Validation of a New Method of Sampling Poultry Carcasses and Parts for Pathogen Testing using a Manual Sampling Mitt Approach

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Data should be considered preliminary until published in a peer-reviewed scientific journal.

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Establishing Equivalency of Poultry Carcass and Parts Sampling using the MicroTally Mitt

Summary

A series of trials were conducted with a total of 567 sets of matched samples comparing manual sampling of poultry carcasses and parts using the MicroTally® Mitt (MT-Mitt) and either turkey carcass sponge sampling or chicken carcass and parts rinses. These data come from samples collected on numerous days across seven processing plants. The results of these trials collectively demonstrate that sampling poultry carcasses and parts using the MT-Mitt provides organism recovery that is equivalent to or better than that of carcass sponge sampling and carcass and parts rinses. Thus, the MT-Mitt method provides an alternative sampling method with at least equivalent organism recovery and some implementation advantages pertaining to labor and ease of use relative to other approved methods for sampling poultry products. In addition, while this study used single carcass and small batch parts sampling, the MT-Mitt is amenable to sampling multiple carcasses and much larger quantities of parts, which would be a distinct sampling improvement for the implementation of the *Salmonella* Framework for Raw Poultry Products.

Objective

The objective of this experiment was to demonstrate that the novel sampling method for poultry carcasses and parts using the MT-Mitt provides equivalent organism recovery compared to previously recognized as efficacious methods.

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 MT-Mitt prior to and after insertion of the user's hand. The MT-Mitt allows the user to collect the sample using one hand with more scrubbing force without concern for grip strength. The MT-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 MT-Mitt for use on poultry carcasses and parts was evaluated.

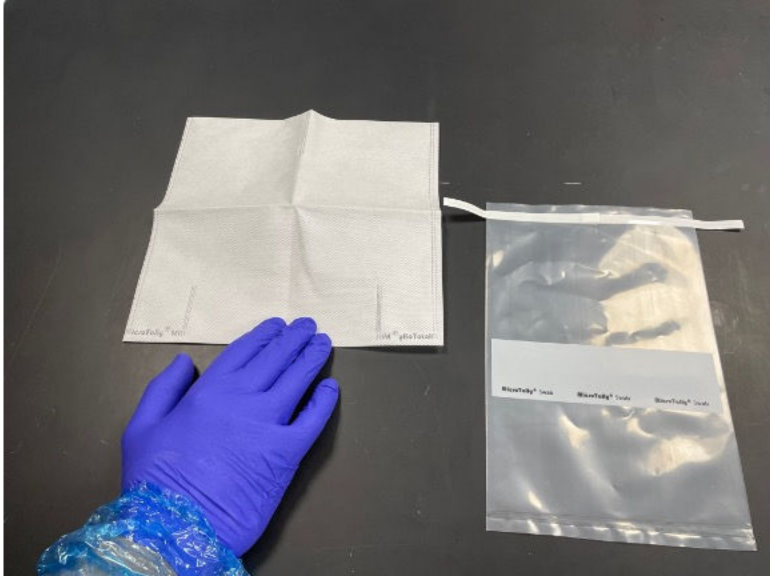

Figure 1. MT-Mitt – removed from bag and unfolded.

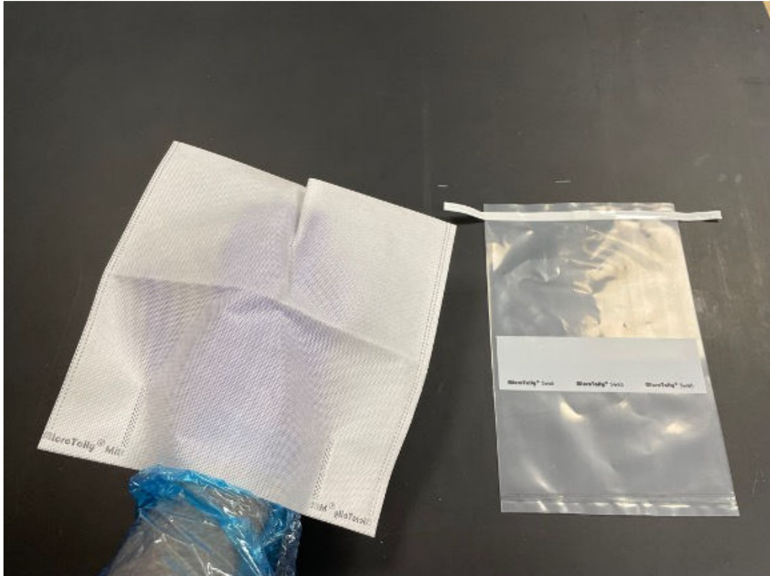

Figure 2. MT-Mitt – hand inserted

Figure 3. MT-Mitt – hand inserted (side view)

MATERIALS and METHODS

The results contained herein are the culmination of seven comparison trials with 567 sets of matched samples of the MT-Mitt versus current sampling methods used for chicken and turkey products. The two previously established sampling methods used for comparison were the carcass swabbing with a cellulose sponge for turkey carcass sampling and the carcass and parts rinse for chicken sampling. The trials were conducted in seven commercial poultry processing facilities (three turkey plants and four chicken plants) in collaboration with industry partners. Carcass samples were collected from rehang and post-chill locations across multiple days at each processing plant. For parts samples, chicken wings were chosen as the parts product to be used for this study. Samples were collected by USMARC scientists.

Carcasses within the same lot were removed from the line. One sample method was performed on each carcass and the carcasses were placed back on the line. The methods were repeated with additional matched carcasses. The order of sample methods was rotated through sampling positions to minimize bias. For each carcass, APC and EBC were performed for each method. For each carcass, prevalence was determined for *Salmonella*, *Campylobacter*, and several pathogen index PCR targets for each method. Each day for three days at each of three turkey processing plants, 23 carcasses (12 rehang & 11 post-chill) per method were sampled. For chickens, each day for two days at each of four chicken processing plants, 30 carcasses (15 rehang and 15 post-chill) and 15 batches of parts (4 lbs) were sampled by each method. Following sample collection, the samples were shipped to the lab on ice overnight. All turkey carcasses, chicken carcasses, and chicken parts were returned to the processing line after sampling.

TURKEY:

MT-Mitt sampling for turkey carcasses: Two MT-Mitts were used for sampling. One MT-Mitt was processed for indicator counts, Salmonella prevalence and pathogen index PCR target prevalence. The other MT-Mitt was processed for Campylobacter prevalence. Prior to sampling, 25 ml of neutralizing buffered peptone water (nBPW) was added to the *Salmonella* and *Campylobacter* sampling MT-Mitts. Plastic sleeves and gloves were sanitized by applying an alcohol-based sanitizer not containing any quaternary ammonium compounds. The MT-Mitt was vigorously rubbed over half of one turkey carcass. The carcass was hung by the legs prior to sampling. Sampling began at the leg and finished at the neck. Sampling was done on one side of the carcass either left or right, from midline on the breast to midline on the back, so each sample covered a leg and wing on the same side of the carcass. For eviscerated carcasses, the body cavity was included in the sampling with the MT-Mitt. One side of the MT-Mitt was used to scrub the sample area for 15 sec then the swab was flipped over and the other side of the MT-Mitt used to for an additional 15 sec. 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 MT-Mitt. After sample collection, the MT-Mitt was placed in an appropriately identified sterile bag for transport to the lab. For rehang carcasses, only the outside of the carcass was sampled. For post-chill carcasses, the inside cavity and external surface of the carcass were sampled.

Cellulose sponge sampling of turkey carcasses: Two cellulose sponges ("Speci-Sponge" measures 1.5 × 3 × 0.63 in when wet) were used for sampling. One sponge was processed for indicator counts, *Salmonella* prevalence and pathogen index PCR target prevalence. The other for *Campylobacter* prevalence. Prior to sampling, 10 ml of nBPW was added to the *Salmonella* sampling sponges and 25 ml of BPW was added to the *Campylobacter* sampling MT-Mitts. Nitrile gloves were sanitized by applying an alcohol-based sanitizer not containing any quaternary ammonium compounds. A template (5 cm x 10 cm) was place on the back of the carcass just to the side of the vertebral column. The template was held with one gloved hand and the other gloved hand was used to wipe the area with sponge. Sampling consisted of 10 vertical wipes over entire sample surface; then 10 horizontal wipes over entire sample surface. The sampling procedure was repeated using the *Campylobacter* sponge on the other side of the carcass.

CHICKEN:

MT-Mitt sampling for chicken carcasses and parts: Only one MT-Mitt was used for collecting chicken carcass and parts samples. Each MT-Mitt was processed for indicator counts, *Salmonella*, *Campylobacter*, and pathogen index PCR target prevalence. Prior to sampling, 25 ml of neutralizing buffered peptone water (nBPW) was added to the MT-Mitts. Plastic sleeves and gloves were sanitized by applying an alcohol-based sanitizer not containing any quaternary ammonium compounds. The MT-Mitt was vigorously rubbed over an entire chicken carcass. An individual MT-Mitt was also used to sample 4 lbs of parts held in a poly bag. 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 MT-Mitt was used to scrub for 15 sec then was flipped over and the

other side of the MT-Mitt used for an additional 15 sec. After sample collection, the MT-Mitt was folded and placed in an appropriately identified sterile bag for transport to the lab. For rehang carcasses, only the outside of the carcass was sampled. For post-chill carcasses, both the inside cavity and external surface of the carcass was sampled.

Chicken carcass and parts rinse: A chicken carcass or 4 lbs of chicken parts were placed in a poly bag (carcass placed neck first). Sampling broth (400 ml of nBPW) was poured into the bag. The broth was mixed through the carcass cavity and outside of the carcass or through the parts for one minute. The bag was opened and 100 ml of rinsate was aseptically poured into a sample container. The chicken carcass or parts were returned to the processing line.

Sample and Data Analysis

Sample processing:
Turkey:

Indicator counts, *Salmonella* and index target prevalence: MT-Mitt samples were diluted in 150 ml of ISO-BPW broth (Thermo-Fisher). Cellulose sponge samples received 50 ml of ISO-BPW. Samples were homogenized by stomaching for 30 sec, then 2.5 ml of homogenate was removed from each sample for indicator count analyses. Samples were then incubated for 20-24 h at 35°C. Following incubation, the samples were analyzed for *Salmonella* using the MDS system (Neogen). Index target PCR was performed as described below.

Campylobacter: MT-Mitt samples were diluted with 100 ml of Hunt's broth (Thermo-Fisher). Cellulose sponge samples received 25 ml of Hunt's broth. Samples were homogenized by gentle hand massage. Samples were then incubated for 24 h at 42°C. Following incubation, the samples were analyzed for *Campylobacter* using the MDS system (Neogen).

Chicken:

MT-Mitts: Each MT-Mitt received 200 ml of ISO-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 MT-Mitt sample was incubated for 18-24 h at 42°C for *Salmonella* and pathogen index PCR target enrichment.

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

Analyses: Analyses performed were split into enumeration of indicator bacteria counts (Aerobic Plate Counts: APC and Enterobacteriaceae counts: EBC) 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 and EBC by plating on Petrifilm (Neogen) and enumerated using an automated PetriFilm reader (Neogen).

Salmonella: For turkey samples, *Salmonella* prevalence was determined using the MDS system (Neogen). For chicken samples *Salmonella* prevalence was determined by screening for invA gene as described by Rahn et al. (5) using real time PCR (Bio-Rad).

Campylobacter: For turkey samples, *Campylobacter* prevalence was determined using the MDS system (Neogen). 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).

Pathogen Index targets: PCR was performed on enrichments after incubation to determine the prevalence of PCR pathogen index targets: **Intimin PCR**: Hemolysin and intimin are factors associated with EHEC. **Virulence Factors PCR**: Additional virulence factors associated with STEC are the heme receptor (*chu*A) and adhesion siderophore (*iha*A). **O serogroup PCR**: The O group PCR data were obtained from three individual, non-STEC-specific O serogroup PCRs: O113, O117, and O146. **Flagella PCR**: The *fli*CH7 gene is found in STEC and generic *E. coli*. **Tet PCR**: The tetracycline resistance genes (tetA and tetB) are commonly found in *E. coli* and *Salmonella*. **Generic *E. coli***: ybbW is a putative gene commonly found in *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 and EB 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

Turkey:

At rehang, the MT-Mitt had higher ($P < 0.05$) recoveries of APC and EBC than the cellulose sponge method (Table 1). Similarly, for post-chill sampling, the MT-Mitt had higher ($P < 0.05$) recovery of APC than the cellulose sponge sampling method. The differences between indicator count values for the MT-Mitt and cellulose sponge were all more than 0.5 log CFU/sample, a limit of equivalence based on previous data (Appendix A). The EBC at post-chill had too many samples that were below the limit of detection to allow for analysis.

The MT-Mitt had higher ($P \leq 0.05$) recovery of *Campylobacter* (Table 2) than the cellulose sponge sampling method at both the rehang and post-chill sampling sites. *Campylobacter* prevalence for the MT-Mitt was approximately double that of the cellulose sponge at rehang and triple at post-chill. For *Salmonella* recovery at rehang, the MT-Mitt had a higher ($P \leq 0.05$) prevalence than the cellulose sponge method, with the MT-Mitt prevalence being double that of

the cellulose sponge. *Salmonella* was detected in only one sample post-chill, hence no analyses could be performed.

The recovery of pathogen index targets by each method was compared. At rehang, the trends were similar to the results of the indicator counts and pathogen prevalence where the MT-Mitt had higher recoveries than the cellulose sponge method. Three of the five pathogen index target (generic *E. coli*, virulence genes, and O serogroup assay) results showed while the mitt had the higher numerical prevalence, it was equivalent ($P > 0.05$) to the cellulose sponge method prevalence (Table 3). For the other two targets (tetracycline resistance and flagella) the MT-Mitt had higher ($P \leq 0.05$) recoveries than the cellulose sponge. At post-chill, MT-Mitt had higher ($p \leq 0.05$) target recoveries than cellulose sponge methods for all targets except the O serogroups (Table 3), which were equivalent ($P > 0.05$).

Chicken:
At rehang, the rinse method had higher ($P < 0.05$) recoveries of APC and EBC than the MT-Mitt method (Table 4), but were within 0.5 log CFU/sample. For post-chill sampling, the MT-Mitt had higher ($P < 0.05$) recovery of APC than the rinse method, but were within 0.5 log CFU/sample. For parts sampling, the MT-Mitt and rinse methods had equivalent ($P > 0.05$) recoveries of APC. The EBC at post-chill and parts sampling sites had too many samples that were below the limit of detection to allow for analysis.

The MT-Mitt had higher ($P \leq 0.05$) recovery of *Salmonella* (Table 5) than the rinse method at both the rehang, but the methods had equivalent ($P > 0.05$) recovery of *Salmonella* from post-chill and parts samples. *Campylobacter* prevalence at rehang, post-chill and parts sample sites was equivalent ($P > 0.05$) for the MT-Mitt and rinse methods.

The recovery of pathogen index targets by each method was compared. At rehang, the MT-Mitt method had higher ($P \leq 0.05$) recoveries than the rinse method for two (intimin and generic *E. coli*) of the four pathogen index targets (Table 6). The methods showed equivalent ($P > 0.05$) recoveries for the flagella and tetracycline resistance targets. For post-chill and rehang, the methods had equivalent ($P > 0.05$) recoveries for all four index targets.

DISCUSSION

The main goal of this project was to evaluate the MT-Mitt as an alternative sample collection method for poultry. The largest differences in organism recoveries between method were observed for turkey. From the turkey sampling results it can be concluded that increasing the surface area sampled increases the bacterial recovery and prevalence of pathogen detection. The current cellulose sponge sampling method employed by FSIS uses two 50 cm^2 sampling areas, whereas the MT-Mitt method evaluated here sampled the one half of the carcass. Also, the current FSIS method does not sample the interior cavity of the carcass at post-chill, while the MT-Mitt method did include sampling of the interior cavity. For the chicken sampling methods, the differences in bacterial recovery were not as great as for the turkey methods, but the MT-Mitt results showed a tendency to provide better pathogen and pathogen index target sensitivity than the rinse methods.

The current study was performed using single carcass and small batch parts sampling. The MT-Mitt was shown to perform well under those conditions, but the MT-Mitt also is amenable to multi-carcass and large batch parts sampling, which would not be achievable using the rinse method. As the *Salmonella* Framework for Raw Poultry Products is being implemented, we believe it will require sampling methods that are more representative of a lot than one carcass or 4 lb of parts. Both Component 2 and Component 3 of the Framework would be better served by methods that can collect more robust samples representing larger percentages of lots. The current study was designed to show equivalence with current methods. The next step will be to determine how sampling of multiple carcasses and larger batches of parts can be used for effective process control and finished product testing.

In conclusion, the data reported herein, collected from 567 sets of matched samples on numerous days across multiple companies, processing plants, and sample types, collectively demonstrate that sample collection using the MT-Mitt would provide equivalent or better performance for recovering bacteria and detecting pathogen contamination as previously established methods (turkey carcass sponge swab, chicken carcass rinse, and chicken parts rinse methods) in turkey and chicken sampling programs.

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

Method	n	log APC/sample	log EBC/sample
Rehang			
Mitt	108	6.7 a	5.7 a
Sponge	108	5.7 b	4.0 b
Post-chill			
Mitt	99	3.7 a	bld
Sponge	99	3.1 b	bld

[1]Means in a column with different letters differed ($P \leq 0.05$).
[2]Abbreviations: APC- aerobic plate counts, EBC – Enterobacteriaceae counts, bld: below limit of detection.

Table 2. Pathogen prevalence for turkey carcass sampling methods[1].

Method	n	*Salmonella*	*Campylobacter*
Rehang			
Mitt	108	28.7% a	89.3% a
Sponge	108	13.9% b	44.9% b
Post-chill			
Mitt	99	1.0%	23.4% a
Sponge	99	0.0%	8.1% b

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

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

Method	n	Virulence factors	Tetracycline resistance	O serogroups	Flagella	Generic *E. coli*
Rehang						
Mitt	108	100.0% a	100.0% a	52.8% a	94.4% a	99.1% a
Sponge	108	99.1% a	93.8% b	39.8% a	76.9% b	94.4% a
Post-chill						
Mitt	99	90.9% a	89.8% a	17.2% a	45.5% a	96.0% a
Sponge	99	40.4% b	46.6% b	10.1% a	5.1% b	56.6% b

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

Table 4. Indicator organism recovery for chicken sampling methods[1,2].

Method	log APC/sample (n=120)	log EBC/sample (n=105)
Rehang		
Mitt	6.3 b	5.8 b
Rinse	6.8 a	6.2 a
Post-chill		
Mitt	3.9 a	bld
Rinse	3.4 b	bld
Parts		
Mitt	3.8 a	bld
Rinse	3.9 a	bld

[1]Means in a column with different letters differed ($P \leq 0.05$).
[2]Abbreviations: APC- aerobic plate counts, EBC – Enterobacteriaceae counts, bld: below limit of detection.

Table 5. Pathogen prevalence for chicken sampling methods[1].

Method	***Salmonella*** **(n=120)**	***Campylobacter*** **(n=90)**
Rehang		
Mitt	77.5% a	72.2% a
Rinse	65.0% b	73.3% a
Post-chill		
Mitt	10.8% a	18.9% a
Rinse	9.2% a	14.4% a
Parts		
Mitt	17.5% a	23.3% a
Rinse	11.7% a	33.3% a

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

Table 6. Pathogen index target prevalence for chicken samples[1].

Method	n	Intimin	Flagella	Tet	Generic *E. coli*
Rehang					
Mitt	120	97.5% a	95.8% a	98.3% a	100.0% a
Rinse	120	90.0% b	91.7% a	100.0% a	92.5% b
Post-chill					
Mitt	120	46.7% a	31.7% a	84.2% a	91.7% a
Rinse	120	25.0% a	20.0% a	83.3% a	83.3% a
Parts					
Mitt	120	44.2% a	37.5% a	89.2% a	95.0% a
Rinse	120	26.7% a	31.7% a	93.3% a	91.7% a

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

Appendix A.

United States Department of Agriculture

Food Safety and Inspection Service
Office of Policy and Program Development
Risk Management and Innovations Staff
Stop Code 3782, Patriot's Plaza III
1400 Independence Avenue, SW
Washington, D.C. 20250-3700

March 12, 2020

By Electronic Mail
Terrance.arthur@usda.gov

Terrance Arthur, Ph.D.
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Dear Dr. Arthur:

On December 9, 2019 you requested FSIS concurrence regarding a standardized HACCP validation protocol for the MicroTally™ Manual Sampling Device (MSD) swab technique used for sampling raw beef trim (15-SMP-1064-N-B) in lieu of an N60 sampling approach. The data you provided indicates that on occasion, use of the MicroTally™ MSD swab may result in lower concentration of indicator organisms, but the results for pathogen genetic indicator targets are consistently at least equal to the N60 excision or IEH N60+ sampling methods.

You requested Agency concurrence that if establishment in-plant HACCP validation data supported that indicator organisms results were no more than 0.5 log lower than either N60 excision or IEH N60+ and/or that the pathogen indicator targets presence was at least equal to that observed with either N60 excision or IEH N60+, that the MicroTally™ MSD swab sampling procedure could be considered equivalent.

FSIS' Technical Review Team (TRT) reviewed the data you submitted and found that the data showed that the MicroTally™ based sampling methods may result in lower indicator organism concentrations than the N60 methods. However, the MicroTally™ based sample methods consistently obtained results with higher percent positive of pathogen index targets and inoculated surrogates.

The data presented supports equivalency criteria of indicator organisms within 0.5 log CFU/sample. Going forward, FSIS has no objection to plants performing in-plant HACCP validation using the MicroTally™ based sampling results using only indicator organism concentration data.

Sincerely,

MELVIN CARTER Digitally signed by MELVIN CARTER Date: 2020.03.12 11:41:56 -04'00'

Melvin Carter, Ph.D.
Director
Risk Management and Innovations Staff
Office of Policy and Program Development