GFP *E. coli* biotype 1 culture was struck for isolation, and one colony from each of the isolated cultures was transferred to separate tubes of 10.0 mL of brain heart infusion (BD, Sparks, MD) supplemented with 0.1 g/L ampicillin (BHI+AMP) and incubated at 35°C for 18 h. After incubation, 0.5 mL of each 10-mL BHI+AMP tube was transferred to a fresh 40 mL of BHI+AMP and incubated at 35°C for 18 h. Following incubation, cultures were transferred to centrifugation tubes and centrifuged for 15 min at 8,000 × *g*, and the supernatant discarded. Pellets were resuspended in 20 mL of 0.85% saline (sodium chloride, Fisher Scientific, Fair Lawn, NJ), vortexed, and centrifuged a second time for 15 min at 8,000 × *g* to perform a wash of the culture. This step was repeated again to provide a second rinse of the culture. After the third and final

FIGURE 2. *Photograph of manual sampling device (MSD).*

centrifugation, each pellet was resuspended in 20 mL of 0.85% saline and vortexed for 15 to 30 s. Suspensions were combined into one sterile container and vortex mixed again for 15 to 30 s. Serial dilutions were performed by transferring 10 mL of each subsequent dilution into new 90-mL containers of sterile 0.85% saline, until a 10^{-8} dilution was reached. Aliquots (100 μL) of resulting dilutions were plated on tryptic soy agar supplemented with ampicillin, incubated at 35°C for 18 h, and enumerated. The saline dilution mixtures were held refrigerated (4 to 8°C) until use.

For the two surrogate inoculation experiments, a 9.0-kg bolus of trim was obtained approximately 30 min prior to the start of each combo fill and inoculated with the GFP *E. coli* biotype 1 cultures at 2.0 log CFU/g. This inoculation level was designed to target an inoculated concentration of 1.0 CFU/g for the entire combo, which would be consistent with the typical *E. coli* levels in raw beef trim as 84.3% of FSIS samples were found to harbor *E. coli* at concentrations less than 10 CFU/g *(12)*. The 2.0-log CFU/g inoculation level was obtained by diluting 0.9 mL of a 1×10^6 CFU/mL inoculum into 4.1 mL of sterile 0.85% saline, and all 5 mL was applied to individual pieces of the 9.0-kg trim bolus and hand mixed with sanitized gloves to spread the inoculum throughout the 9.0 kg of product. The inoculated bolus was held at processing room temperature (7°C) until it could be added to the beef trim conveyor feeding into the test combo bin at the specified time for that combo. The average time to fill each combo for each target trim line was determined and divided into 20 evenly spaced increments (e.g., 50% trim line: fill time 5 min ÷ 20 increments = 15 s per increment; 93% trim line: fill time 30 min ÷ 20 increments = 1.5 min per increment). Because the location within the combo of the inoculated surrogate organism was expected to impact the ability of different sampling methods to detect it and to simulate low concentration, nonuniform pathogen contamination, a random number generator was used to select a random, unbiased fill time when the inoculated trim bolus was introduced into each combo. This ensured that across all the test combos within an experiment, the inoculated trim could be located anywhere throughout the combo. A random number generator with 0 as the lower limit and 19 as the upper limit was used to generate 20 or 25 time points to determine the time into each combo fill that the inoculated bolus was added to the trim conveyor. For example, the random number generator indicated the first 50% lean combo should have the inoculated trim bolus added at the sixth time increment (15 s × 6 = 1.5 min), so the bolus was added to the trim line so that it would enter the combo 1.5 min after it started filling. FSIS provided a letter of no objection for this protocol.

Sample analysis for surrogate-inoculated beef trim. Samples from surrogate-inoculated trim from experiments 2 and 4 were held refrigerated (2 to 8°C) until tested. Samples from inoculated trim were analyzed for APC, coliforms, *E. coli,* and GFP *E. coli* enumeration and GFP *E. coli* prevalence, as appropriate for specific experiment objectives. N60 samples (375 ± 5.0 g) each received 375 mL of Butterfield's diluent and were stomached for 1 min. N60 Plus samples received a 1:1 dilution with Butterfield's diluent based on sample weight (≈165 g) and were stomached for 1 min. CSD and MSD samples each received 50 mL of Butterfield's diluent and were stomached for 1 min. Aliquots from each sample were plated on APC Petrifilm, *E. coli*/Coliform Petrifilm (3M, St. Paul, MN), and violet red bile agar plus 100 μg/mL ampicillin plates for enumeration of *E. coli* O157:H7. For prevalence detection of GFP *E. coli,* buffered peptone water and 100 μg/mL ampicillin was added to the 50 mL of Butterfield's dilution to achieve a final dilution volume of 1:10. Samples were incubated at 35°C for 18 to 24 h. After incubation, samples were swab streaked for isolation onto violet red bile agar plus 100 μg/mL ampicillin plates to determine presence or absence of GFP organisms.

Sample analysis for noninoculated beef trim. Samples from noninoculated trim (experiments 1, 3, 5, and 6) were held refrigerated (2 to 8°C) until tested. Samples were analyzed for APC, coliforms, and *E. coli* enumeration and *E. coli* O157:H7 and *Salmonella* prevalence, as appropriate for specific experiment objectives. For N60 Excision samples, 375 g of beef trim was diluted 1:3 with mEHEC media (BioControl, Seattle, WA). For N60 Plus samples, 165 g of beef trim was mixed with 300 mL of mEHEC media. CSD and MSD samples were enriched with 200

TABLE 1. *Prevalence of natural* E. coli *O157:H7 and* Salmonella *and enumeration for indicator organisms by sampling method using a sponge as the CSD sampling material (experiment 1)*[a]

	Experiment 1A[b]			Experiment 1B[b]	
	CSD	N60 Plus	N60 Excision[c]	CSD	N60 Excision
n	100[d]	100[d]	100[d]	214[e]	214[e]
E. coli O157:H7 (%)	0	0	0		
Salmonella (%)				9.2	6.0
APC[f]					
No. quantifiable[g]	95/100	100/100	100/100	214/214	214/214
Mean[h]	4.65	4.73	4.50	2.57	2.58
Enterobacteriacea					
No. quantifiable				108/214	112/214
Mean				1.17	1.15
Coliforms					
No. quantifiable	74/100	98/100	72/100		
Mean	2.82	3.02	3.10		
E. coli					
No. quantifiable	21/100	25/100	20/100		
Mean	2.40	2.49	2.71		

[a] CSD, continuous sampling device.
[b] There was no significant difference ($P > 0.05$) between methods for any measurement.
[c] All N60 Excision samples weighed 365 g.
[d] Fifty 93% lean trim combos and fifty 50% lean trim combos. All paired single-combo lots.
[e] All 90% lean trim combos. All paired single-combo lots.
[f] APC, aerobic plate count.
[g] No. quantifiable, the number of samples with quantifiable data/total number of samples. The mean was calculated only from quantifiable data.
[h] Mean, reported in log CFU per sample.

mL of mEHEC media. Samples were stomached for 1 min. APC, coliforms, and *E. coli* counts were obtained by plating 1-mL aliquots of the stomached sample, removed prior to sample incubation, onto the appropriate PetriFilm (3M). Samples were incubated for 8 h at 42°C and then analyzed for *E. coli* O157:H7 or *Salmonella* by using the Assurance GDS assays (BioControl, Seattle, WA).

Statistical analyses. Count data were log transformed prior to analysis. Many samples had values that fell below the assay limit of detection for enumeration. The data are reported as the number of samples with quantifiable data out of the total. The mean was calculated only from quantifiable data. A few samples ($n = 4$) had colonies that were too numerous to count. Values for samples where colonies were too numerous to count were recorded as 1.0 log greater than the upper limit of the dilution level (i.e., >250 was equated to 2,500).

One-way statistical analysis (analysis of variance) was performed by using the general linear model procedure of SAS (SAS Institute Inc., Cary, NC) for main effects of sampling method and lean type and their interaction. Least-squares means were calculated, and pairwise comparisons of means were determined by using the Tukey-Kramer test method, with the probability level at $P < 0.05$. Comparison of pathogen prevalence was performed by using Prism (GraphPad Software, La Jolla, CA).

RESULTS

Experiment 1 included two proof-of-concept tests. In experiment 1A comparing CSD to N60 Plus, there were no positive *E. coli* O157:H7 tests from any method, but APC, coliforms, and *E. coli* were not different ($P > 0.05$) between the two methods (Table 1). In experiment 1B comparing CSD to N60 Excision, the APC and *Enterobacteriaceae* counts were not different ($P > 0.05$) between the two methods, but the CSD found *Salmonella* in 9.2% of the samples compared with 6.0% for N60 Excision, although this difference was not significant ($P > 0.05$; Table 1). These preliminary results led to refinement of the concept (e.g., sponge was replaced with spunbond cloth material to reduce costs) and initiation of additional experiments to determine whether the continuous sampling approach was as good or better than existing methods of trim sampling.

Experiment 2 compared the prevalence of inoculated surrogate organisms and the recovery of indicator organisms from beef trim combos among N60 Excision, N60 Plus, and CSD sampling methods. The CSD detected the GFP *E. coli* in 100% of samples, which was greater ($P < 0.05$) than the prevalence detected by N60 Excision in either lean type and greater ($P < 0.05$) than the prevalence detected by N60 Plus for 50% lean (Table 2). The CSD recovered a slightly lower ($P < 0.05$) level of APC than N60 Plus for 93% lean but similar ($P > 0.05$) levels for 50% lean. The CSD recovered the same ($P > 0.05$) level of APC as N60 Excision regardless of lean type. CSD and N60 Plus recovered quantifiable APC from all samples. The CSD recovered similar ($P < 0.05$) levels of *E. coli* as N60 Plus regardless of

TABLE 2. *Prevalence or enumeration for each microorganism class by lean type and sampling method using surrogate inoculation (experiment 2)*

Lean type (%)	Microorganism	N60 Excision ($n = 4$)[a]	N60 Plus ($n = 20$)[b]	CSD ($n = 20$)[c]
93	GFP *E. coli* (%)[d]	50 B[e]	90 A	100 A
	APC[f]			
	No. quantifiable[g]	4/4	20/20	20/20
	Mean[h]	4.96 AB	5.64 A	4.65 B
	E. coli			
	No. quantifiable	0/4	17/20	19/20
	Mean	NQ[i]	3.37 A	2.59 A
50	GFP *E. coli* (%)	75 B	70 B	100 A
	APC			
	No. quantifiable	4/4	20/20	20/20
	Mean	4.67 A	4.96 A	4.32 A
	E. coli			
	No. quantifiable	0/4	13/20	18/20
	Mean	NQ[i]	3.10 A	2.33 A

[a] Five combo lots, but the same 20 matched combos.
[b] N60 Plus mean sample weights were 50%, 176.8 g (range was 163 to 192 g); 93%, 180.5 g (165 to 206 g). The same 20 matched single-combo lots.
[c] CSD, continuous sampling device. The same 20 matched single-combo lots.
[d] GFP *E. coli,* green fluorescent protein–labeled *E. coli.*
[e] Means within the same row with different letters differed ($P < 0.05$).
[f] APC, aerobic plate count.
[g] No. quantifiable, the number of samples with quantifiable data/total number of samples. The mean was calculated only from quantifiable data.
[h] Mean, reported in log CFU per sample.
[i] NQ, not quantifiable.

lean type. Across both lean types, CSD recovered a greater number of quantifiable *E. coli* samples than did N60 Plus (38 versus 30 of 40). None of the N60 Excision samples had quantifiable *E. coli*.

Experiment 3 compared the prevalence of naturally occurring *E. coli* O157:H7 from beef trim combos between the N60 Plus and CSD sampling methods across three processing plants and two lean types (Table 3). From a total of 578 samples, the CSD found one and N60 Plus found two *E. coli* O157:H7–positive combos from the same plant and lean type. One of the combos was positive with both sampling methods.

Experiment 4 compared the prevalence of inoculated surrogate organisms and the recovery of indicator organisms from beef trim combos between the N60 Plus and MSD sampling methods. The MSD detected the GFP *E. coli* in 44 and 52% of samples from 50 and 80% lean types, respectively, which were both numerically higher, but not different ($P > 0.05$) from the 36 and 32% prevalence detected by N60 Plus for the two lean types, respectively (Table 4). The MSD recovered lower ($P < 0.05$) levels of APC and coliforms than N60 Plus regardless of lean type. CSD and N60 Plus recovered quantifiable APC from all 50 samples. Across both lean types, CSD recovered a similar number of quantifiable coliform samples as N60 Plus (48 versus 42 of 50).

TABLE 3. *Prevalence of natural* E. coli *O157:H7 by plant, lean type, and sampling method (experiment 3)*

Plant	Lean type (%)	n[a]	N60 Plus (%)[b]	CSD (%)[c]
A	50	26	0	0
	80	50	0	0
B	50	45	0	0
	80	50	0	0
C	50	217	0.9	0.5
	80	190	0	0

[a] All paired single-combo lots.
[b] There was no significant difference ($P > 0.05$) between methods for any measurement.
[c] CSD, continuous sampling device.

Experiment 5 compared the prevalence of naturally occurring *E. coli* O157:H7 and indicator organisms from beef trim combos with two lean types between the N60 Plus and MSD sampling methods. The MSD found 7.4% *E. coli* O157:H7–positive combos with 50% lean and 2.3% positive combos with 80% lean, which were not different ($P > 0.05$) from N60 Plus with 6.3% *E. coli* O157:H7–positive combos for 50% lean and 0.6% positive combos for 80% lean (Table 5). Ten (5.7%) of the 50% lean combos were *E. coli* O157:H7 positive with both methods. The MSD found higher ($P < 0.05$) levels of APC for both lean types than did N60 Plus. Both sampling methods detected similar ($P > 0.05$) levels of coliforms and *E. coli* regardless of lean type. Across both lean types and three indicator organisms, CSD recovered a similar number of quantifiable samples as N60 Plus (125 versus 128 of 147).

Experiment 6 compared the prevalence of naturally occurring *E. coli* O157:H7 and *Salmonella* from 182 beef trim combos (paired single-combo lots) between the N60

TABLE 4. *Prevalence or enumeration for each microorganism class by lean type and sampling method using surrogate inoculation (experiment 4)*

Lean type (%)	Microorganism	N60 Plus ($n = 25$)[a,b]	MSD ($n = 25$)[b,c]
50	GFP *E. coli* (%)[d]	36 A[e]	44 A
	APC[f]		
	No. quantifiable[g]	25/25	25/25
	Mean[h]	5.26 A	4.29 A
	Coliforms		
	No. quantifiable	23/25	20/25
	Mean	3.26 A	2.04 A
80	GFP *E. coli* (%)	32 A	52 A
	APC		
	No. quantifiable	25/25	25/25
	Mean	6.07 A	4.93 B
	Coliforms		
	No. quantifiable	25/25	22/25
	Mean	3.74 A	2.64 B

[a] N60 Plus mean sample weights were 50%, 161.6 g (range was 147 to 180 g; two samples below 150 g); 80%, 166.8 g (137 to 181 g; two samples below 150 g).
[b] The same 25 paired single-combo lots.
[c] MSD, manual sampling device.
[d] GFP *E. coli,* green fluorescent protein–labeled *E. coli*.
[e] Means within the same row with different letters differed ($P \leq 0.05$).
[f] APC, aerobic plate count.
[g] No. quantifiable, the number of samples with quantifiable data/total number of samples.
[h] Mean, reported in log CFU per sample.

TABLE 5. *Prevalence of natural* E. coli *O157:H7 and enumeration for indicator organisms by sampling method and lean type (experiment 5)*

Lean type (%)	Microorganism	n[a]	N60 Plus	n	MSD[b]
50	*E. coli* O157:H7 (%)	175	6.3 A[c]	175	7.4 A
	APC[d]				
	No. quantifiable[e]		25/25		25/25
	Mean[f]		4.65 B		5.57 A
	Coliforms				
	No. quantifiable		24/25		25/25
	Mean[g]		3.39 A		3.38 A
	E. coli				
	No. quantifiable		16/25		13/25
	Mean[g]		2.88 A		2.65 A
80	*E. coli* O157:H7 (%)	174	0.6 A	175	2.3 A
	APC				
	No. quantifiable		24/24		24/24
	Mean		5.07 B		5.79 A
	Coliforms				
	No. quantifiable		23/24		22/24
	Mean[h]		3.28 A		3.14 A
	E. coli				
	No. quantifiable		16/24		16/24
	Mean[h]		2.82 A		2.84 A

[a] All paired single-combo lots. Indicator organisms measured on a subsample of the 175 combos.
[b] MSD, manual sampling device.
[c] Means within the same row with different letters differed ($P \leq 0.05$).
[d] APC, aerobic plate count.
[e] No. quantifiable, the number of samples with quantifiable data/total number of samples.
[f] Mean, reported in log CFU per sample.
[g] N60 Plus had two samples "too numerous to count." For analysis, those samples were assigned values 1 log above the upper limit of detection.
[h] N60 Plus had one sample "too numerous to count." For analysis, that sample was assigned values 1 log above the upper limit of detection.

Excision and MSD sampling methods for 90% lean trim. Neither method detected *E. coli* O157:H7 in any combo. *Salmonella* detection was not different ($P > 0.05$) between methods in which N60 Excision found 5.5% and MSD found 4.9% *Salmonella*-positive combos (Table 6). Of the 182 combos, 15 were *Salmonella* positive by at least one method. Of the 15, 10 were positive by N60 Excision and 9 were positive by MSD, but only 4 combos were positive by both methods.

DISCUSSION

U.S. beef processing companies have implemented extensive food safety systems designed to minimize the risk of pathogen contamination in final products *(5)*. A part of that safety system is the process of conducting test-and-hold sampling and pathogen testing of all lots of beef trim that are destined for raw, nonintact products. The standard for trim sampling is N60 Excision *(1, 4)*. It was first used in 2003, and by 2007, most large U.S. beef processors had implemented the N60 Excision sampling approach for testing lots of beef trim for *E. coli* O157:H7 *(7)*. In addition, the N60 Plus method has facilitated the desire of some producers of beef trimmings to move from a five-combo lot to a single-combo lot sampling and testing program. Industry concerns with the current sampling methods center around employee safety, time and labor requirements, and product loss due to sample collection. The new methods do not involve the use of a knife or difficulties associated with the N60 Plus drilling through dry ice–frozen meat. The CSD method samples a vast majority of the meat pieces in the combo, and the MSD samples ~11,000 cm^2, while the current methods sample ~1,100 cm^2 (N60 Excision) or slightly less (N60 Plus).

Numerous studies have been published describing sampling methods for beef, primarily carcass surfaces. Sampling the purge that collects in the bottom of the combo

TABLE 6. *Prevalence of naturally occurring* E. coli *O157:H7 and* Salmonella *by sampling method (experiment 6)*

Organism[a]	n[b]	N60 Excision (%)	MSD (%)[c]
E. coli O157:H7	182	0	0
Salmonella	182	5.5	4.9

[a] There was no significant difference ($P > 0.05$) between methods for either organism.
[b] All paired single-combo lots.
[c] MSD, manual sampling device.

bin has been shown to be effective but not feasible for routine testing *(8)*. Excision of surface tissue has been considered the standard, and many of these studies have compared excision sampling to various approaches to sampling the meat surface. Various materials for swabbing a meat surface to sample the bacterial population have been studied *(9, 11, 12, 17)*. The general conclusion has been that quantification of bacteria by any swab method depends on the conditions of the sample (e.g., hot or chilled and wet or dry) *(11)*. It also has been concluded that excision generally yields a greater number of bacteria *(2),* but for purposes of presence or absence testing, cellulose sponge swabbing has performed adequately (*9–12, 17, 22)*. Based on those studies and the historical use of cellulose sponge sampling of carcasses, the preliminary CSD testing in experiments 1A and 1B used cellulose sponge as the sampling material, and although promising results were obtained, it proved too expensive for commercial implementation. That realization led us to the spunbond olefin polymer cloth material that was absorbent but strong and slightly abrasive with food-grade approval that was used in all subsequent experiments for CSD and MSD.

In situations in which the trim is not conveyed to a combo bin and, thus, the CSD is not an option, the MSD provides an alternative sampling method that also provides a sample of a greater amount of the trim in the combo than current methods, with many of the same benefits as the CSD. Consistent with previous comparisons of excision and swabbing sampling approaches, the CSD and MSD provided similar organism recovery overall and detected pathogens or their surrogates at least as accurately as excision sampling. As expected, not all experiments resulted in consistent outcomes. It is generally accepted that there is variation in effectiveness of any sampling method. Thus, to achieve a robust comparison of these sampling methods, they were compared in multiple experiments, under a variety of conditions across multiple companies, plants, and lean percentages to detect a variety of organisms. The CSD and MSD approaches provide opportunities to significantly increase the amount of trim sampled within each combo at potentially lower cost than current methods, depending on ultimate commercial implementation. These cost savings may be achieved through safer practice, less labor, nondestructive sampling, and decreased media for enrichment.

In conclusion, these data collected from over 1,400 samples on numerous days across multiple companies, processing plants, and lean percentages collectively demonstrate that both the CSD and MSD would provide the same or better level of performance for detecting pathogen contamination in beef trim as that currently provided by the N60 Excision and N60 Plus sampling methods. In agreement with this conclusion, FSIS has evaluated these data and provided a letter of no objection allowing use of CSD and MSD sampling of beef trim combos for microbiological testing purposes.

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