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Research Paper

Determination of Verification Parameters for Using the Manual Sampling Device for Fresh Raw Beef Trim

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ABSTRACT

Multifaceted food safety systems are used by the beef processing industry to minimize risk of bacterial contamination of the finished product. These systems are comprised of several parts including the conditional release of product requiring a sample to produce a negative result on a pathogen test prior to sending the product into the food supply. The methods of sample collection require verification activities that ensure the sampling protocols are performed adequately. The research described herein was done to determine the parameters for use in verifying adequate beef trim sampling for the Manual Sampling Device (MSD) method. In addition, the efficacy of repeated sampling via multiple applications of the MSD procedure on a fresh raw beef trim combo was investigated. The results show that MSD sample collection that covers less than the entire combo surface, but at least one-half of the combo surface and is collected for a minimum of 90 s, is adequate for the recovery of organisms and prevalence targets from fresh raw beef trim. In addition, the evidence that MSD sample collection that occurs for less than the recommended time, but not less than 30 s per side of the swab, is adequate for the recovery of organisms and prevalence targets from raw beef trim. Finally, results show that in a scenario where an in-plant MSD sample and a regulatory MSD sample are required from the same combo, two MSD samples can be collected from the same combo bin with similar test results for both samples. While the recommended MSD protocol specifications will not be changed, the data presented herein provide support for some flexibility in accepting test results when verification activities indicate that sampling did not occur as specified in the recommended procedure.

Beef processing companies employ complex food safety systems to minimize the risk of bacterial contamination of the finished product. These systems are comprised of several parts including employee training on best practices for proper carcass dressing techniques, application of multiple antimicrobial interventions to inactivate and remove bacterial contamination, and conditional release of product requiring a negative result on a pathogen test prior to sending the product into the food supply. The collection of the beef trim sample for pathogen testing had previously been done by either excision of 60 pieces of carcass surface material (N60 method) or shaving material from the surfaces of trimmings using a drill-like tool (N60+ method). Recently, nondestructive sample collection methods using the MicroTally® swab (MT-Swab) were developed (Arthur and Wheeler, 2021; Wheeler and Arthur, 2018). The Continuous Sampling Device (CSD) and Manual Sampling Device (MSD) methods employ passive and active abrasion, respectively, of fresh raw beef trimmings using the MT-Swab to collect the sample.

One element common to all these sample collection methods is the necessity of verification activities that ensure the sampling protocols are performed adequately. For the N60 and N60+ methods, that verification typically was done by weighing the excised meat sample. However, the simple sample weight measurement does not provide a true verification of sample collection adequacy. For the N60 excision method, proper sample collection entails the excision of sixty strips of material (U.S. Department of Agriculture, F. S. I. S. Date, 2014). Each strip is to come from a separate piece of beef trim and the excised surface should be that which originated from the carcass surface. The carcass surface is targeted as it is the main site of bacterial contamination deposition during hide removal (Arthur et al., 2007, 2004; Nou et al., 2003). For the N60+ method, the manufacturer's instructions specify a certain number of insertions into the trim combo bin with

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the N60+ sampling device and specific locations for each insertion. Verification of these less definitive parameters has been done by performance audits, either in-person or via video monitoring. However, if performance is observed during an audit to not follow the sampling instruction to completion, there is no data to determine how much variation in the sample collection process would be allowed before the sample collection procedure would be deemed inadequate. For the MSD method, sample verification can be performed in a similar fashion. Sample weights with in-person or video monitoring are currently in use at commercial production facilities, but there was again no data to determine how much variation in the sample collection procedure could be tolerated before the sample would be deemed inadequate. The research described herein was done to determine the parameters for use in verifying adequate sampling for the MSD method. In addition, the efficacy of repeated sampling of a fresh raw beef trim combo with multiple MT-Swabs was determined.

Materials and methods

Sampling procedures

Samples were collected in a commercial beef processing plant during normal plant operations. Processing plant antimicrobial spray applications were not altered for any experiment. Peroxyacetic acid was utilized by the processing plant as an antimicrobial spray on the trim conveyors. No neutralizers were added to the samples in this study other than the enrichment media added at the laboratory. All samples were shipped overnight to the laboratory for processing.

Trial 1: Verification of sample area

This trial was designed to compare the MSD method, using less than the entire surface area of a beef combo bin, to the N60 method to determine if there is some flexibility when plant audit activities indicate the top of the combo was not completely sampled using the MSD method. Each sampling method was performed on single combo lots. Each combo was sampled by the MSD-half surface, MSD-quarter surface, and N60 methods.

MSD-half surface: The MSD samples were collected using the MT-Swab (FREMONTA Corp.) following the MSD protocol presented below with the following exception. Instead of the sampler working around the combo and sampling the entire top surface of beef trim, only half of the top surface was sampled for a minimum of 90 s, 45 s per side of the MT-Swab. The samples were collected by plant employees trained by USMARC scientists.

MSD-quarter surface: The MSD samples were collected using the MT-Swab following the MSD protocol presented below with the following exception. Instead of the sampler working around the combo and sampling the entire top surface of beef trim, only one quarter of the top surface was sampled for a minimum of 90 s, 45 s per side of the MT-Swab. The samples were collected by plant employees trained by USMARC scientists.

N60: The N60 excision samples were collected by plant personnel according to the plant's standard operating procedure.

The Trial 1 comparison used eighty combos and both quantitative and qualitative analyses. For each combo, the N60 method was collected first. The MSD samples were typically conducted simultaneously as they did not require any overlap in surface area to be sampled. Samples were shipped overnight at 4°C to the USMARC laboratory for processing. Upon arrival at the laboratory, samples were enriched by adding 42°C mEHEC broth (BioControl) and incubating for 12 h at 42°C then held at 4°C until further processing. Analyses performed were (Arthur et al., 2007) enumeration of indicator bacteria counts (aerobic plate counts: APC and Enterobacteriaceae counts: EB) prior to sample incubation and (Arthur et al., 2004) prevalence for PCR pathogen index targets representative of STEC-like organisms and *Salmonella* (intimin, heme receptor, O-groups, H7 flagella, *sdi*A, and tetracycline resistance genes) following incubation (Arthur and Wheeler, 2021). Intimin is a virulence factor associated with STEC. The heme receptor (*chu*A) is an outer membrane protein found in STEC. The O-group PCR data were obtained from three individual, non–STEC-specific O serogroup PCRs. The H7 flagella are found in many *E. coli* including pathogenic and nonpathogenic strains. The tetracycline resistance genes (*tet*A and *tet*B) are commonly found in *E. coli* and *Salmonella*. The *sdi*A gene is involved in quorum sensing for *E. coli* and *Salmonella*.

Trial 2: Verification of repeated sampling

The trial was designed to determine the efficacy of a repeated MSD sampling of the same beef combo. This could occur, for example, when plant employees have sampled a combo using the MSD method and FSIS would need to collect an MSD sample as well, or vice versa. The protocol for sampling required two MSD samples to be collected on single combo lots following the MSD protocol below. The samples were collected by plant employees trained by USMARC scientists. Immediately after collecting an MSD sample of the entire combo surface, a second MSD sample of the entire combo surface was collected.

The Trial 2 comparison used sixty combos for quantitative analyses and one hundred twenty combos for qualitative analyses. Samples were shipped at 4°C to the USMARC laboratory for processing. Upon arrival at the laboratory, samples were enriched by adding 42°C mEHEC broth (BioControl) and incubating for 12 h at 42°C then held at 4°C until further processing. Analyses performed were (Arthur et al., 2007) enumeration of indicator bacteria counts (aerobic plate counts: APC and Enterobacteriaceae counts: EB) prior to sample incubation and (Arthur et al., 2004) prevalence for PCR pathogen index targets representative of STEC-like organisms and *Salmonella* (intimin, heme receptor, O-groups, H7 flagella, *sdi*A, and tetracycline resistance genes) following incubation (Arthur and Wheeler, 2021).

Trial 3: Verification of sampling time

The trial was designed to compare the variations in the total sampling time using the Manual Sampling Device (MSD) method to determine if there is some flexibility when processing plant audit activities indicate that sampling for the recommended 90 s minimum did not occur. In the recommended MSD Sampling Protocol, sampling is to occur for a minimum of 90 s, which is split into two 45 s periods for each side of the MT-Swab. However, a data gap existed regarding the potential detrimental impact to MSD sampling if not done for the prescribed length of time. For this study, two sampling deviations thought to be plausible in day-to-day sampling at commercial beef plants were compared to the recommended procedure. From each combo, the following three samples were collected: 1) standard 90-s MSD (45 s per side), 2) a 60-s MSD (30 s per side), and 3) a 45-s MSD (45 s one side only). The order of sample collection was rotated so each sample method has an equal number of rotations at first, second, and third sample order.

Standard 90-s MSD sample (45 s per side): The MSD samples were collected using the MT-Swab following the MSD protocol presented below. The samples were collected by plant employees trained by USMARC scientists.

60-s MSD sample (30 s per side): The MSD samples were collected using the MT-Swab following the MSD protocol presented below with the following exception. Instead of sampling the entire top surface of beef trim for 45 s per side of the MT-Swab, the sample duration was shortened to 30 s per side. The samples were collected by plant employees trained by USMARC scientists.

One side only of MSD for 45 s: The MSD samples were collected using the MT-Swab following the MSD protocol presented below with

the following exception. Instead of sampling the entire top surface of beef trim for 45 s per side of the MT-Swab, the entire surface of each combo was sampled for only 45 s using one side of the MT-Swab. The samples were collected by plant employees trained by USMARC scientists.

The Trial 3 comparison used eighty-four combos and both quantitative and qualitative analyses. All eighty-four combos were tested using each of the three sample collection methods. For each combo, the order of sample collection was rotated so each sample method had an equal number of rotations at first, second, and third sample order. Samples were shipped at 4°C to the USMARC laboratory for processing. Upon arrival at the laboratory, samples were enriched by adding 42°C mEHEC broth (BioControl) and incubating for 12 h at 42°C then held at 4°C until further processing. Analyses performed were (Arthur et al., 2007) enumeration of indicator bacteria counts (aerobic plate counts: APC and Enterobacteriaceae counts: EB) prior to sample incubation and (Arthur et al., 2004) prevalence for PCR pathogen index targets representative of STEC-like organisms and *Salmonella* (intimin, heme receptor, O-groups, H7 flagella, *sdi*A, and tetracycline resistance genes) following incubation (Arthur and Wheeler, 2021).

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 61 by 25.4 cm (ca. 24 in × 10 in) MT-Swab (FREMONTA Corp.) 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 (Wheeler and Arthur, 2018). 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 for 45 s; then, the swab was flipped over and the other side of the swab was used to scrub the other half of meat exposed on top of the combo bin for an additional 45 s. 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 were aseptically excised from individual combo bins (U.S. Department of Agriculture, F. S. I. S. Date, 2014, 2016). Each sample slice was 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.

Sample analysis

Samples were shipped to the laboratory overnight and held refrigerated (2–8°C) until tested. Samples were analyzed for indicator organism counts and pathogen index target prevalence as described in the individual experiment methods. N60 Excision samples were processed according to standard laboratory procedures used by the collaborating companies. MSD samples were enriched with 200 mL of growth media appropriate for the detection platform implemented by the individual processing company and analyzed using the same procedures as for the other sampling methods. Samples were stomached for 30 s to homogenize. Samples were incubated for 12 h at 42°C in prewarmed mEHEC media (BioControl) for enrichment and then held at 4°C until processing.

Indicator counts

After stomaching, but prior to incubation, aliquots were removed for enumeration of indicator counts. Indicator counts were enumerated using PetriFilm (3M) according to the manufacturer's instructions.

Pathogen index targets

Pathogen index targets were detected following enrichment. Initial targets were chosen to represent Shiga Toxin-Producing *E. coli* (STEC) (Bosilevac and Koohmaraie, 2011; Paton and Paton, 1998). 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 (U.S. Department of Agriculture, F. S. I. S. Date, 2010). 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. While 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 (*hly*A) and intimin (*eae*) - virulence factors associated with EHEC (Paton and Paton, 1998); O serogroups- five individual, non-Top 6 STEC O serogroup PCRs: O55, O113, O117, O126, and O146 (each O serogroup includes STEC, but are not specific for STEC) (Bosilevac and Koohmaraie, 2011); heme receptor (*chu*A) and adhesion siderophore (*iha*A) - virulence factors associated with STEC; and tetracycline resistance genes (Hoffmann et al., 2001; Tarr et al., 2000); (*tet*A and *tet*B) - commonly found in STEC and *Salmonella* (Vikram et al., 2017).

Statistical analyses

Colony counts were transformed to $\log_{10}$ CFU/sample before analyses. Repeated measures, t test, or one-way statistical analysis of variance (ANOVA) for paired samples was performed using Prism 8 (GraphPad). Prevalence data were tallied as positive or negative for the specific PCR pathogen index targets. Comparison of pathogen index target prevalence was performed using Fishers Exact Test in Prism 8 (GraphPad) with the probability level at $P < 0.05$.

Results

Trial 1: Minimum area for MSD sampling

Data comparisons for the MSD-half surface, MSD-quarter surface, and N60 methods from 80 combos can be seen in Table 1. Both APC and EB counts were significantly higher ($P \leq 0.05$) for the N60 as compared to either MSD method (Table 1). The MSD-half was within 0.5 log CFU/sample for both APC and EB counts when compared to the N60 method, while the MSD-quarter was within 0.5 log CFU/sample for EB counts, but not APC. The MSD-half and MSD-quarter methods had equivalent ($P > 0.05$) recoveries for APC and EB counts. Both MSD methods had higher ($P \leq 0.05$) prevalence for the *sdi*A pathogen index target than the N60 method, while the three methods had equivalent ($P > 0.05$) prevalence for the H7, O-group, intimin, heme, and Tet target assays (Table 1). The prevalence results for the MSD-half and MSD-quarter were not different from each other ($P > 0.05$) for any index targets.

Table 1
Indicator Counts and Prevalence of Pathogen Index Targets for Trial 1[1,2]

Methods	Count	log APC/sample	log EB/sample	H7	O-group	Intimin	Heme	Tet	*sdi*A
N60	80	5.5 a	3.6 a	37.5% a	23.8% a	6.3% a	18.8% a	91.3% a	7.5% b
MSD-half	80	5.0 b	3.3 b	38.8% a	22.5% a	2.5% a	26.3% a	88.8% a	21.3% a
MSD-quarter	80	4.9 b	3.2 b	35.0% a	16.3% a	5.0% a	26.3% a	87.5% a	25.0% a

[1]Means in a column with different letters differed ($P \leq 0.05$).
[2]Abbreviations: APC- aerobic plate count, EB-Enterobacteriaceae, MSD – manual sampling device.

Trial 2: Repeated MSD sampling

Data comparisons for the MSD-1st and 2nd samples can be seen in Tables 2 and 3. APC recovery was 0.1 log APC/sample higher ($P \leq 0.05$) for the first sample than the second sample (Table 2). The first and second samples were equivalent ($P > 0.05$) for recovery of EB counts. The first and second MSD samples had equivalent ($P > 0.05$) performance in recovering all the pathogen index PCR targets (Table 3).

Trial 3: Minimum time of MSD sampling

Data comparisons for the MSD time variation method Standard 90 s, 60 s (30/30), and 45 s one side from the 84 combos can be seen in Table 4. Both APC and EB counts were significantly higher ($P \leq 0.05$) for the Method A as compared to either Method B or C (Table 4). The results for MSD methods B and C differed ($P \leq 0.05$) for APC, but were equivalent for EB counts ($P > 0.05$). All methods were within the 0.5 log CFU/sample both APC and EB counts. All MSD methods had equivalent ($P > 0.05$) prevalence results for the O-group, intimin, heme, and Tet PCR assays (Table 4). MSD method A resulted in higher ($P \leq 0.05$) prevalence than method C for the H7 assay, but was equivalent ($P > 0.05$) to method B. Methods B and C were equivalent ($P > 0.05$) in their recoveries of the H7 target.

Discussion

Effective food safety programs are of utmost importance in meat and poultry production. Processing plants go to great lengths to train their employees, utilize multiple antimicrobial interventions, and test the finished product. Recent estimates for annual food safety expenditures at large-scale beef processing plants exceed five million dollars per plant with additional capital costs for food safety equipment costing up to two million dollars per installation (Viator et al., 2017). In order to maximize the return on investment, the food safety systems employed at processing plants are constantly monitored to ensure operational parameters are being met. These parameters include carcass dressing procedure specifications (how often to sanitize knives, line speed, proper hide removal techniques, etc.), threshold temperatures and pressures for antimicrobial sprays, minimum concentrations and dwell times for organic acids, and criteria for proper sample collection for use in microbial testing. If any of these parameters are found to be outside of specification during routine monitoring or audit, the plant personnel will take action, which may include condemning product produced since the last time the particular parameter was recorded as meeting specifications. Therefore, it is necessary to know how much tolerance around a specification is allowed in a treatment or procedure before it is no longer adequate for the intended purpose.

Table 2
Indicator counts for Trial 2[1,2]

MSD	Count	log APC/sample	log EB/sample
1st	60	5.2 a	3.8 a
2nd	60	5.1 b	3.8 a

[1]Means in a column with different letters differed ($P \leq 0.05$).
[2]Abbreviations: APC- aerobic plate count, EB-Enterobacteriaceae, MSD – manual sampling device.

In the case of MSD sample collection with an MT-Swab, the specifications for proper performance of the method have been established. The MSD sample protocols specify that the MSD sample is to cover the entire top surface of a raw beef trim 907-kg (2,000-lb) combo bin and be collected for a minimum of 90 s using each side of the MT-Swab for 45 s (Arthur and Wheeler, 2021; Wheeler and Arthur, 2018). However, it was not known how much variation could occur when implementing the method before the procedure was no longer adequate for informing product disposition decisions. The studies described herein were designed to provide data for determining at what point deviations from sampling specifications should be considered unacceptable.

The Trial 1 evaluation provided evidence that MSD sample collection that covers less than the entire combo surface, but at least one-half of the combo surface and is collected for a minimum of 90 s, is equivalent to the N60 method for recovery of organisms and prevalence targets from raw beef trim. The Trial 3 evaluation provided evidence that MSD sample collection that occurs for less than the recommended time, but not less than 30 s per side of the MT-Swab, is equivalent to the recommended MSD method for recovery of organisms and prevalence targets from raw beef trim. When sample collection occurred for 45 s using only one side of the MT-Swab, the deviation was determined to be detrimental to the sampling procedure and thus unacceptable. Therefore, sampling must occur using both sides of the MT-Swab for a minimum of 30 s per side.

Despite these findings, it is recommended to sample the entire top surface of the combo for a minimum of 90 s, which is split into two 45 s periods for each side of the MT-Swab, as recommended (Wheeler and Arthur, 2018). While the recommended MSD protocol will not be changed, this data set provides support for some flexibility in accepting test results when verification activities indicate that sampling did not occur as specified in the recommended procedure.

On multiple occasions in this report, the indicator organism counts, APC and or EB, were significantly different for one method vs. another despite the numerical difference being quite small. In many instances, the significant differences in indicator organism counts did not correspond to significant differences in pathogen index target prevalence. In fact, in the case of Trial 1 where the N60 was compared to the MSD-half and MSD-quarter, the indicator counts for the N60 method were significantly higher than the MSD methods, while the prevalence of the *sdi*A target was significantly lower for the N60 method as compared to the MSD-half and MSD-quarter samples. Similar observations were made previously (Arthur and Wheeler, 2021). As the MT-Swab-based sampling methods were designed for prevalence testing with the purpose of detecting the presence of bacterial pathogens, it was expected there would be times where the recovery of indicator organism counts for the MT-Swab-based methods would be less than that of the N60 methods. Based on previous data (Arthur and Wheeler, 2021), that was shown to be the case, hence, more weight was given to the prevalence data. A threshold for indicator count data to be biologically significant was determined to be 0.5 log CFU/sample, as that was the

Table 3
Prevalence of Pathogen Index Targets for Trial 2[1]

MSD	Count	H7	O-group	Intimin	Heme	Tet	*sdi*A
1st	120	42.5% a	92.5% a	6.7% a	21.7% a	70.0% a	1.7% a
2nd	120	32.5% a	88.3% a	5.0% a	16.7% a	72.5% a	2.5% a

[1]Means within the same PCR assay with different letters differed ($P \leq 0.05$).
[2]Abbreviations: MSD – manual sampling device.

Table 4
Indicator Counts and Prevalence of Pathogen Index Targets for Trial 3[1,2]

Methods	Count	log APC/sample	log EB/sample	H7	O-group	Intimin	Heme	Tet
Standard 90 s	84	4.6 a	3.7 a	28.6% a	15.5% a	3.6% a	4.8% a	75.0% a
60 s, 30/30	84	4.4 b	3.5 b	22.6% a,b	13.1% a	0.0% a	1.2% a	76.2% a
45 s one side	84	4.2 c	3.4 b	11.9% b	7.1% a	2.4% a	7.1% a	69.0% a

[1]Means in a column with different letters differed ($P \leq 0.05$).
[2]Abbreviations: APC- aerobic plate count, EB-Enterobacteriaceae, MSD – manual sampling device.

usual breakpoint where pathogen index target prevalence also began to decline.

Another purpose of this study was to determine if two MSD samples, collected from the same combo, would provide equivalent bacterial recovery for pathogen detection. As there is no practical way to avoid sampling the same material when multiple MSD samples are collected from the same combo of beef trim, data were needed to ensure a second MSD sample would provide an equivalent test result. From the data presented herein, the results indicate that the second MSD sample provides the same level of detection as the first MSD sample. The Trial 2 evaluation provided evidence that two MSD sample collections from the same beef trim combo bin do not result in lower sensitivity of the second MSD sample for detecting pathogens when compared to the first MSD sample. These results show that in a scenario where an in-plant MSD sample and a regulatory MSD sample are required from the same combo, they can be collected with equivalent efficacy.

This study provides beef processors with guidance on tolerance parameters when deviations in MSD sample collection specifications occur for the decision-making process regarding product disposition.

Declaration of Competing Interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Terrance M. Arthur and Tommy L. Wheeler have a patent with royalties paid to USDA.

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