Validation of Combo Bin Sampling of Poultry Parts Using a Manual Sampling Mitt Approach

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Summary

A series of trials were conducted with a total of 160 sets of matched samples comparing manual sampling of poultry parts contained within a 2,000-lb combo bin using the MicroTally® Mitt (Mitt) to parts rinses. These data come from samples collected on numerous days across four commercial processing plants. The results of these trials collectively demonstrate that sampling poultry parts contained within combo bins using the Mitt provides organism recovery that is equivalent to or better than that of 4-lb parts rinses. Thus, the Mitt method provides an alternative sampling method for poultry parts that is more representative of large batches than 4-lb parts rinses. Mitt sampling of combo bins provides multiple implementation advantages pertaining to labor and ease of use relative to the current parts rinse method. This project demonstrates that the Mitt is amenable to sampling much larger quantities of parts, which will be a distinct sampling improvement for poultry process control and finished product testing.

Objective

The objective of this experiment was to demonstrate that a combo bin sampling method for poultry parts using the MicroTally® Mitt provides equivalent or better organism recovery compared to the previously recognized as efficacious method, a 4-lb parts rinse.

Background

Sampling for detection of foodborne pathogens is a key component of food safety plans for meat and poultry processors. We have developed a more robust and representative sampling device using a spunbond polymer cloth and validated it for various approaches to sampling beef trimmings for pathogen detection [1, 2, 3]. We further have validated an improved version of the sampling cloth by configuring it as a Mitt that fits on one hand to improve the ease of sampling beef trimmings [4]. Figures 1-3 show the Mitt prior to and after insertion of the user's hand. The Mitt allows the user to collect the sample using one hand with more scrubbing force without concern for grip strength. The Mitt dimensions are 10" x 10" and is made of the same food-grade, spunbond polymer as the MicroTally Swab. In the current experiments, the application of the sampling Mitt for use on combo bins of poultry parts was evaluated.

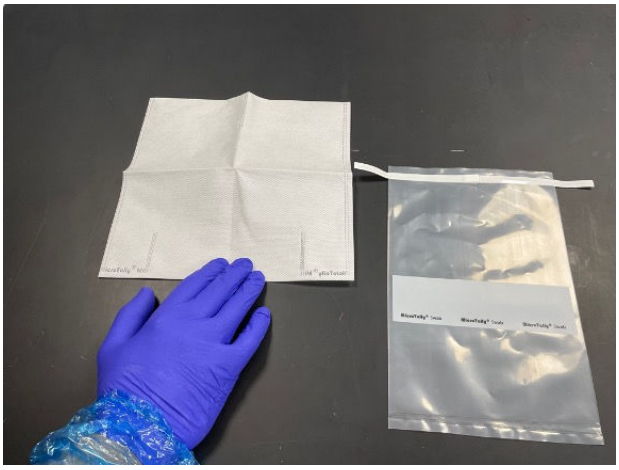

Figure 1. Mitt – removed from bag and unfolded.

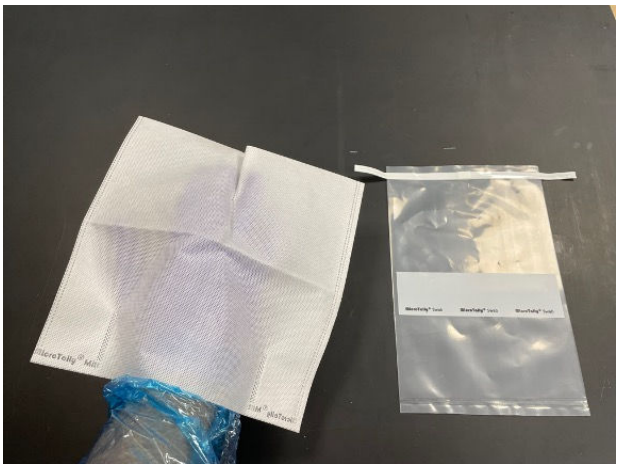

Figure 2. Mitt – hand inserted

Figure 3. Mitt – hand inserted (side view)

MATERIALS and METHODS

The results contained herein are the culmination of comparison trials with 160 sets of matched samples of the Mitt versus the current sampling method used for poultry parts, a 4-lb rinse. The trials were conducted in four commercial poultry processing facilities in collaboration with industry partners. Parts samples were spread across multiple types of parts (i.e. boneless thighs, beast tenders, whole breasts, wings, etc.).

Each day for two days at each of four chicken processing plants, twenty 2,000-lb combo bins of parts were sampled by each method. Typically, 4-lb of parts were collected from the combo for the rinse sample prior to sample collection using the Mitt. Following sample collection, the samples were shipped to the lab on ice overnight.

Mitt sampling: Only one Mitt was used for collecting poultry parts samples. Each Mitt was processed for indicator counts, *Salmonella*, *Campylobacter*, and pathogen index PCR target prevalence. Plastic sleeves and gloves were sanitized by applying an alcohol-based sanitizer not containing any quaternary ammonium compounds. Note: Preliminary results indicated that adding 25 ml of neutralizing buffered peptone water (nBPW) immediately after sample collection, as opposed to prewetting the Mitt prior to sampling, would provide better recovery of bacteria. Hence, Mitts were used dry and 25 ml of nBPW or equivalent was added immediately after sampling the combo bin.

An individual Mitt was used to sample across the entire top surface of the product in a combo bin. Individuals collecting the samples were instructed to use enough pressure to ensure that microbiological organisms present on the poultry surfaces would be dislodged from the product and captured on the swab. One side of the Mitt was used to scrub for 45 sec across one half of the surface of the combo bin, then was flipped over and the other side of the Mitt used for an additional 45 sec to sample the remaining half of the combo bin surface. During sampling, the Mitt was used to scrub the surface poultry parts and in the combo bin and inserted down in between the parts to achieve a thorough sample. After sample collection, the Mitt was folded and placed in an appropriately identified sterile bag and 25 ml of nBPW or equivalent was added to the Mitts to neutralize any antimicrobial collected during sampling. The bags were closed and placed on ice for transport to the lab.

Poultry parts rinse: A batch of 4 lbs of parts was placed in a poly bag. Sampling broth (400 ml of nBPW) was poured into the bag. The bag opening was twisted to close. The broth was mixed through the parts for one minute. The bag was opened and 100 ml of rinsate was aseptically poured into a sample container. The sample container was capped and placed on ice for transport to the lab.

Sample and Data Analysis

Sample processing:
Mitts: Each Mitt received 200 ml of BPW (prewarmed to 42°C). Samples were homogenized by stomaching for 30 sec, then 2.5 ml of homogenate was removed from each sample for indicator

count analyses. Another 30 ml was removed for Campylobacter enrichment. The 30 ml aliquot of homogenate was mixed with 30 ml of *Campylobacter* Enrichment Medium (Neogen) for *Campylobacter* detection and incubated for 22-26 h at 42°C. For chicken samples, *Campylobacter* prevalence was determined by screening for *cje*-ATP gene described by Lanzl et al. (6) using real time PCR (Bio-Rad). The remaining Mitt sample was incubated for 18-24 h at 42°C for enrichment prior to *Salmonella* and pathogen index target detection by PCR.

Rinsates: Aliquots (2.5 ml) of parts rinsates were used for indicator count analyses. Portions (30 ml) of rinsates were mixed with 30 mL BPW (prewarmed to 42°C) for enrichment. Samples were homogenized by stomaching for 30 sec, then incubated for 18-24 h at 42°C prior to *Salmonella* and pathogen index target detection by PCR. For *Campylobacter* detection, another 30 ml of rinsate was mixed with 30 ml of *Campylobacter* Enrichment Medium (Neogen) and incubated for 22-26 h at 42°C.

Analyses: Analyses performed were split into enumeration of indicator bacteria counts (Aerobic Plate Counts: APC) and determination of prevalence for *Salmonella*, *Campylobacter* and PCR pathogen index targets representative of STEC-like and *Salmonella*-like organisms.

Indicator counts: Counts were determined for APC by plating on Petrifilm (Neogen).

Salmonella: *Salmonella* prevalence was determined by screening for *inv*A gene as described by Rahn et al. (5) using real time PCR (Bio-Rad).

Campylobacter: *Campylobacter* prevalence was determined by screening for *cje*-ATP gene described by Lanzl et al. (6) using real time PCR (Bio-Rad).

Pathogen Index targets: PCR was performed on enrichments after incubation to determine the prevalence of PCR pathogen index targets: **Intimin PCR**: intimin is an attachment factor associated with EHEC. **Flagella PCR**: The *fli*CH7 gene is found in STEC and generic *E. coli*. This comprehensive list of targets was used because they were expected to yield a range of positives across all samples, thus increasing the probability of having at least one target in the desired range of 20-80% positive.

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 10, GraphPad Software). Prevalence data were tallied as positive or negative for the specific pathogen or 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.

RESULTS and DISCUSSION

Results for indicator organisms (APC) can be found in Table 1. The Mitt and rinse methods had equivalent ($P > 0.05$) recoveries of APC, with the Mitt recovering 0.1 log APC/sample more than the rinse. The Mitt had higher ($P \leq 0.05$) recovery of *Salmonella* and *Campylobacter* (Table 2) than the rinse method. The prevalence for both *Salmonella* and *Campylobacter* was almost twice that for the rinse method. The recovery of pathogen index targets by each method was compared. The Mitt method had higher ($P \leq 0.05$) recoveries than the rinse method (Table 3). for both pathogen index targets (intimin and H7-flagella).

Our previous study (7) was performed using single carcass and small batch parts sampling demonstrated that the Mitt performed well under those conditions compared to the rinse methods. However, the Mitt also is amenable to multi-carcass and large batch parts sampling, which would not be achievable using the rinse method. As FSIS and the poultry industry move forward to control *Salmonella*, we believe it will require sampling methods that are more representative of a lot than one carcass or 4 lb of parts. Food safety systems benefit from sampling methods that can collect more robust samples representing larger percentages of lots. The current study was designed to show that sampling larger surface areas and contacting more product will provide results that better represent the lot of product being produced, than current methods, which are limited in the amount of product that can be sampled.

In conclusion, the data reported herein, collected from 160 combo bins on numerous days across multiple companies, processing plants, and sample types, collectively demonstrate that sample collection using the Mitt would provide equivalent or better performance for recovering bacteria and detecting pathogen contamination as previously established 4-lb parts rinse method in poultry sampling programs.

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Table 1. Indicator organism recovery for parts sampling methods[1,2].

Sample type	n	log APC/sample
Mitt	160	5.1 a
Rinse	160	5.0 a

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

Table 2. Pathogen prevalence for parts sampling methods[1].

Sample type	n	*Salmonella*	Campy
Mitt	160	31.9% a	46.9% a
Rinse	160	18.8% b	24.4% b

[1]Means in a column with different letters differed ($P \leq 0.05$).

Table 3. Pathogen index target prevalence for parts sampling methods[1].

Sample type	n	Intimin	Flagella
Mitt	160	38.1% a	49.4% a
Rinse	160	20.0% b	28.1% b

[1]Means in a column with different letters differed ($P \leq 0.05$).