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Research Paper

Validation of Additional Approaches and Applications for Using the Continuous and Manual Sampling Devices for Raw Beef Trim

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ABSTRACT

In this work, the goal was to determine the efficacy of MicroTally-based sampling in scenarios commonly encountered in the commercial beef processing industry, but outside of the parameters evaluated during the initial proof-of-concept work. The data were derived from 1,650 matched samples collected from 540 individual combo bins at six commercial beef processing plants, comparing MicroTally-based sampling (continuous and manual sampling devices [CSD and MSD]) to N60 Excision and/or N60 Plus methods. Mounting a 61-cm CSD cartridge to a 30-cm-wide conveyor provided sampling that is equivalent to N60 Excision and N60 Plus methods. Mounting a CSD to a chute instead of a conveyor was equivalent to the N60 Plus sampling method. The CSD was shown to be effective for sampling when used in conjunction with a "swinging arm trim diverter" and receiving product in batch mode as opposed to continuous flow. MSD sampling of oval combo bins with trim surface area (≈0.93 m^2 [≈1,439 in^2]) less than 1 m^2 (1,600 in^2) was shown to be equivalent to the N60 Plus sample collection method. Peracetic acid applied at the end of the trim conveyor did not negatively impact pathogen index target detection of the CSD even if the samples were shipped overnight before analysis. Pathogen index targets were demonstrated to be useful tools for validating methods designed to measure pathogen prevalence. The data presented herein support equivalency criteria of within 0.5 log CFU per sample for indicator organism counts. These data collectively support various alternative applications of MicroTally-based trim sampling and the application and interpretation of alternative methods for pathogen detection.

HIGHLIGHTS

- CSD cartridges used in a variety of mountings were equivalent to N60 methods.
- MSD sampling of combo bins with surface area <1 m^2 was equivalent to N60 Plus.
- Pathogen index targets are useful alternatives for detection of rare pathogens.

Key words: Beef trim; Continuous sampling device; Manual sampling device; MicroTally; N60; Pathogen

Escherichia coli O157:H7 is an adulterant in raw beef products *(16),* so beef processing companies test 900-kg combo bins of beef trimmings and divert any combo bins that produce positive test results for *E. coli* O157:H7, preventing them from being used for the production of raw ground beef *(1, 2, 8)*. The previous standard methods for sampling raw beef trim, N60 Excision and N60 Plus, were tedious, labor-intensive, and destructive methods that only sampled a very small fraction (∼0.45 kg) of a 900-kg lot. MicroTally-based sampling, used in both continuous sampling device (CSD) and manual sampling device (MSD) formats, recently was proven to be at least as good as, if not better than, the previous standard methods for sample collection of raw beef trimmings *(24)*.

Because there is little to no consistency with layout and design of commercial beef processing plants, it is difficult to implement a "one size fits all" validation for finished product testing. When applications implemented by processing plants deviate substantially from the previously validated methods, those changes must be validated as well. As validated in the proof-of-concept trials, each CSD was mounted to the end of a conveyor at a 45° angle down from the horizontal *(24)*. The conveyors used in the initial validation were 61 cm (ca. 24 in.) wide or wider. Whereas the CSD is only available in a 61-cm width, processing plant conveyors used to fill 900-kg combo bins often can be found as narrow as 30 cm (ca. 12 in.) wide. Due to space constraints, some combo bin fill stations have a substantial vertical gap between the end of the conveyor and the combo bin and use a chute to bridge the gap. In these scenarios, the CSD would be mounted at the end of the chute to collect the sample. For the MSD, there are fewer variables among plants. However, one important factor is the surface area of the combo bin. The combo bins used in the initial MSD validation were square with a surface area of 1 m^2 (ca. 1,600 in^2), but many combo bins utilized by the beef

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processing industry have an oval opening with a slightly smaller surface area (0.93 m^2 [ca. 1,439 in^2]).

In addition, the initial validation utilized a combination of testing assays (pathogen prevalence, indicator organism concentrations, and surrogate organism inoculation) to evaluate efficacy. In this study, detection of pathogen index targets, an indirect measure of pathogen prevalence, was utilized as well. Buchanan *(4)* defined index organisms as "a microorganism or group of microorganisms that is indicative of a specific pathogen." Similarly, a pathogen index target would be a gene or group of genes that is indicative of a specific pathogen, but not absolutely confined to that pathogen. When performing qualitative testing for genes harbored by the pathogen of interest as well as similar organisms, the prevalence increases to a more useful range *(17)* than direct pathogen testing, and the results are a better fit to the purpose of the sampler's intended application than quantitative analyses like indicator organism counts. Furthermore, these target genes would be similar to those currently used in many commercial, PCR-based pathogen detection tests.

The research described herein was done to extend the validation of the CSD and MSD to include additional scenarios encountered in many commercial beef processing plants during normal operations. In addition, this article illustrates the usefulness of pathogen index targets and interpretation of indicator organism count data to provide a more appropriate measure of sampling efficacy.

MATERIALS AND METHODS

Sampling procedures. CSD sampling occurred as individual trim combo bins were filled to provide single combo bin samples. N60 Excision sampling was conducted by trained personnel according to approved Food Safety and Inspection Service procedures for single combo bin sampling *(18, 19)*. N60 Plus sampling was conducted by trained personnel according to IEH Laboratories and Consulting procedures to provide single combo bin samples. MSD sampling was conducted using the MicroTally swab (Fremonta Corporation, Fremont, CA) on single combo bins after filling *(16)*. When used, the order of sampling collection by MSD, N60 Excision, and/or N60 Plus was rotated to avoid bias. Antimicrobial spray applications were not altered for any experiment. Most plants sprayed peracetic acid (PAA) 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 added at the laboratory. Samples for experiments 1, 3, 4, 5, and 6 were shipped overnight to the laboratory for processing. Samples for experiment 2 were processed in-house.

Experiment 1. Samples were collected on four separate days at a commercial beef processing plant to compare the efficacy of all four sample collection methods: CSD, MSD, N60 Excision, and N60 Plus. In the initial publication *(24)* of the validation of the CSD and MSD, the two new methods (CSD and MSD) were not compared with each other but only to the established N60 Excision and N60 Plus methods. Experiment 1 of the current work was performed so all methods could be compared with one another. One hundred eighty-five combo bins (94 of 50% and 91 of 80% lean trim) were tested for indicator organism counts, aerobic plate counts (APC), and *Enterobacteriaceae* counts (EBC), as well as for prevalence of pathogen index targets: intimin, hemolysin, and O serogroups. The 50% lean point conveyor was 61 cm wide, whereas the 80% lean point conveyor was 30 cm wide. Both conveyors had PAA spraying trim pieces just prior to exiting the conveyor (50% line, PAA was 2 m from end of line; 80% line, PAA was 0.5 m from end of line). Samples were shipped at 4°C overnight to the laboratory for processing the next day.

Experiment 2. The goal of experiment 2 was to collect additional data on the efficacy of using a 61-cm-wide CSD to collect a sample from a 30-cm-wide belt. For this experiment, the CSD was compared with single combo bin N60 Excision sampling for 115 combo bins. The conveyor had PAA spraying on trim pieces approximately 1 m prior to the CSD mounting. Samples were processed on-site. Samples were analyzed for pathogen index targets using the GeneDisc Plate STEC Top 7 (Pall Corporation, Port Washington, NY).

Experiment 3. A MeatMaster sorting (Foss Analytics, Hilleroed, Denmark) on percentage fat with a swinging arm diverter was used for an evaluation of the CSD's sampling efficacy in batch mode. The CSD was compared with N60 Excision sampling for 40 combo bins. Samples were analyzed for indicator organism counts (APC) and for prevalence of pathogen index targets: intimin, hemolysin, and O serogroups.

Experiment 4. Samples were collected on 2 days at a processing plant from 40 combo bins filled from one trim line (85:15 lean point) using a CSD mounted at the bottom of a chute. MSD and N60 Plus samples were collected for comparison. The surface area of the trim combo bins used for experiments 4, 5, and 6 was approximately 0.93 m^2 per combo bin, as opposed to combo bins in the initial validation, which had 1 m^2 of surface area per combo bin. Forty combo bins were tested for indicator organism counts (APC and EBC) and for prevalence of pathogen index targets: intimin, hemolysin, O serogroups, heme receptor, *tetA,* and *tetB*. PAA spray was applied to trim approximately 6 m upstream of the CSD mounting at the combo bin fill station. Samples were shipped overnight to the laboratory for processing.

Experiment 5. Samples were collected on 2 days at a processing plant using a CSD mounted at the bottom of a chute. MSD and N60 Plus samples were collected for comparison. Eighty combo bins total from two fill stations (lean points ranged from 65:35 to 85:15) were tested for indicator organism counts (APC and EBC) and for prevalence of pathogen index targets: intimin, hemolysin, O serogroups, heme receptor, adhesion siderophore, *tetA,* and *tetB*. PAA spray was applied to trim approximately 6 m upstream of the CSD mounting at the combo bin fill station. Samples were shipped overnight to the laboratory for processing.

Experiment 6. Samples were collected on 2 days from two trim lines each day at a processing plant using two conveyor-mounted CSDs. MSD and N60 Plus samples were collected for comparison. Eighty combo bins total from two fill stations (lean points ranged from 50:50 to 81:19) were tested for indicator organism counts (APC and EBC) and for prevalence of pathogen index targets: intimin, hemolysin, O serogroups, heme receptor, adhesion siderophore, *tetA,* and *tetB*. PURAC CL 21-80 (Corbion, Lenexa, KS) was applied as an antimicrobial spray to trim approximately 9 m upstream of the CSD mounting at the combo bin fill station. Samples were shipped overnight to the laboratory for processing.

CSD sampling procedure. The stainless steel CSD baseplates were installed as described in Supplemental Figure S1, with the specifics described in each of the experiment method summaries. Prior to starting to fill a combo bin, a MicroTally swab (61 by 25.4 cm [ca. 24 by 10 in.], Fremonta Corporation) was installed into the removable plastic cartridge such that an ≈1,549-cm^2 (ca. 144-in^2) sampling area of the swab was exposed for contacting with the trim to collect the sample. The trim contacted the swab per normal flow and fall as the combo bin filled. Once the combo bin was full of trimmings, and after the combo bin was pulled away, the cartridge was removed and a new cartridge previously loaded with a MicroTally swab was inserted into the baseplate to sample the next combo bin. When a new empty combo bin was in place, the belt was restarted as normal to fill the combo bin with trimmings and collect the next sample. The used cartridge was disassembled, and the MicroTally swab was aseptically removed, folded, and placed into a sterile bag.

MSD sampling procedure. Plastic sleeves and gloves were sanitized by applying an alcohol-based sanitizer not containing any quaternary ammonium compounds. After the combo bin was full of trimmings, and after the combo bin was pulled away to a staging area, the MicroTally swab (61 by 25.4 cm [ca. 24 by 10 in.]) was vigorously rubbed over the entire top surface of the trim meat in the combo bin and pushed into the crevices between trim pieces by trained personnel, as previously described *(24)*. Individuals collecting the samples were instructed to use enough pressure to ensure that microbiological organisms present on the surfaces of the beef trimmings were dislodged from the product and captured on the swab. One side of the swab was used to scrub one half of the meat exposed on top of the combo bin, and then the swab was flipped over and the other side of the swab used to scrub the other half of the meat exposed on top of the combo bin. Swabbing was conducted for a minimum of 90 s. Sanitized gloves were worn during sample collection, and care was taken so that gloved hands did not contact anything but the swab and the meat being tested. After sample collection, the swab was placed in an appropriately identified sterile bag for transport to the laboratory.

N60 Excision sampling procedure. Before each sample, the excision sampling equipment was sanitized. Plastic sleeves and gloves were sanitized by applying chemical sanitizer to all surfaces. Sixty surface slices (approximately 7.6 by 2.5 by 0.32 cm [ca. 3 by 1 by 1/8 in.]) were aseptically excised from individual combo bins *(18, 19)*. Sample slices were each obtained from different individual pieces of trim. The 60 slices per combo bin were obtained with a target weight of ∼375 g and were placed into a sample bag.

N60 Plus sampling procedure. Plastic sleeves and gloves were sanitized by applying chemical sanitizer to all surfaces. A sterile sampling bag and hot water–sanitized IEH N60 Plus sampler were carried to the combo bin to be sampled. Samples were collected from five areas (four corners and center) of each combo bin 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 a sanitized sample removal tool was used to push the collected sample out of the sampling head and into the bag.

Sample analysis. Samples for experiments 1, 3, 4, 5, and 6 were shipped to the laboratory overnight and held refrigerated (2 to 8°C) until they were tested. Samples for experiment 2 were processed in-house the same day as they were collected. Samples were analyzed for indicator organism counts and pathogen index target prevalence as described in the individual experiment summaries. N60 Excision and N60 Plus samples were processed according to standard laboratory procedures used by the collaborating companies. CSD and MSD samples were enriched with 200 mL of growth media appropriate for the detection platform implemented by the individual processing company and were analyzed using the same procedures as for the other sampling methods. Samples were stomached for 30 s to homogenize them. For experiments 1, 3, 4, 5, and 6, all samples were incubated for 9 h at 42°C in prewarmed tryptic soy broth for enrichment and then were held at 4°C until they were processed. Experiment 2 used buffered peptone water as the enrichment media and used incubation conditions in accordance with the GeneDisc Plate STEC Top 7 (Pall Corporation) instructions.

Indicator counts. After stomaching, but prior to incubation, aliquots were removed for enumeration of indicator counts. Indicator counts were enumerated using PetriFilm (3M, Minneapolis, MN) according to the manufacturer's instructions. Indicator count data with values below the limit of detection (LOD) were recorded as LOD/2 for statistical analysis except for experiment 1 EBC data. Experiment 1 EBC data are based only on samples with quantifiable data because the number of zero counts was greater than 15% *(20)*.

Pathogen index targets. Pathogen index targets were detected following enrichment. Initial targets were chosen to represent Shiga toxin–producing *E. coli* (STEC) *(3, 13)*. Additional targets were added to include genes also commonly found in *Salmonella*. The goal was to have targets that varied in prevalence from low to high. Optimally, multiple assays would achieve prevalences between 20 and 80% within each experiment *(17)*. As suitable candidate genes were identified, they were added to the repertoire; thus, not all targets were used in all experiments. The targets were chosen so that they would not require regulatory action if present. Whereas index targets are indicative of pathogenic bacteria, they are not specific for pathogens. Therefore, none of the individual targets or any combination of multiple targets chosen for this study impacted disposition decisions of the product for pathogens. The targets consisted of hemolysin (*hlyA*) and intimin (*eae*), virulence factors associated with enterohemorrhagic *E. coli (13)*; O serogroups, five individual, non–Top 6 STEC O serogroup PCRs (O55, O113, O117, O126, and O146; each O serogroup includes STEC but is not specific for STEC) *(3)*; heme receptor (*chuA*) and adhesion siderophore (*ihaA*), virulence factors associated with STEC; and tetracycline resistance genes *(9, 15)* (*tetA* and *tetB*), commonly found in STEC and *Salmonella (22)*.

Statistical analyses. Colony counts were transformed to log CFU per sample before analyses. Repeated measures, *t* test, or one-way statistical analysis of variance for paired samples was performed using Prism 8 (GraphPad, San Diego, CA). Comparison of pathogen prevalence was performed using Fisher's Exact Test in Prism 8 (GraphPad Software Inc., San Diego, CA) with the probability level at $P < 0.05$.

RESULTS

In experiment 1, all four methods were compared for two different lean points. For the 50:50 lean point indicator

TABLE 1. *Sample method evaluation using indicator organism counts and pathogen index target prevalence*[a]

Lean point[b]	Assay[c]	CSD	MSD	N60 Plus	N60 Excision
50:50[d]	*n*	94	94	94	94
	Indicator counts				
	Log APC/sample	4.1 B	4.1 AB	4.1 B	4.3 A
	Log EBC/sample	3.5 AB	3.4 B	3.4 B	3.8 A
	Sum of APC zero	2 A	0 A	0 A	3 A
	Sum of EBC zero	14 A	18 A	14 A	31 B
	Index target prevalence (%)				
	Intimin	8.5 A	3.2 A	2.1 A	3.2 A
	Hemolysin	22.3 A	11.7 AB	8.5 B	16.0 AB
	O group	18.2 A	25.0 A	9.1 A	20.5 A
80:20[e]	*n*	91	91	91	91
	Indicator counts				
	Log APC/sample	3.6 C	4.0 B	4.2 A	4.2 A
	Log EBC/sample	3.4 B	3.4 B	3.5 AB	3.9 A
	Sum of APC zero	5 A	1 A	0 A	4 A
	Sum of EBC zero	29 BC	20 AB	11 A	37 C
	Index target prevalence (%)				
	Intimin	4.4 A	3.3 A	3.3 A	7.7 A
	Hemolysin	13.2 A	7.7 A	6.6 A	15.4 A
	O group	17.4 A	17.4 A	8.7 A	19.6 A

[a] CSD, continuous sampling device; MSD, manual sampling device; APC, aerobic plate count; EBC, *Enterobacteriaceae* counts. Means in a row with different letters differ ($P \leq 0.05$).
[b] Lean point is given as % lean:% fat.
[c] Sum of APC zero: number of samples with counts below the limit of detection. Index target prevalence, index targets: hemolysin (*hlyA*), intimin (*eae*), and O serogroups O55, O113, O117, O126, and O146. O group: O serogroup assay was performed only for 44 samples of 50:50 and 46 samples of 80:20.
[d] Standard 61-cm-wide conveyor line.
[e] Cartridge mounted on 30-cm-wide conveyor line.

counts (Table 1), the CSD, MSD, and N60 Plus had similar counts ($P > 0.05$). The N60 Excision method indicator counts were higher than those of the CSD, MSD, and N60 Plus methods by approximately 0.2 log APC per sample; however, the differences were only statistically significant ($P < 0.05$) between the N60 Excision method and both the CSD and N60 Plus methods, but not ($P > 0.05$) the MSD method. Because of the high number of samples that were below the EBC LOD for the individual methods in experiment 1, particularly the N60 Excision method, the EBC results should be interpreted with caution. Because interpretation of data below the LOD is subjective, there will always be a certain degree of bias. We chose to analyze only samples that were above the LOD for EBC data and to show the number of samples for each method that fell below the LOD. For EBC, the N60 Excision method was higher ($P < 0.05$) than MSD and N60 Plus methods, but not different ($P > 0.05$) from CSD. The 80:20 lean point came from a 30-cm-wide conveyor. The CSD had the lowest ($P < 0.05$) APC recovery, at 3.6 log APC per sample (Table 1). Whereas the MSD was 0.4 log APC per sample higher ($P < 0.05$) than the CSD, it was 0.2 log APC per sample lower ($P < 0.05$) than both the N60 Plus and N60 Excision methods, which were not different ($P < 0.05$) from each other for APC. For EBC, the N60 Excision method was 0.5 log EBC per sample higher ($P > 0.05$) than the CSD and MSD methods, but only 0.4 log EBC per sample higher than, and not significantly different ($P > 0.05$) from, the N60 Plus method. The CSD, MSD, and N60 Plus methods were not different ($P > 0.05$) from each other for EBC.

The pathogen index target prevalence results from experiment 1 (Table 1) differed significantly ($P < 0.05$) for only one of the six comparisons: the prevalence of the hemolysin gene in the 50:50 samples. For that data set, the CSD hemolysin prevalence was higher ($P < 0.05$) than the N60 Plus prevalence (22.3 versus 8.5%), whereas the MSD and N60 Excision methods were equivalent ($P > 0.05$) to each other and to the N60 Plus method for hemolysin detection. For the other five prevalence comparisons, the CSD had the highest prevalence values in 50:50 samples for intimin, and MSD had the highest recovery values for O groups in 50:50 samples; however, these did not differ significantly ($P > 0.05$) from the other methods. In 80:20 samples, N60 Excision had the highest prevalence values for all three index targets, but none of these were statistically different ($P > 0.05$) from the other three sampling methods.

In experiment 2, only CSD and N60 Excision from one combo bin fill station (30-cm-wide conveyor) were compared and only prevalences of index targets were evaluated. The CSD and N60 Excision methods each had numerically higher prevalence results from one of the three

TABLE 2. *Sample method evaluation using pathogen index target prevalence for a CSD mounted on a 30-cm-wide conveyor*[a]

Assay	CSD	N60 Excision
n	115	115
Index target prevalence (%)[b]		
O serogroups	27.8 A	36.5 A
Shiga toxin 1 and 2	7.8 A	6.1 A
Virulence factors	10.4 A	10.4 A

[a] CSD, continuous sampling device. Means in a row with different letters differ ($P \leq 0.05$).
[b] Index targets: GeneDisc Plate STEC Top 7 kit (Pall Corporation).

targets (Table 2), but there were no statistically significant differences ($P > 0.05$) between the two methods for any of the three targets.

Also in experiment 3, only CSD and N60 Excision were compared. This experiment utilized a swinging arm system to feed trim off the side of a conveyor line across the CSD in batches, as opposed to continuous flow of separate trim pieces along a conveyor line. The average APC recovered was 0.3 log APC per sample lower ($P < 0.05$) for the CSD than for the N60 Excision method (Table 3). However, the prevalence of pathogen index targets was numerically higher for the CSD than for the N60 Excision method for all three targets. However, only the difference in prevalence of the hemolysin gene (40.0 versus 17.5%) was statistically significant ($P < 0.05$) between the two sample collection methods.

Experiment 4 was the first time the CSD had been mounted at the end of a chute and the first experiment in which the MSD was used to sample combo bins with trim surface area (≈0.93 m^2 [ca. 1,439 in^2]) less than 1 m^2 (ca. 1,600 in^2). The CSD had lower ($P < 0.05$) recoveries for APC and EBC than the MSD and N60 Plus sample collection methods (Table 4). The recovery of APC was higher ($P < 0.05$) for the MSD compared with N60 Plus, but for EBC there was no statistical difference ($P > 0.05$) between the two methods. When evaluating the methods using pathogen index targets, there was only one target of the six, heme receptor, that had a statistically significant difference. For the heme receptor, the MSD had a higher prevalence than the N60 Plus method (52.5 versus 25.0%). The CSD was equivalent ($P > 0.05$) to both the MSD and N60 Plus prevalence for the heme receptor gene.

TABLE 3. *Sample method evaluation using indicator organism counts and pathogen index target prevalence for a CSD receiving beef trim from a swinging arm system*[a]

Assay	CSD	N60 Excision
n	40	40
Indicator counts		
Log APC/sample	5.6 B	5.9 A
Index target prevalence (%)[b]		
Intimin gene	30.0 A	17.5 A
Hemolysin gene	40.0 A	17.5 B
O serogroups	75.0 A	67.5 A

[a] APC, aerobic plate count; CSD, continuous sampling device. Means in a row with different letters differ ($P \leq 0.05$).
[b] Index targets: hemolysin (*hlyA*), intimin (*eae*), and O serogroups O55, O113, O117, O126, and O146.

TABLE 4. *Sample method evaluation using indicator counts and pathogen index targets on combo bins with slightly smaller surface area*[a]

Assay	CSD	MSD	N60 Plus
n	40	40	40
Indicator counts			
Log APC/sample	4.9 C	5.7 A	5.4 B
Log EBC/sample	4.0 B	4.7 A	4.5 A
Index target prevalence (%)[b]			
Intimin	2.5 A	15.0 A	5.0 A
Hemolysin	0.0 A	5.0 A	0.0 A
Heme receptor	45.0 AB	52.5 A	25.0 B
O serogroups	37.5 A	47.5 A	37.5 A
tetA	57.5 A	70.0 A	57.5 A
tetB	40.0 A	50.0 A	50.0 A

[a] APC, aerobic plate count; EBC, *Enterobacteriaceae* counts; CSD, continuous sampling device; MSD, manual sampling device; N60 Plus, N60 Plus sampler. Means in a row with different letters differ ($P \leq 0.05$).
[b] Index targets: hemolysin (*hlyA*), intimin (*eae*), O serogroups O55, O113, O117, O126, and O146, heme receptor (*chuA*), and tetracycline resistance genes (*tetA* and *tetB*).

In experiment 5, samples from 80 combo bins at a separate beef processing plant from experiment 4 were analyzed, and also CSD-chute mounting and combo bin surface areas of ≈0.93 m^2 (ca. 1,439 in^2) were evaluated. The recovery of APC using the N60 Plus method did not differ ($P > 0.05$) from either the CSD or MSD methods, whereas the CSD had a 0.1-log APC per sample lower ($P < 0.05$) average APC yield than the MSD method (Table 5). For EBC, the CSD and MSD were both 0.2 log EBC per sample higher ($P < 0.05$) than the N60 Plus method but were not different from each other ($P > 0.05$).

Seven pathogen index targets were used in experiment 5 to determine the prevalence of recovery for each sample collection method. Of the seven pathogen index targets, only two (O serogroups and *tetA*) had differences that were statistically significant. For the O serogroups index target, the prevalence of the target in the N60 Plus samples was lower ($P < 0.05$) than the prevalences for both the CSD and MSD methods (63.8% versus 82.5 and 86.3%). The CSD and MSD O serogroup prevalences were equivalent ($P > 0.05$). The N60 Plus method had a lower ($P < 0.05$) *tetA* prevalence compared with the CSD, but it was similar to the MSD ($P > 0.05$). The MSD *tetA* prevalence was also similar ($P > 0.05$) to the CSD *tetA* prevalence.

In experiment 6, CSD, MSD, and N60 Plus methods were compared for 80 combo bins using conveyor-mounted CSDs and the smaller combo bin surface area of ≈0.93 m^2 (ca. 1,439 in^2). The N60 Plus method had the highest ($P < 0.05$) APC and EBC compared with both the CSD and MSD methods (Table 6). The MSD had higher ($P < 0.05$) APC

TABLE 5. *Sample method evaluation using indicator counts and pathogen index targets on combo bins with slightly smaller surface area*[a]

Assay	CSD	MSD	N60 Plus
n	80	80	80
Indicator counts			
Log APC/sample	4.6 B	4.7 A	4.7 AB
Log EBC/sample	3.8 A	3.8 A	3.6 B
Index target prevalence (%)[b]			
Hemolysin gene	13.8 A	12.5 A	7.5 A
Intimin gene	30.0 A	30.0 A	22.5 A
Adhesion siderophore	28.8 A	22.5 A	26.3 A
Heme receptor	55.0 A	53.8 A	47.5 A
O serogroups	82.5 A	86.3 A	63.8 B
tetA	66.3 A	60.0 AB	47.5 B
tetB	78.8 A	70.0 A	70.0 A

[a] CSD, continuous sampling device; MSD, manual sampling device; N60 Plus, N60 Plus sampler; APC, aerobic plate count; EBC, *Enterobacteriaceae* counts. Means in a row with different letters differ ($P \leq 0.05$).

[b] Index targets: hemolysin (*hlyA*), intimin (*eae*), O serogroups O55, O113, O117, O126, and O146, heme receptor (*chuA*), adhesion siderophore (*ihaA*), and tetracycline resistance genes (*tetA* and *tetB*).

TABLE 6. *Sample method evaluation using indicator counts and pathogen index targets on combo bins with slightly small surface area*[a]

Assay	CSD	MSD	N60 Plus
n	80	80	80
Indicator counts			
Log APC/sample	4.2 C	4.3 B	4.7 A
Log EBC/sample	3.0 B	3.1 B	3.4 A
Index target prevalence (%)[b]			
Hemolysin gene	2.5 A	2.5 A	1.3 A
Intimin gene	5.0 A	5.0 A	5.0 A
Adhesion siderophore	12.5 A	6.3 A	11.3 A
O serogroups	17.5 A	11.3 A	12.5 A
Heme receptor	23.8 A	20.0 A	28.8 A
tetA	31.3 A	25.0 A	23.8 A
tetB	41.3 A	35.0 A	46.3 A

[a] CSD, continuous sampling device; MSD, manual sampling device; N60 Plus, N60 Plus sampler; APC, aerobic plate count; EBC, *Enterobacteriaceae* counts. Means in a column with different letters differ ($P \leq 0.05$).

[b] Index targets: hemolysin (*hlyA*), intimin (*eae*), O serogroups O55, O113, O117, O126, and O146, heme receptor (*chuA*), adhesion siderophore (*ihaA*), and tetracycline resistance genes (*tetA* and *tetB*).

than the CSD but had equivalent ($P > 0.05$) EBC. Seven pathogen index targets were used, but there were no significant differences ($P > 0.05$) among the three sample collection methods for any of the targets.

DISCUSSION

Presented herein are data sets amassed from 1,650 matched samples collected from 540 individual combo bins at six commercial beef processing plants. In this work, the goal was to collect data for determining efficacy of MicroTally-based sampling in scenarios commonly encountered in the commercial beef processing industry, but outside of the parameters evaluated during the initial proof-of-concept work *(24)*. The four main deviations from the initial publication were (i) using a 61-cm-wide CSD cartridge to collect samples from 30-cm-wide conveyor belts, (ii) mounting CSDs at the ends of chutes, (iii) CSD sampling of trim in small batches instead of continuous flow, and (iv) collecting MSD samples from combo bins with slightly less trim surface area than 1 m^2.

In addition, performance of this work provided the opportunity for all four sample collection methods (CSD, MSD, N60 Excision, and N60 Plus) to be compared as a group in one experiment. In the proof-of-concept publication *(24),* the CSD had been compared with the N60 Excision and N60 Plus methods, and the MSD had been evaluated similarly. However, all four methods had not been performed in the same comparison group until now. Experiment 1 provides a representative snapshot of the entirety of the data collected in this work. The N60-based methods are as good as, or better at, total bacterial collection, as demonstrated by having more instances of significantly higher counts of indicator organisms. However, the MicroTally-based methods are as good as, or better at, pathogen detection, as evidenced by more instances of significantly higher pathogen index target prevalences. Whereas these trends were observed when all four methods were compared in experiment 1, they continued throughout the study in the other experiments.

On only two occasions, APC in experiment 4 and EBC in experiment 5, were there instances of MicroTally-based sampling with significantly higher indicator organism counts than at least one of the N60 methods (N60 Plus in both cases). In contrast, the N60-based methods had significantly higher indicator counts than at least one of the MicroTally-based methods on 8 of the 11 occurrences of indicator count testing performed for this work. Interestingly, the trends for the qualitative tests were opposite of those for the quantitative tests. There were no instances of the N60-based methods having significantly better recovery of any index target than the MicroTally-based methods (0 of 32 assays). The MicroTally-based sample collection methods resulted in index target prevalences that were significantly higher than those for the N60-based methods on six occasions (6 of 32 assays).

This phenomenon, that excision-based methods typically recover more indicator organisms than swab-based methods but have similar prevalence results to swab-based methods, has been reported previously *(6, 7, 10, 14)*. Ghafir and Daube *(6)* reported significantly lower recovery of APC and *E. coli* counts by swabbing compared with excision sampling, but they found no significant difference between the swabbing and excision sampling methods for the prevalence of *Salmonella* or *Campylobacter*. Similarly, Martinez et al. *(12)* reported total viable bacteria counts and EBC that were significantly higher for excision sampling

compared with sponge swabbing; however, the percentage of carcasses from which *Enterobacteriaceae* were detected when sampled by swabbing exceeded that obtained when sampling was done by excision.

This improvement in qualitative results versus quantitative results for swab sampling is believed to be due in part to the increased surface sampling area available for swabbing compared with excision sampling. Gill and Jones *(7)* posited that when *E. coli* pathogens on carcasses are sparse, the incidence of their recovery will approximately double for each 10-fold increase in the surface area sampled. Of the four methods evaluated in the current work, the CSD method samples a vast majority of the meat pieces in the combo bin; and the MSD samples ~11,000 cm^2, the N60 Excision ~1,100 cm^2, and the N60 Plus slightly less surface area than the N60 Excision method *(24)*.

If surface area sampled was the key factor, it was necessary to determine whether using less surface area of the sampler would be acceptable. To address conveyors less than 61 cm wide, experiment 1 (the 80:20 line) and experiment 2 each had a CSD mounted on a 30-cm-wide conveyor. For both lines, there was no significant difference in prevalence of index target organisms between the CSD and any other sample collection method tested. The 80:20 line of experiment 1 was the only one of the 30-cm lines that had indicator organism counts analyzed. The CSD had lower APC than the MSD and had lower recoveries for both APC and EBC than the N60-based methods, but the differences were 0.5 log CFU per sample or less. Based on these observations, we concluded that mounting a 61-cm-wide CSD cartridge to a 30-cm-wide conveyor provides sampling that is equivalent to MSD and N60-based methods for determination of pathogen prevalence. This result was not unexpected because, even on 61-cm-wide conveyors, the beef trimmings do not always occupy the full width of the conveyor and frequently are contacting 50 to 80% of the sampling cartridge width, depending on how trim is positioned onto the final conveyor.

Similarly, the CSD was shown to be effective for sampling when used in conjunction with a "swinging arm diverter" and when receiving product in small batch mode as opposed to continuous flow, for which it was initially designed. The swinging arm diverter was designed to deliver pulsed batches of approximately 22.7 kg (ca. 50 lb) at a time to the CSD and then to the combo bin. For experiment 3, each CSD collected samples from approximately 40 of these small batches throughout the fill of each 900-kg combo bin. Although a deviation from the original continuous sampling intent, the CSD was still sampling throughout the combo bin filling period and was sampling much more trim surface area than previous methods, although it was likely slightly less area than for a continuous fill, end of conveyor line CSD mounting.

Mounting a CSD to a chute instead of a conveyor is a dramatic physical change from the standard conveyor mounting of the CSD, but it does change the manner and speed of the trim pieces as they encounter the CSD. As trim pieces fall off of a conveyor vertically, they impact the CSD, mounted at a 45° angle from horizontal; thus, the trim impacts the MicroTally swab as the trajectory is diverted. When the CSD is attached to a chute, which is frequently angled 45° from horizontal as well, the physical impact of the trim pieces as they cross the MicroTally swab is reduced when the CSD is mounted in the same plane as the chute. The directions for chute mounting a CSD specify that the CSD should be mounted at an angle that is at least 10° less from horizontal than the angle of the chute to increase trim contact with the swab (Fig. S1). Nonetheless, experiments 4 and 5 show that chute mounting CSDs provided equivalent sample collection as the MSD and N60 Plus methods.

As stated previously, there are fewer physical variations associated with the MSD method. The MSD method was developed to obtain a representative sample by contacting the entire surface of the trim in a filled combo bin with the MicroTally swab. However, even though most combo bins are filled to the same weight (900 kg), not all combo bins are the same dimensions, which results in different effective surface areas for sampling. Experiments 4, 5, and 6 collected MSD samples from combo bins with less surface area than the 1-m^2 surface area from combo bins in the initial validation *(24)*. With the exception of the indicator counts in experiment 4, the MSD method used to collect samples from the combo bins with slightly less surface area was shown to be equivalent to or better than the N60 Plus method for both quantitative and qualitative tests. It was not surprising that limiting the surface area of the sample by about 10% did not result in dramatically lower bacterial recoveries. As stated previously, Gill and Jones *(7)* reported that when *E. coli* pathogens on carcasses are sparse, the incidence of their recovery will approximately double for each 10-fold increase in the surface area sampled. The corollary would state that, to reduce the incidence of recovery by half, one would need to reduce the surface area sampled by 90%. In addition, the surface area from these combo bins is still in vast excess of that for both N60 Excision and N60 Plus, even though slightly less than for combo bins from the original proof-of-concept data *(24)*. This work demonstrated that MSD sampling of rounded combo bins with trim surface area less than 1 m^2 (≈0.93 m^2) was shown to be equivalent to the N60 Plus sample collection method.

For this study and the proof-of-concept study *(24),* all normally applied antimicrobial interventions were applied as per each plant's normal operating procedures. The PAA application varied among plants in this study from ≈0.5 to 6 m from the end of the conveyor CSD location. No intervention applied at the end of the trim conveyor was observed to negatively impact pathogen index target detection of the CSD even if the samples were shipped overnight (five of six experiments shipped samples) before analysis.

This research utilized pathogen index targets as additional measures for validating methods that were initially designed to measure pathogen prevalence. Detection of pathogen index targets provides a few essential benefits compared with direct pathogen testing and enumeration of generic indicator organisms when evaluating qualitative pathogen detection methods. Direct detection of pathogens is the best test of efficacy for a sample method

designed to be used for determination of pathogen prevalence; however, pathogen prevalence in commercial beef trimmings is typically less than 0.1% *(5, 21)*. Hence, pathogen detection typically does not provide sufficient data points for analysis without collecting thousands of samples, which is cost and time prohibitive. Indicator organism counts have been the traditional measure of bacterial contamination in the beef processing industry. Enumeration of indicator organisms provides a rapid, low-cost, and user-friendly option for estimating how changes in a process affect bacterial load. However, such assays may not provide the best evaluation of a qualitative test that is designed to detect the presence of a specific strain of bacteria rather than collect all bacteria it encounters. Had only indicator counts been used, it would not have been possible to fully observe the potential of the MicroTally-based methods, specifically the CSD, for collecting samples for pathogen detection. With the increase in molecular testing, pathogen index target testing is roughly similar to the current single target or multiplexed target assays commonly used for the detection of *Salmonella* and STEC by the beef processing industry *(1, 23)*.

On 9 of 26 occasions, the CSD differed from the MSD, N60 Excision, or N60 Plus methods in recovery of indicator counts by 0.5 log CFU per sample or more; yet, the CSD was never significantly lower than any method for prevalence of any of the index targets. There were six occasions on which one of the methods was significantly higher than another method for index target prevalence. For all six, a MicroTally-based method was the higher prevalence method (CSD = 4, MSD = 2).

With a total of 80 replicates per method for some experiments, there were instances in which small differences in indicator counts (e.g., 0.1 log CFU per sample) were statistically significant. It is difficult to imagine that such small differences would produce any biologically relevant results in pathogen detection *(11)*; thus, it has been difficult historically to determine the importance of small differences in indicator counts when used to assess a method to detect pathogens. The addition of the pathogen index target prevalence data has allowed determination of the relationship between the indicator count differences and prevalence data differences among methods. It was found that whereas the MicroTally-based sampling methods occasionally may result in lower indicator organism counts than the N60 methods, the MicroTally-based sample methods consistently obtained results with higher prevalences of pathogen index targets, which implies that the 0.1 to 0.5 log CFU per sample differences in indicator organism counts were not biologically important. Thus, the data presented herein support equivalency criteria of within 0.5 log CFU per sample for indicator organism counts to be the threshold for biological importance when used for assessing pathogen detection. Further support for this metric was seen from comparing indicator organism count differences relative to pathogen index target differences between N60 Excision and N60 Plus in Table 1.

In conclusion, the MicroTally-based sampling methods have been proven to be robust and have been shown to perform adequately in a variety of configurations. Modifications that cause small changes in the surface area sampled by the MicroTally-based methods would not appear to have any large negative impacts on pathogen detection. This is likely due the fact that these changes are relatively small in comparison to the large amount of surface area typically sampled by these methods. In addition, pathogen index targets were demonstrated to be useful tools for evaluating qualitative methods in lieu of direct pathogen testing. Finally, differences of up to 0.5 log CFU per sample of indicator organism counts may not have any biological significance when evaluating qualitative methods for pathogen detection.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/JFP-20-345.s1

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