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Research Note

# Validation of a New Method of Sampling Beef Manufacturing Trimmings for Pathogen Testing Using a Manual Sampling Mitt Approach

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ABSTRACT

The USDA Food Safety Inspection Service has declared *Escherichia coli* O157:H7, and six additional Shiga toxin-producing *E. coli* (STEC) are adulterants for nonintact raw beef products. The U. S. beef processing industry has implemented several antimicrobial intervention technologies throughout the carcass dressing process to remove or destroy foodborne pathogens present on beef carcasses. Despite these efforts, STEC have been shown to cause finished product contamination, albeit at prevalences typically $<0.5\%$. Recent work described the development and validation of improved methods for collecting samples from raw beef trimmings. One of the methods, the Manual Sampling Device (MSD) method, uses the manual implementation of the MicroTally® Swab (MT-Swab) to vigorously scrub the surface of raw beef manufacturing trimmings for pathogen detection. The work described herein reports the data from an evaluation of a novel MSD method using the MicroTally® Mitt (MT-Mitt). The MT-Mitt provides a more user-friendly option for sample collection than the MT-Swab. A series of trials were conducted with a total of 360 matched samples comparing manual sampling of raw beef manufacturing trimmings using the MT-Swab, N60-excision, or N60-plus methods to a novel method using the MT-Mitt. The results of these trials collectively demonstrate that manual sampling of raw beef manufacturing trimmings using the MT-Mitt provides organism recovery that is not significantly different from that of the MT-Swab, N60-excision, and N60-plus methods. Thus, the MT-Mitt method provides an alternative sampling method with organism recovery that is not significantly different from previous methods for sampling beef manufacturing trimmings for pathogen detection and some implementation advantages pertaining to labor and ease of use.

In 1994, public notice was given by USDA Food Safety Inspection Service (FSIS) that ground beef harboring *Escherichia coli* O157:H7 would be considered adulterated and in 1999 broadened the category of potentially adulterated beef material to include nonintact beef cuts and intact beef cuts destined for nonintact use (U.S. Department of Agriculture, 1999). In 2011, FSIS announced that six additional Shiga toxin-producing *E. coli* would be included as adulterants in nonintact raw beef (U.S. Department of Agriculture, 2011). To mitigate the risk of STEC contamination on the finished product, the U. S. beef processing industry has implemented several antimicrobial intervention technologies throughout the carcass dressing process (Arthur et al., 2008; Arthur et al., 2007; Bosilevac et al., 2006; Bosilevac et al., 2004; Kalchayanand et al., 2009; Kalchayanand et al., 2008; Wang et al., 2014; Wheeler et al., 2014). In spite of these efforts, STEC have been shown to cause finished product contamination with prevalences reported recently for *E. coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* to be 0.07% and 0.36%, respectively (Vial et al., 2019). With the potential for the finished product to be contaminated with an adulterant, U.S. beef processors have implemented test-and-hold systems, whereby samples of every lot of finished product destined for nonintact use are tested and the product lot is not released into the food chain if adulterating STEC are detected in the sample. Hence, finished-product testing has become a centerpiece of food safety programs implemented by commercial beef processing companies and verification programs implemented by regulatory agencies (U.S. Department of Agriculture, 2014, 2023).

Recently, improved methods for sample collection from raw beef manufacturing trimmings, the Continuous and Manual Sampling Devices (CSD and MSD, respectively) were developed (Wheeler & Arthur, 2018) by the USDA Agricultural Research Service (ARS) in a cooperative research agreement with FREMONTA Corp. The CSD and MSD methods were subsequently validated and implemented as

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standard sampling practices (Arthur et al., 2023; Arthur & Wheeler, 2021; U.S. Department of Agriculture, 2023; Wheeler & Arthur, 2018). These new methods provided time and labor savings, lowered risk of employee injury, no product loss due to sample collection, and an increase in the finished product surface area sampled as compared to previously used N60-type methods.

Both the CSD and MSD utilize the MicroTally® Swab (MT-Swab) for sample collection. While the CSD and MSD methods were demonstrated to be as good or better than the previously established sampling methods for raw beef trimmings (Arthur & Wheeler, 2021; Wheeler & Arthur, 2018), some users found the MT-Swab a bit cumbersome when performing the MSD technique. To provide a more user-friendly sampling alternative, the MicroTally® Mitt (MT-Mitt) was developed by ARS and FREMONTA Corp. The MT-Mitt still provides the same sample collection advantages of the MT-Swab, but is easier to manipulate by the user than the MT-Swab. The objective of this work was to validate that the novel beef trim sampling method using the MT-Mitt provides organism recovery that is equivalent to other approved methods for sampling raw beef manufacturing trimmings.

## Materials and Methods

The results contained herein are the culmination of 360 matched samples from three comparison trials of the MSD method using MT-Mitt (FREMONTA Corp., San Jose, CA). In each trial, the MT-Mitt was compared to one previously established method. The three previously established sampling methods used for comparison were the MSD method using the MT-Swab, the N60-excision method, and the N60-plus method. The trials were conducted in six commercial beef processing facilities in collaboration with industry partners. Samples were collected from multiple lean-to-fat ratios across multiple days at each processing plant. Samples were collected by scientists from the U.S. Meat Animal Research Center (USMARC) and processing plant employees trained by USMARC scientists.

**MT-Mitt details:** Figure 1 shows the MT-Mitt prior to and after insertion of the user's hand. The MT-Mitt allows the user to collect the sample with more scrubbing force without concern for grip strength. The MT-Mitt is made of the same food-grade, spun-bond polymer material as the MT-Swab. The MT-Mitt dimensions are 25.4 cm by 25.4 cm (10 in by 10 in). The sampling procedure for the MT-Mitt follows the same guidance parameters as the MT-Swab.

**MT-Swab comparison:** For the comparison with the MT-Swab (FREMONTA Corp.), the surface material of 240 combo-bins containing 2,000-lbs of raw beef manufacturing trimmings was sampled (Wheeler & Arthur, 2018). Twenty combo bins were sampled per day for 4 days at each of three commercial beef processing plants. One MT-Mitt and one MT-Swab sample were collected from each combo-bin. The order of sample collection was rotated so each sample method had an equal number of rotations at the 1st and 2nd sample order (Arthur et al., 2023).

**N60 excision comparison:** For the comparison with the N60-excision method, 80 lots of beef manufacturing trimmings were sampled using the MT-Mitt and the N60 excision methods at two commercial beef processing plants (40 matched samples/plant). Lots consisted of subprimals and trimmings destined for grinding. Samples were collected over 4 days. One MT-Mitt and one N60-excision sample were collected from each lot. Due to processing plant procedures, the N60 excision method was collected first and the MT-Mitt sample second.

**N60-plus comparison:** For the comparison with the N60-plus method, 40 lots of raw beef manufacturing trimmings were sampled using the MT-Mitt and the N60-plus methods at one commercial beef processing plant. Samples were collected over 2 days. One MT-Mitt and one N60-plus sample were collected from each lot.

**MSD Sampling Procedure for MT-Mitt and MT-Swab.** Plastic sleeves and gloves were sanitized by applying an alcohol-based sanitizer not containing any quaternary ammonium compounds (Alpet D3 Quat-free, Best Sanitizers, Penn Valley, CA). The MT-Mitt or MT-Swab was vigorously rubbed over the entire top surface of the raw beef manufacturing trimmings by trained personnel. Individuals collecting the samples were instructed to apply downward pressure on the Mitt/Swab while vigorously scrubbing the top surface of the combo-bin as well as driving the Mitt/Swab into the nooks and crannies between the trimmings. One side of the Mitt/Swab was used to scrub for 45 s; then, the swab was flipped over and the other side of the Mitt/Swab was used for an additional 45 s as previously described (Wheeler & Arthur, 2018). Vigorous scrubbing was conducted for a minimum of 90 s. After sample collection, the MT-Mitt or MT-Swab was placed in an appropriately identified sterile bag for transport to the lab.

**N60 Excision Sampling Procedure.** Before each sample, the excision sampling equipment was sanitized. Plastic sleeves and gloves were sanitized by applying an alcohol-based sanitizer not containing any quaternary ammonium compounds (Alpet D3 Quat-free) to all surfaces. Sixty carcass surface slices were aseptically excised from individual pieces (U.S. Department of Agriculture, 2014, 2023). Excised slices were approximately 7.6 cm long by 2.5 cm wide by 0.32 cm thick (ca. 3 by 1 by 1/8 in). Sample slices targeting the carcass surface were obtained from 60 different pieces of beef product. The 60 slices per lot 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 an alcohol-based sanitizer not containing any quaternary ammonium compounds (Alpet D3 Quat-free) to all surfaces. A hot water-sanitized IEH N60 Plus Sampler (Microbiologique, Seattle, WA) was used to collect the samples from raw beef manufacturing trimmings. 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

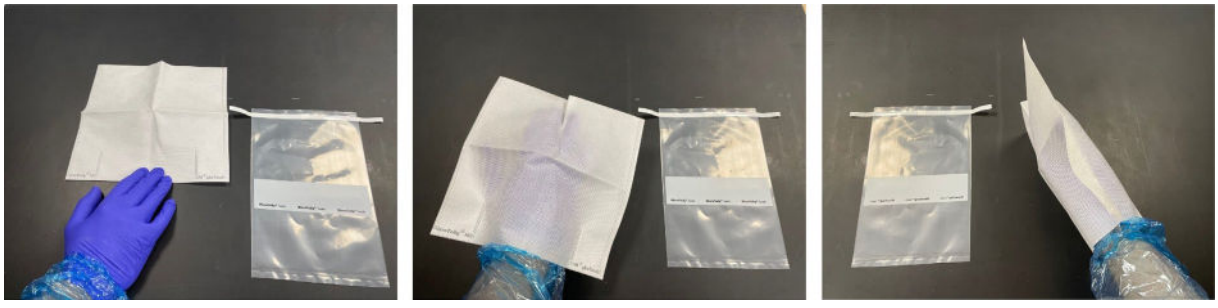

**Figure 1.** Shows the MT-Mitt prior to and after insertion of the user's hand.

sample removal tool was used to push the collected sample out of the sampling head and into the bag.

**Sample processing:** MT-Mitt, MT-Swab, and N60 samples were shipped overnight on ice to the lab. Upon arrival at the lab, MT-Mitt and MT-Swab samples were diluted in 200 ml of mEHEC broth (MilliporeSigma, Burlington, MA) prewarmed to 42°C. N60-excision and N60-plus samples were diluted 1:3 in mEHEC broth (MilliporeSigma) prewarmed to 42°C. Samples were homogenized by stomaching for 30 s on speed setting 7 with a BagMixer 400 (Interscience, Woburn, MA) then 2.5 ml of homogenate was removed from each sample for indicator count analyses. Samples were then incubated for 12 h at 42°C and then held at 4°C until analysis.

**Analyses**: Analyses performed were split into the enumeration of Aerobic Plate Counts: APC and determination of prevalence for PCR pathogen index targets representative of STEC-like and *Salmonella*-like organisms. Indicator counts were determined for APC by plating 1 ml dilutions of the stomached homogenate, described above, on Petrifilm (Neogen, Lansing, MI) and enumerated using an automated PetriFilm reader (Neogen). PCR was performed on enrichments after incubation to determine the prevalence of PCR pathogen index targets: **Intimin PCR**: Hemolysin (*hly*A) and intimin (*eae*) are virulence factors associated with EHEC (Paton & Paton, 1998). **Virulence Factors PCR**: Additional virulence factors associated with STEC are the heme receptor (*chu*A) and adhesion siderophore (*iha*A) (Hoffmann et al., 2001; Tarr et al., 2000). **O group PCR**: The O group PCR data were obtained from three individual, non-Top 6 STEC O serogroup PCRs: O113, O117, and O146 (each O serogroup includes STEC, but are not specific for STEC)(Bosilevac & Koohmaraie, 2011). **H7 PCR**: The H7 gene is found in STEC and generic *E. coli*. **Tet PCR**: the tetracycline resistance genes (*tet*A and *tet*B) are commonly found in *E. coli* and *Salmonella*. (Vikram et al., 2017)*;* and **SdiA PCR**: a transcriptional regulator (*sdi*A) – found in *Salmonella* and *Citrobacter* (Volf et al., 2002).

**Data**: Enumeration data were calculated on a per-sample basis and reported as log CFU/sample. APC data were analyzed using a t test with the probability level at $P \leq 0.05$ (Prism, GraphPad Software, La Jolla, CA). Prevalence data were tallied as positive or negative for the specific PCR pathogen index targets and reported as the proportion of positive samples. Prevalence data were analyzed with a two-sided Fisher's exact test using Prism 10 (La Jolla, CA).

## Results and Discussion

The indicator organism counts and pathogen index target prevalence results for comparison of the MT-Mitt and MT-Swab are shown in Table 1. Recovery of total aerobic bacteria was not significantly different ($P > 0.05$) for the two methods. Pathogen index target analyses similarly showed that the sample collection methods were not significantly different (Table 1). The MT-Mitt had numerically higher prevalence results for three pathogen index targets (O serogroup, tetracycline resistance genes, and *sdi*A), while the MT-Swab had numerically higher prevalence results for the other three pathogen index targets (Virulence factors, H7, and Intimin). However, none of these differences were statistically significant ($P > 0.05$). These data collected from 240 Mitt and Swab samples over numerous days across multiple companies, processing plants, and lean-to-fat ratios collectively demonstrate that MSD sample collection using the MT-Mitt would provide equivalent performance for detecting pathogen contamination in beef trim as that currently provided by the MT-Swab.

Data for the comparison of MT-Mitt to N60-excision using eighty matched samples are presented in Table 2. The average recovery of APC was 0.1 log CFU/sample higher for the MT-Mitt as compared to the N60-excision method. This difference was not significant ($P > 0.05$) at this level of sampling and was within the 0.5 log CFU/sample threshold for indicator count data equivalence (Arthur & Wheeler, 2021). Pathogenic Index target prevalence values of four (Intimin, O group, H7, and Tet) of the five targets were numerically higher for the MT-Mitt as compared to the N60-excision method, but none of these differences were significant ($P > 0.05$). The N60-excision method had a numerically higher prevalence for the Vir factor

**Table 1**
Indicator counts and pathogen index target prevalence for MT-Mitt with MT-Swab methods[1,2]

| Method | n | log APC/sample | Intimin | O group | Vir factor | H7 | Tet | *sdi*A |
|---|---|---|---|---|---|---|---|---|
| MT-Mitt | 240 | 4.70 a | 10.0% a | 23.3% a | 31.3% a | 36.7% a | 68.3% a | 33.8% a |
| MT-Swab | 240 | 4.74 a | 12.1% a | 20.8% a | 32.5% a | 40.4% a | 65.0% a | 32.9% a |

[1] Abbreviations: APC – aerobic plate counts.
[2] Means in a column with different letters differed ($P \leq 0.05$).

**Table 2**
Indicator counts and pathogen index target prevalence for MT-Mitt with N60-excision methods[1,2]

| Method | N | log APC/sample | Intimin | O group | Vir factor | H7 | Tet |
|---|---|---|---|---|---|---|---|
| Mitt | 80 | 6.49 a | 50.0% a | 65.0% a | 61.3% a | 68.8% a | 83.8% a |
| N60-excision | 80 | 6.44 a | 36.3% a | 50.0% a | 67.5% a | 62.5% a | 81.3% a |

[1] Abbreviations: APC – aerobic plate counts.
[2] Means in a column with different letters differed ($P \leq 0.05$).

**Table 3**
Indicator counts and pathogen index target prevalence for MT-Mitt with N60-plus methods[1,2]

| Method | n | log APC/sample | Intimin | O group | Vir factor | H7 | Tet |
|---|---|---|---|---|---|---|---|
| Mitt | 40 | 4.36 a | 5.0% a | 15.0% a | 62.5% a | 92.5% a | 57.5% a |
| N60+ | 40 | 4.34 a | 15.0% a | 17.5% a | 60.0% a | 80.0% a | 50.0% a |

[1] Abbreviations: APC – aerobic plate counts.
[2] Means in a column with different letters differed ($P \leq 0.05$).

PCR as compared to the MT-Mitt, but again this difference was not significant ($P > 0.05$). Based on the data presented in Table 2, it can be concluded that the MT-Mitt and N60-excision methods are equivalent for sampling raw beef manufacturing trimmings.

Table 3 contains the data resulting from a comparison to the MT-Mitt to the N60-plus method using forty matched samples. Similar to the MT-Mitt and N60-excision comparison, the average recovery of APC was 0.1 log CFU/sample higher for the MT-Mitt as compared to the N60-plus method. This difference was not significant ($P > 0.05$) at this level of sampling and was within the 0.5 log CFU/sample threshold for indicator count data equivalence (Arthur & Wheeler, 2021). The prevalence values of three (Vir factor, H7, and Tet) of the five pathogen index targets were numerically higher for the MT-Mitt as compared to the N60-plus method, but none of these differences were significant ($P > 0.05$). The N60-plus method had numerically higher Intimin and O group prevalence values as compared to the MT-Mitt, but again these differences were not significant ($P > 0.05$). Based on the data presented in Table 3, it can be concluded that the MT-Mitt and N60-plus methods are equivalent for sampling raw beef manufacturing trimmings.

## Conclusion

The data reported herein collected from 360 matched samples on numerous days across multiple companies, processing plants, and lean-to-fat ratios collectively demonstrate that MSD sample collection using the MT-Mitt would provide performance that is not significantly different for recovering bacteria and detecting pathogen contamination in raw beef manufacturing trimmings as the previously established methods (MT-Swab, N60-excision, and N60-plus). The MT-Mitt maintains the same sampling advantages (time and labor savings, lowered risk of employee injury, no product loss due to sample collection, and an increase in the finished product surface area sampled) as compared to previously used N60-type methods. In addition, the MT-Mitt design provides easier manipulation by the user than the MT-Swab during MSD sample collection.

## CRediT Author Contribution Statement

TMA and TLW conceptualized the project. TMA and FJR were responsible for labratory method development, sample analyses, and data curation. TMA wrote the draft. TMA, FJR, and TLW reviewed and edited the manscript.

## Declaration of competing interest

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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