FSIS Poultry Exploratory Sampling Program Report

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Abstract

FSIS conducted a paired sampling *Salmonella* study to examine the microbial profile of young chicken carcasses from the rehang step to the end of the slaughter process, *i.e*., post-chill step. This study found that both *Salmonella* incidence and aerobic indicator levels decreased significantly from rehang to post-chill, with reduction rates of 92% and 99.9%, respectively. When present, *Salmonella* was most often found at low levels. Eighteen point four percent of rehang carcass samples and 15.7% of post-chill carcass samples had *Salmonella* concentrations above the 10 CFU/mL limit of quantification (LOQ), with median values of 58 CFU/mL and 27 CFU/mL, respectively. Given the recent guidance from the National Advisory Committee on Microbiological Criteria for Foods (2023), FSIS conducted an additional study to evaluate the variability in *Salmonella* enumeration test results using traditional most probable number (MPN) methods compared to using newer quantitative polymerase chain reaction (qPCR) test methods. Both MPN and qPCR *Salmonella* enumeration methods exhibited variability at the low microbial levels typically found in poultry products. FSIS will continue to collaborate with researchers and test kit manufacturers to evaluate new methods of *Salmonella* quantification as they become available to identify the best method for a high-throughput regulatory laboratory environment.

Introduction

The Food Safety and Inspection Service (FSIS) is the regulatory agency within the United States Department of Agriculture (USDA) responsible for ensuring that domestic and imported meat, poultry,

and egg products are safe, wholesome and accurately labeled. FSIS advances food safety through inspection verification of Pathogen Reduction, Hazard Analysis and Critical Control Point (PR/HACCP) systems utilized by federal establishments to produce meat, poultry and egg products. The agency gathers data that is used to establish science-based policy and programs aimed at reducing foodborne illness, such as qualitative performance standards that set limits on pathogen occurrence in raw products. These policies are continually evaluated and updated to protect public health.

Recently, FSIS shared a comprehensive framework under consideration for reducing foodborne salmonellosis associated with poultry products (FSIS, 2022b). According to the Centers for Disease Control and Prevention (CDC), *Salmonella* is responsible for more than 1 million cases of foodborne illness each year in the United States (Scallan et al., 2011). Over 20% of these cases are attributed to poultry products (IFSAC, 2022). Since the establishment of *Salmonella* performance standards, FSIS has seen a 50% decrease in the prevalence of *Salmonella* on poultry produced in the U.S. (Williams et al., 2022). Despite this decline in *Salmonella* contamination, the incidence of salmonellosis attributed to poultry consumption has remained steady (Delahoy et al., 2023). The U.S. Department of Health and Human Services Healthy People 2020 food safety goal to lower foodborne *Salmonella* illness by 25% was not met and has now been set as the Healthy People 2030 goal (HHS, 2021). While this is the target for reducing *Salmonella* infection from all sources, FSIS has set this same goal for FSIS-regulated products subject to *Salmonella* performance standards.

Understanding the current efficacy of process control and overall microbial load reduction during poultry processing across the industry provides valuable data to inform policy development. Previously, quantification of indicator organisms such as aerobic counts (AC) and Enterobacteriaceae (EB) have been used to estimate the microbial status of a population, but there is no clear correlation between levels of indicator organisms and levels of *Salmonella* (Bueno Lopez et al., 2022; De Villena et al., 2022). More

recently, methods of direct *Salmonella* quantification have become commercially available and a method was introduced into FSIS laboratories (FSIS, 2022a).

The Agency conducted this paired sampling study to examine microbiological data beyond the *Salmonella* presence/absence data currently generated to evaluate establishment performance and generate data to help guide development of a revised strategy. Data generated to inform risk management strategies targeted to achieve the Healthy People 2030 target included:

i. *Salmonella* occurrence early in the slaughter process, before significant microbial interventions have been applied (i.e., rehang), and after most, if not all, microbial interventions have been applied (i.e., post chill) to determine the change during the slaughter process. Both qualitative and quantitative data were generated.
ii. Serotype characterization, to identify *Salmonella* serotypes more commonly associated with human illnesses, and concordance of serotypes between processing steps.
iii. Levels of indicator organisms, which may be useful for monitoring process control.

A paired, two-point exploratory study was established in parallel with FSIS verification of young chicken carcasses. Carcass rinsate samples were collected at the pre-evisceration step of the slaughter process (rehang step) in addition to the standard PR/HACCP regulatory samples taken after the application of microbial interventions (post-chill). The paired nature of the program allows direct comparison of the microbiological status of a slaughtered flock, providing critical information to evaluate process control. In addition, FSIS subsequently gathered additional data on further processed raw chicken parts as well as raw comminuted chicken and turkey products.

Materials and Methods

Study Design and Carcass Rinsate Sample Collection

FSIS determined that a minimum of 12 analyzed paired results were required from each USDA-inspected establishment currently processing young chicken carcasses to achieve a minimal statistical sample size. As current FSIS chicken carcass sampling is limited to either five or two sampling events per month based on establishment size, six months of sampling was necessary to reach the target for small establishments. Here, small establishment refers to those processing fewer than 10,000,000 birds/year. Currently FSIS analyzes roughly 9600 young chicken carcasses yearly, so a 6-month timeframe would yield approximately 4800 rehang samples paired with 4800 post-chill samples total. Notably, this sample size exceeds the prior FSIS chicken carcass baseline performed in 2008 that analyzed 3275 paired samples.

Young chicken carcasses were sampled as described in FSIS Directive 10250.1 and further detailed in FSIS Notice 44-22. For this project, inspection program personnel selected one young chicken carcasses at the rehang step prior to evisceration and a second young chicken carcass at the post-chill step after the application of microbial interventions. These two carcasses were selected from the same flock and sampled during the same slaughter timeframe, pairing them for comparison. Each carcass was rinsed in 400 mL of neutralizing buffered peptone water (nBPW). The resultant carcass rinsates were sent for analysis to an FSIS field service laboratory. For data gathered during the subsequent expansion of *Salmonella* quantification and indicator testing, these analyses were added to post-intervention regulatory chicken parts rinsate samples and comminuted poultry samples collected as part of regularly scheduled verification sampling programs.

Salmonella Detection and Isolation

FSIS Microbiology Laboratory Guidebook (MLG) Chapter 4, “Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, Siluriformes (Fish) Products and Carcass and Environmental Sponges,” was followed for the detection and cultural isolation of *Salmonella* (FSIS, 2023b). Briefly,

30mL of rinsate was added to 30mL BPW enrichment buffer and incubated overnight at 35 C. For comminuted samples, 325g of product was added to 1,625mL of BPW enrichment buffer. Enrichments were then screened for the presence of *Salmonella* by loop-mediated isothermal amplification. Positive samples were then incubated in secondary enrichments of Tetrathionate broth (TT, Hajna formulation) and Rappaport-Vassiliadis broth (RV) and incubated overnight at 42 C. Each secondary enrichment was then plated for isolation onto Brilliant Green Sulfa agar (BGS) and Double Modified Lysine Iron agar (DMLIA) and incubated overnight at 35 C. Typical colonies were then transferred to Triple Sugar Iron agar slants (TSI), Lysine Iron Agar slants (LIA), and Trypticase Soy Agar with 5% sheep blood (SBA) and incubated overnight at 35°C. Isolates were then confirmed by matrix assisted laser desorption/ionization (MALDI) biotyping.

Salmonella Quantification

For *Salmonella* quantification, FSIS MLG Chapter 4, "Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, Siluriformes (Fish) Products and Carcass and Environmental Sponges," was again followed (FSIS, 2023b). Briefly, samples that screened positive for *Salmonella* presence were enumerated using the commercially available and AOAC 061801 PTM approved *bioMérieux* GENE-UP QUANT *Salmonella* test kit (QUANT).

Salmonella Quantification comparing qPCR and Most Probable Number (MPN)

Salmonella quantification result discrepancies between this study and previous baselines highlighted the need to examine method variability. For this variability study of the QUANT method, FSIS inoculated three sets of 20 samples at 5 CFU/mL, 10 CFU/mL and 50 CFU/mL respectively, one set at each of the three FSIS field service laboratories, for *n*=60 samples at each dilution. These inoculum standards were quantified using 3M Aerobic Plate Count Petrifilm™. Each of the inoculated samples were then quantified using the previously described *Salmonella* quantification method. Subsequently, FSIS

inoculated another three sets of 20 samples at 5 CFU/mL, 10 CFU/mL and 50 CFU/mL respectively, one set at each of the three FSIS field service laboratories, for *n*=60 and MPN analysis. MPN analysis was conducted as described in FSIS MLG Appendix 2.05, "Most Probable Number Procedure and Tables" (FSIS, 2023c). The method described is a three tube method previously used at FSIS. Additional tubes per dilution result in great precision of MPN estimates.

Serotype Identification

FSIS MLG Chapter 42, "Whole Genome Sequencing of Bacterial Isolates," was followed to identify the serotype of all *Salmonella* isolates (FSIS, 2020). Briefly, genomic DNA was isolated from each isolate and DNA libraries prepared. Library preparation consisted of tagmentation, indexing, size-selection of processed DNA, and pooling. Libraries were sequenced with Illumina MiSeq™, and the generated data was analyzed using SeqSero2 (Zhang et al., 2019) to determine *Salmonella* serotypes.

Indicator Testing

Section 3.12 of FSIS MLG Chapter 3, "Quantitative Analysis of Bacteria in Foods as Sanitary Indicators," was followed to quantify the overall levels of mesophilic aerobic bacteria (AC) and EB present in each sample (FSIS, 2015). Briefly, bacterial counts were generated using the commercially available *bioMérieux* TEMPO® automated system. For data analysis, values below the limit of detection (10 CFU/mL) were substituted with 5 CFU/mL.

Results and Discussion

Salmonella Incidence and Quantification

The overall *Salmonella* percent positive at the rehang production step was 62.6% (2,916/4,660). The overall *Salmonella* percent positive at the post-chill production step was 5.0% (233/4,660) (Figure 1). While the post-chill rate is consistent with the 2008 FSIS baseline study of young chicken carcasses, the

rehang rate was found to be higher (62.6% compared to 40.7%) (FSIS, 2008). The observed decrease in percent positive between processing was found to be highly significant (T-test, $p < .0001$). This 12.5-fold reduction is in line with the previously reported efficacy of microbial interventions and best practices (Stopforth et al., 2007; Thames et al., 2022). These statistics are descriptors of FSIS sampling, and not of true *Salmonella* rates across the industry, which must consider establishment volume. FSIS' 2023 Quantitative Microbial Risk Assessment for Salmonella in Raw Chicken and Raw Chicken Products compares industry wide *Salmonella* rates (e.g., prevalence) from this study and the 2008 FSIS baseline study.

FSIS implemented a newly available high-throughput commercial method for *Salmonella* quantification (FSIS, 2022a) in order to quantify the concentration of *Salmonella* present in positive samples. This method became available midway through the study and was performed on 1,464 and 121 of the total *Salmonella* positive rehang and post-chill samples, respectively. As such, quantification statistics pertain only to a portion of the study sample size. Only 18.4% (269/1464) of rehang samples and 15.7% (19/121) of post-chill positive samples were above the LOQ of 10 CFU/mL, with median values of 58 CFU/mL and 27 CFU/mL respectively (Figure 2). The remaining samples were below the LOQ indicating that most *Salmonella* positives fall between 0 and 10 CFU/mL. While significant differences in *Salmonella* occurrence were seen between the two processing steps, only minor differences were seen in overall *Salmonella* levels, with rehang being generally higher than post-chills.

In this study *Salmonella* was found at low levels when present. These levels were generally higher than those observed in the 2008 young chicken baseline study where MPN methodology was utilized for *Salmonella* enumeration. In this earlier study, only 5.5% of rehang samples were above 10 CFU/mL in comparison to 18.4% in the current study. The 2008 baseline post-chill samples were also lower, with only 1.3% of samples above 10 CFU/mL, and those 5 samples were all 11 CFU/mL.

After conclusion of the paired sample study, FSIS expanded *Salmonella* quantification to chicken parts (January 2023), comminuted chicken (March 2023) and comminuted turkey (April 2023) testing in addition to young chicken carcasses (FSIS, 2023a) (Table 1.) This data includes chicken carcass samples collected after the conclusion of the paired study. Again, most *Salmonella* positives were enumerated at levels lower than 10 CFU/mL. Interestingly, similar to young chicken carcasses, quantified *Salmonella* levels in chicken parts were generally higher than the previous 2012 FSIS raw chicken parts baseline survey with 10.8% over the LOQ compared to 5.8% above 3 CFU/ml found in the baseline (FSIS, 2012). For samples above 10 CFU/mL, the median values were 33, 44, 27, and 20 CFU/ml for carcass rinsates, parts rinsates, comminuted chicken, and comminuted turkey respectively.

Since both baseline studies (FSIS 2008; FSIS 2012) were conducted with the MPN quantification method, FSIS conducted a variability study in a model system to determine if the results observed were due to changes in average *Salmonella* levels over time or whether the changes are simply an artifact of method variability. Due to the higher values seen here compared to previous baselines, an inoculum level of 50 CFU/ml was included in addition to below the LOQ (5 CFU/ml) and at the LOQ (10 CFU/ml) of the new method. The results of this small-scale study can be seen in Figure 3. Using Petrifilm™ counts to quantify standards, the new *Salmonella* quantification method showed high variability at 5 CFU/mL and significantly overestimated the actual *Salmonella* level (12.3 ± 16.0 CFU/mL). In contrast, MPN showed a fair amount of variability at 5 CFU/mL and slightly underestimated the actual level (4.3 ± 3.3 CFU/mL). At 10 CFU/mL, both methods overestimated, with 17.3 ± 24.0 CFU/mL with QUANT and 12.8 ± 14.4 CFU/mL with MPN. At the higher end of 50 CFU/mL, QUANT underestimated with 29.2 ± 23.6 CFU/mL and MPN showed a large degree of deviation at 57.2 ± 64.1 CFU/mL.

Table 2 shows the accuracy of both methodsrelative to 10 CFU/mL (the LOQ for QUANT). When applied to levels below the LOQ, the QUANT method correctly classified samples as <10 CFU/mL 72% of the time, while the MPN did so 100% of the time. For levels at the LOQ, both methods behaved similarly,

with QUANT and MPN detecting only 33% and 42% as ≥10 CFU/mL respectively. At counts above the LOQ, both methods were equal, identifying 75% as ≥10 CFU/mL. While the MPN method outperformed the QUANT method, it is not a suitable means for running the high-throughput volume of poultry samples received each day at FSIS laboratories. The MPN method requires sixteen individual subsamples as opposed to the QUANT only requiring one. FSIS will continue to collaborate with the research and test kit manufacturers to evaluate new methods of *Salmonella* quantification, as they become available, that provide both accuracy and fitness for a high-throughput laboratory environment.

Salmonella Serotypes

The top serotypes among rehang and post-chill samples are listed in Table 3. Kentucky, Infantis, Typhimurium, Enteritidis, and Schwarzengrund are the top five serotypes in both sample sources and combined account for 92.9% of all *Salmonella* from rehang and 93.5% from post-chill. *Salmonella* serotypes including Enteritidis, Typhimurium and Infantis are among the top six serotypes found in laboratory-diagnosed infections according to CDC's FoodNet Fast Pathogen Surveillance Tool, 2022 and account for a combined 33% of *Salmonella* illnesses. These serotypes represent 45.9% and 37.3% of all *Salmonella* from rehang and post-chill samples respectively. While overall *Salmonella* levels are reduced from rehang to post-chill samples, serotype distribution varies. Of the top serotypes found in laboratory-diagnosed infections mentioned above, only Infantis decreased significantly (32.6% to 16.7%) when looking at the overall distribution between rehang and post-chill isolates. Enteritidis and Typhimurium both increased (4.4% to 9.4% and 8.9% to 11.2% respectively, Table 3); however, only significantly for Enteritidis (Table 3a). It is worth noting the minimal occurrence of *Salmonella* serotype I 4,5,12:I:- which is also among the top serotypes attributed to human illness, was identified in only 0.4% of rehang samples and none from post-chill in the time frame of this study. On average, from 2016 through the present, I 4,[5],12:I:- makes up 1% of *Salmonella* positive chicken carcass post-chill samples and is the

seventh most frequently occurring serotype in FSIS PR/HACCP chicken carcass sampling. This serotype is antigenically similar to *Salmonella* Typhimurium but lacking second-phase flagellar antigens.

For paired samples that were *Salmonella* positive in both rehang and post-chill samples, 62.5% had a matching serotype while 37.5% had a differing serotype. The most frequently identified serotypes among paired samples where the same serotype was identified, were Kentucky, Infantis and Typhimurium (Figure 4). While serotype distributions remain similar among the two processing steps, the presence of non-matching serotypes recovered indicates multiple serotypes are likely present in flocks, and potentially in samples.

Indicator Organism Detection and Relevance

The presence of sanitary indicator organisms on animal carcasses is commonly used to evaluate overall operational hygiene and good manufacturing practices (Mataragas et al., 2012). They are often used as measures of statistical process control (SPC) by examining bacterial load pre- and post-intervention. Published research provides conflicting reports on the correlation of these indicators and the presence of *Salmonella* in meat and poultry production (Bueno Lopez et al., 2022; Cason et al., 1997; Matias et al., 2010; Moura-Alves et al., 2022; NACMCF, 2023). Additionally, there is no indicator currently recognized as an industry standard. The two most commonly used are aerobic count, which measures all mesophilic bacteria present, and Enterobacteriaceae, specific to those enteric organisms that would represent contamination during evisceration.

AC and EB indicator levels were examined in each sample pair of the study. At the rehang step, quantifiable values were obtained from 99% of samples for AC and 97% for EB. At post-chill, however, AC counts were quantifiable in 70% of samples while EB was only quantifiable in 16% (Table 4). This drop in recovery of EB was not correlated to *Salmonella*. AC obtained as CFU/mL values were log-transformed and compared between processing steps, showing an average value of 4.4 at rehang and 1.4 at post-chill

(Figure 5). This 1,000-fold drop in bacterial load was consistent with the earlier observation of effective interventions. These results suggest that EB is not suitable for SPC. A process can be considered "under control" when the obtained data falls within an expected statistical range and the variability can be measured (Montgomery, 2013). Since only 16% of post-chill samples provide quantifiable EB data, the true effectiveness of the process would be unknown. The vast majority of post-chill samples do provide quantifiable AC counts, presenting an appropriate tool for SPC.

To determine if AC indicator data could also be used as a marker for *Salmonella*, AC values were compared in samples negative or positive for *Salmonella* presence (Figure 6, top panel). The average log-transformed AC value was 2.4 for *Salmonella* positives and 1.5 for negatives. While the average for negatives was equal to the total population, positives separate out with almost an order of magnitude higher values, indicating a tendency for higher AC values in samples that contain *Salmonella*. This apparent correlation can likely be explained by HACCP system effectiveness. If HACCP programs and the associated intervention strategies are working properly, then post-chill samples should have low indicator counts and, consequently, lower *Salmonella* levels as well. The correlation between the two should be directly related to these strategies. Often, lower volume establishments have fewer resources for intervention programs than higher volume ones, and the data was stratified based on production levels (Figure 6, bottom panel). High production establishments (defined as 10,000,000 birds/year or higher) had an average log-transformed value of 2.0. for *Salmonella* positive, while low production establishments had a higher value of at 2.8. This indicates that *Salmonella* positive samples at higher volume establishments have somewhat higher AC levels than *Salmonella* negative samples, but the nearly 100-fold difference between the average AC values in *Salmonella* positive samples at low volume establishments and average AC values in total negatives is driving the overall correlation. This could be due to less effective process controls and most likely represents that high AC values are indicative of poor HACCP system effectiveness, making the contamination of *Salmonella* more likely.

Conclusion

Examination of poultry products at two points during production is crucial to understanding the underlying effectiveness of well-implemented HACCP programs and intervention strategies. By examining these products pre- and post-intervention, this study has shown that overall, the poultry processing interventions were effective at reducing the presence of *Salmonella* and indicator organism levels when implemented properly. This is consistent with the previously mentioned 50% drop in *Salmonella* entering the U.S. food supply since 2012. Bacterial levels were reduced 1,000-fold, and the presence of *Salmonella* dropped significantly between the rehang and post-chill processing steps. Interestingly, *Salmonella* was present at low levels in both steps, suggesting that even when positive, low levels will typically be found. Serotype data suggest that multiple serotypes may be present in positive samples, in agreement with recent research in serotype diversity (Rasamsetti & Shariat, 2023). A metagenomic approach may be needed to obtain and identify multiple isolates from an individual sample. Identification of appropriate indicator organisms remains difficult, as there is still not clear evidence linking aerobic count values to *Salmonella* detection. Despite this, AC was identified as the better tool for statistical process control than Enterobacteriaceae and can be used to easily estimate overall HACCP plan effectiveness, which can impact whether *Salmonella* will be present in finished products. Data from this study will be used in risk assessments and statistical models to evaluate risk management strategies and monitor process control with the goal of reducing foodborne salmonellosis attributed to poultry consumption.

Figures and Tables

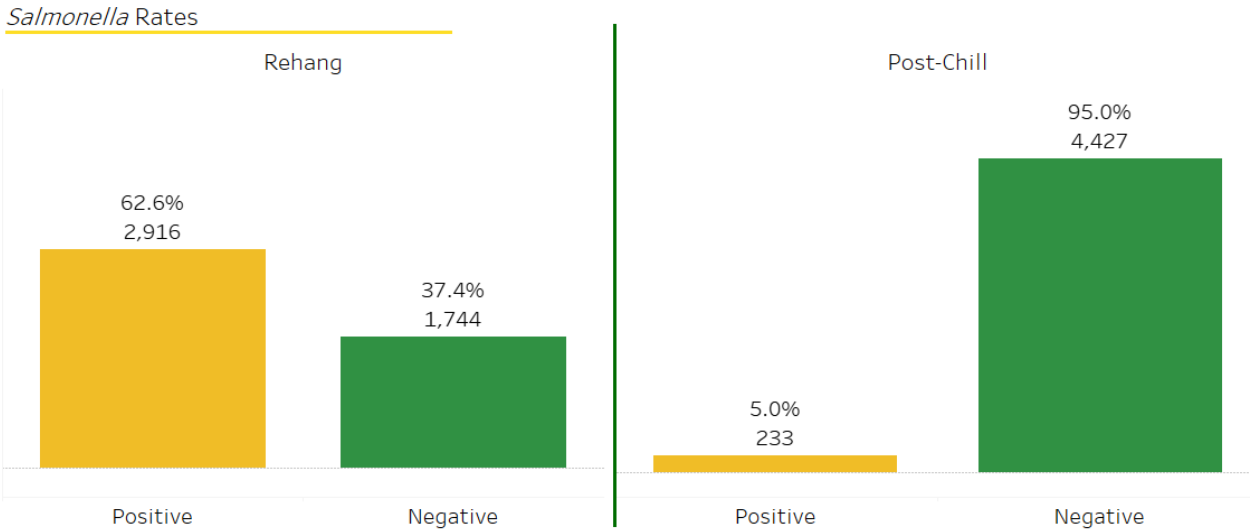


Figure 1. *Salmonella* percent positive rates from the paired, two-point exploratory study. A total of 4,660 sample sets were collected from paired rehang and post-chill production steps at FSIS-inspected poultry processing establishments. The percent positive rate of *Salmonella* in rehang samples was 62.6% with only 5.0% positive in post-chill.

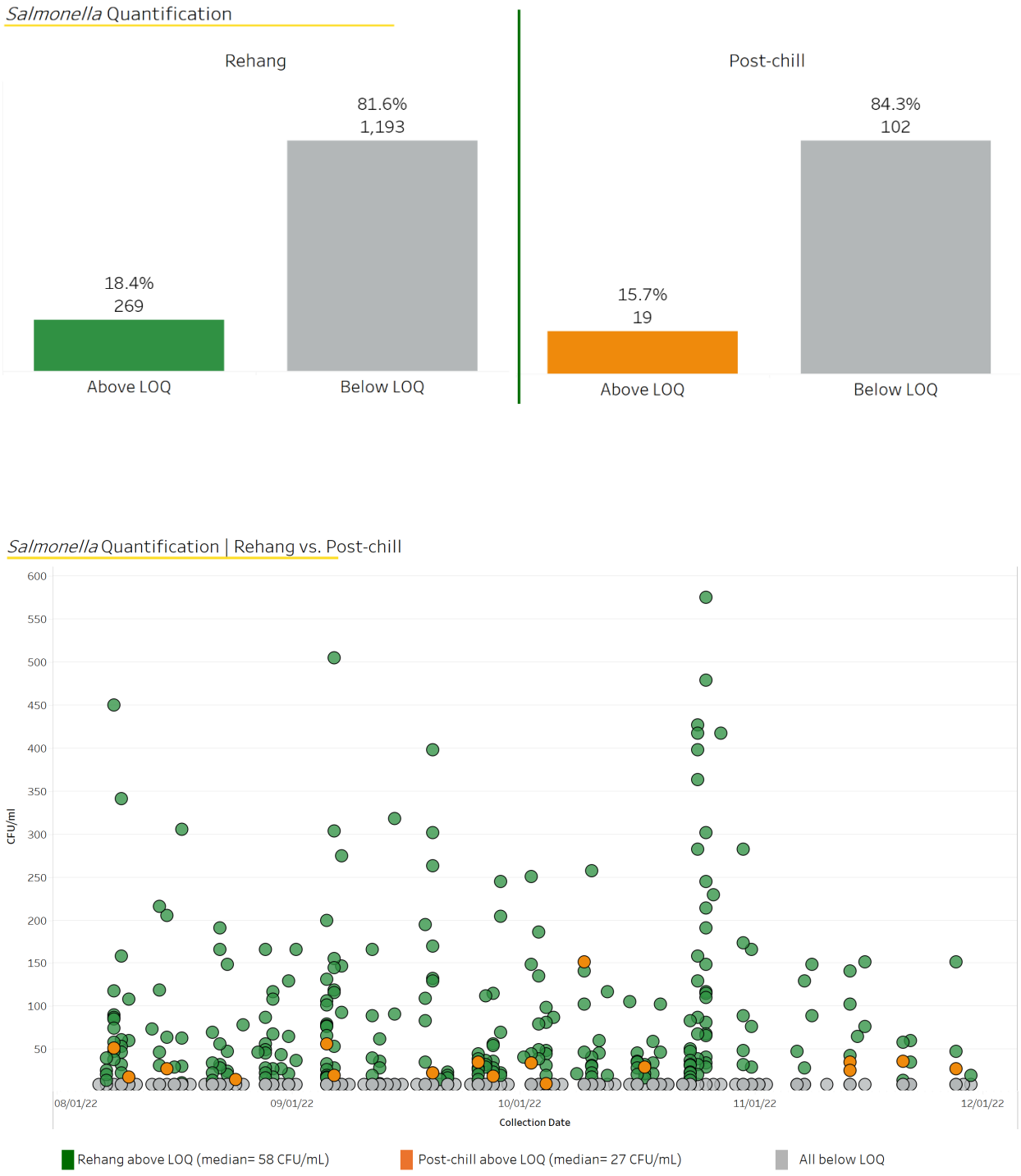


Figure 2. Distribution of *Salmonella* from the paired, two-point exploratory study. A total of 2,933 sample sets were collected from paired rehang and post-chill production steps at FSIS-inspected poultry processing establishments, and samples positive for

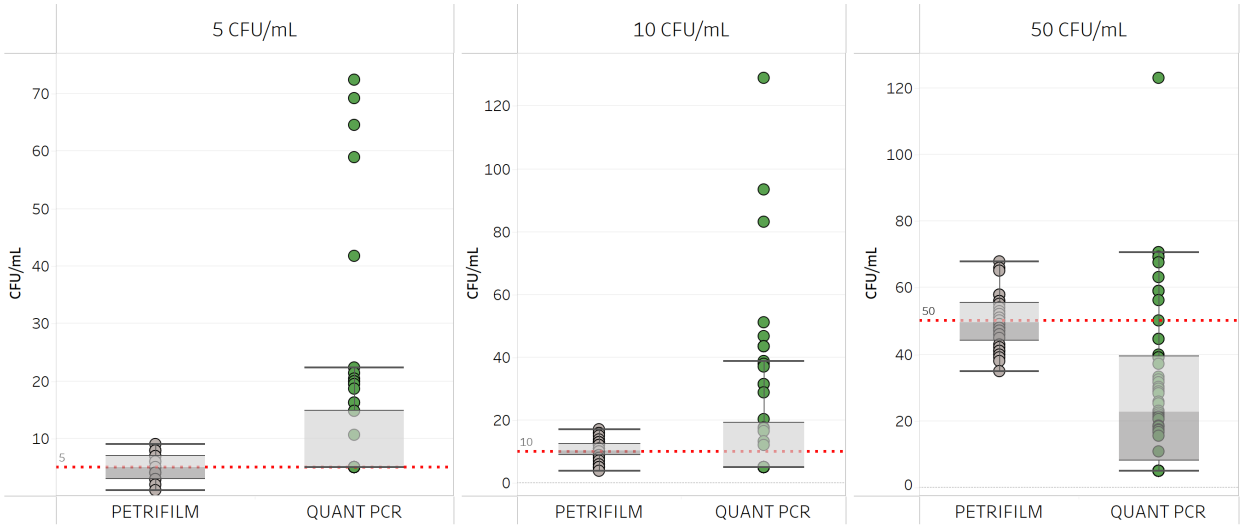
Salmonella were enumerated. Top panel: Over 80% of all *Salmonella* positives fell below the LOQ, with 18.4% having detectable values in rehang samples and 15.7% in post-chills. Bottom panel: Selected value distribution of quantification results. In order to better represent values graphically, eight rehang samples (0.05% of rehang samples) with CFU/mL values over 600 were excluded. For *Salmonella* positives above the LOQ, median values were 58 CFU/mL for rehangs and 27 CFU/mL for post-chills.

Salmonella Quantification | Other Projects

	Chicken Carcass Rinse		Chicken Parts Rinse		Chicken Comminuted		Turkey Comminuted	
	No.	%	No.	%	No.	%	No.	%
	Median: 33 CFU/mL		Median: 44 CFU/mL		Median: 27 CFU/mL		Median: 20 CFU/mL	
Above LOQ	77	15.7%	65	10.8%	13	5.8%	3	4.5%
Below LOQ	415	84.3%	537	89.2%	210	94.2%	63	95.5%
Grand Total	492	100.0%	602	100.0%	223	100.0%	66	100.0%

Table 1. Snapshot of FSIS *Salmonella* quantification data across multiple poultry products between August 2022 and August 2023. *Salmonella* levels above 10 CFU/mL are uncommon. Carcass data shown here does not include non-regulatory rehang samples collected as part of the exploratory project. Median values for samples over 10 CFU/ml are shown. Note *Salmonella* quantification of each commodity began on different dates, as noted in text.

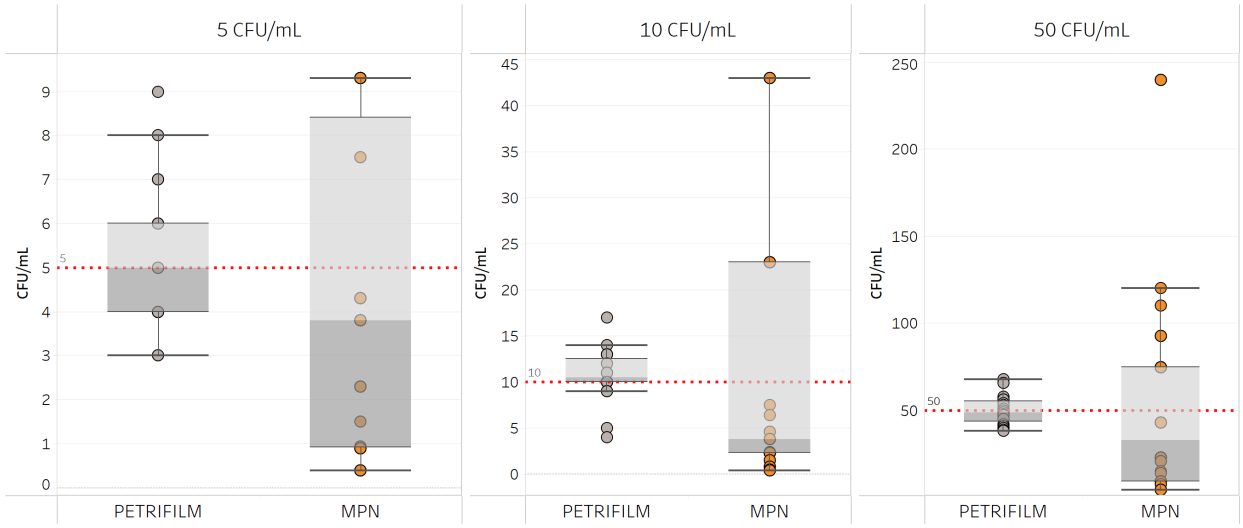
Salmonella Quantification Method Variability | Petrifilm and Quantification PCR



Summary

	5 CFU/mL		10 CFU/mL		50 CFU/mL	
	PETRIFILM	QUANT PCR	PETRIFILM	QUANT PCR	PETRIFILM	QUANT PCR
Avg. CFU/mL	5.1	12.3	10.3	17.3	49.6	29.2
Std dev. of CFU/mL	2.2	16.0	3.3	24.0	7.2	23.6

Salmonella Quantification Method Variability | Petrifilm and MPN



Summary

	5 CFU/mL		10 CFU/mL		50 CFU/mL	
	PETRIFILM	MPN	PETRIFILM	MPN	PETRIFILM	MPN
Avg. CFU/mL	5.3	4.3	10.7	12.8	49.9	57.2
Std dev. of CFU/mL	1.6	3.3	2.8	14.4	8.0	64.1

Figure 3. (Top Panel) Box plots and average values of CFU for variability study of the current qPCR-based FSIS *Salmonella* quantification method (QUANT). Sixty samples were analyzed at each inoculum level (5 CFU/mL, 10 CFU/mL and 50 CFU/mL). QUANT showed degrees of variability compared to each standard quantified by Petrifilm™, overcounting at 5 and 10 CFU/mL and undercounting at 50 CFU/mL. For QUANT, samples with reported values below the detection limit of 10 CFU/mL were substituted with a value of 5 CFU/mL for analysis. (Bottom Panel) Distribution plots and average values of CFU for variability study of MPN. MPN showed greater deviation at the higher target of 50 CFU/mL, but less variability at 5 and 10 CFU/mL. Each y-axis range was optimized for data visualization. Excluding outliers, the box plot shows the middle 50% of values, while the upper and lower whiskers represent scores outside of the middle 50% (i.e., the lower 25% of scores and the upper 25% of scores). The lowest value is represented at the end of the bottom whisker while the highest value is represented at the top of the upper whisker.

QUANT Method				MPN Method			
Actual Salmonella level (CFU/mL)	Counts Below 10 CFU/mL	Counts Above 10 CFU/mL	Method Accuracy	Actual Salmonella level (CFU/mL)	Counts Below 10 CFU/mL	Counts Above 10 CFU/mL	Method Accuracy
5	43/60	17/60	72%	5	60/60	0/60	100%
10	40/60	20/60	33%	10	35/60	25/60	42%
50	15/60	45/60	75%	50	15/60	45/60	75%

Table 2 Accuracy of QUANT and MPN relative to 10 CFU/mL when applied to different Salmonella levels

Top Serotypes

	% Rehang	n Rehang		% Post-chill	n Post-chill
Kentucky	41.1%	1,199	Kentucky	54.1%	126
Infantis [1]	32.6%	952	Infantis [1]	16.7%	39
Typhimurium [1]	8.9%	259	Typhimurium [1]	11.2%	26
Schwarzengrund	5.9%	171	Enteritidis [1]	9.4%	22
Enteritidis [1]	4.4%	127	Schwarzengrund	2.1%	5
Alachua	1.2%	34	Anatum	0.9%	2
Thompson	0.7%	20	Blockley	0.9%	2
Others (Rehang)	5.3%	154	Others (Post-chill)	4.7%	11
Grand Total	100.0%	2,916	Grand Total	100.0%	233

Table 3. Top serotypes identified in rehang and post-chill *Salmonella* positive samples. [1]CDC top serotypes found in laboratory-diagnosed infections.

Serotype	Incidence rate difference between rehang and post-chill	95% Confidence Interval	P-value
Enteritidis	-0.05	[-0.08, -0.02]	0.0006
Typhimurium	-0.02	[-0.06, 0.02]	0.2713
Infantis	0.16	[0.08, 0.23]	< 0.0001

Table 3a. Incidence rate analysis of *Salmonella* serotypes of public health concern between rehang and post-chill samples.

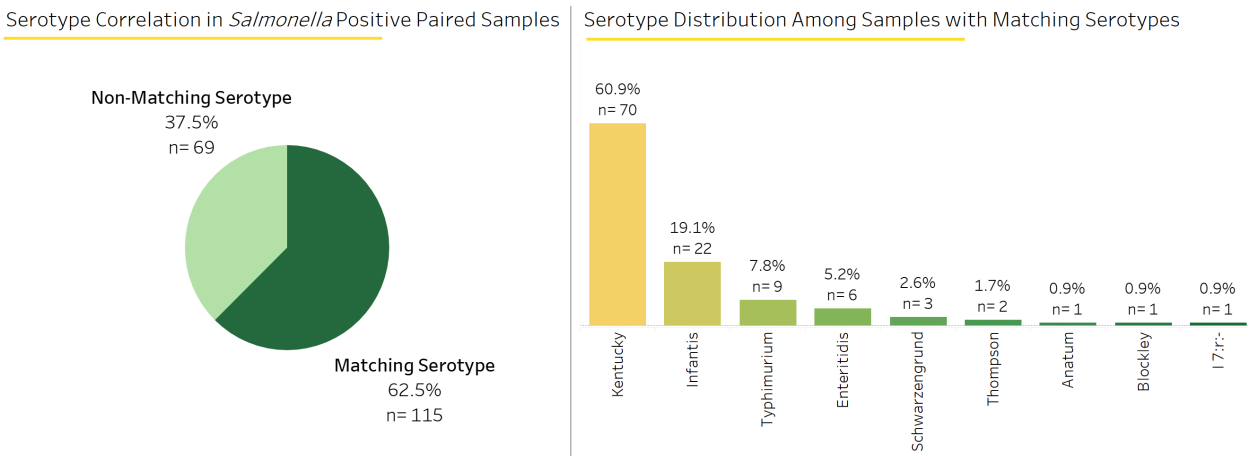


Figure 4. Left panel: Serotype correlation in *Salmonella* positive paired samples (where both the rehang and the post-chill sample from the same sampling event were positive for *Salmonella*). Right panel: Serotype distribution among paired samples with matching serotypes.

Indicator	Step	Detectable		Non-Detectable	
		Count	%	Count	%
AC[1]	Rehang	4,598	99.8%	8	0.2%
	Post-chill	3,222	70.0%	1,384	30.0%
EB[2]	Rehang	4,539	97.4%	57	1.2%
	Post-chill	751	16.1%	3,840	82.4%

Table 4. Indicator results from the paired, two-point exploratory study. AC = Aerobic Count, EB = Enterobacteriaceae

[1] A total of 54 rehang samples and 54 post-chill samples were not analyzed for aerobic count.

[2] A total of 62 rehang samples and 69 post-chill samples were not analyzed for Enterobacteriaceae.

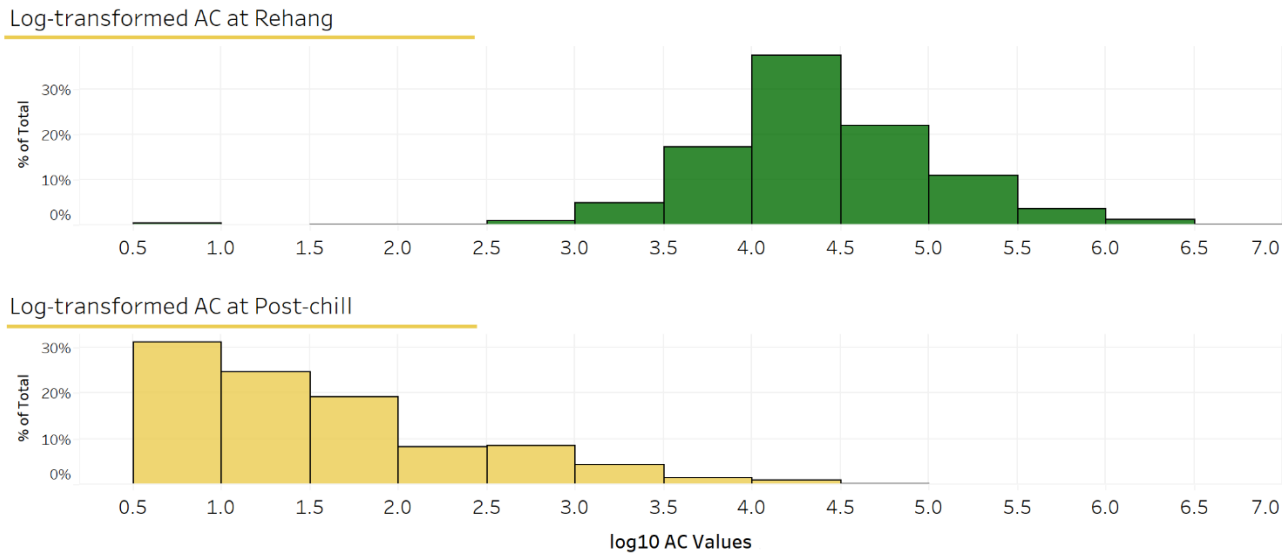


Figure 5. Log-transformed AC indicator data from the paired, two-point exploratory study. The average value was 4.4 at rehang and 1.4 at post-chill, indicating an average log reduction of 2.9. This means that on average 1 out of 1000 aerobic bacterium survive between processing steps. Values below the detection limit (10 CFU/mL) were replaced with 5 CFU/mL for data analysis.

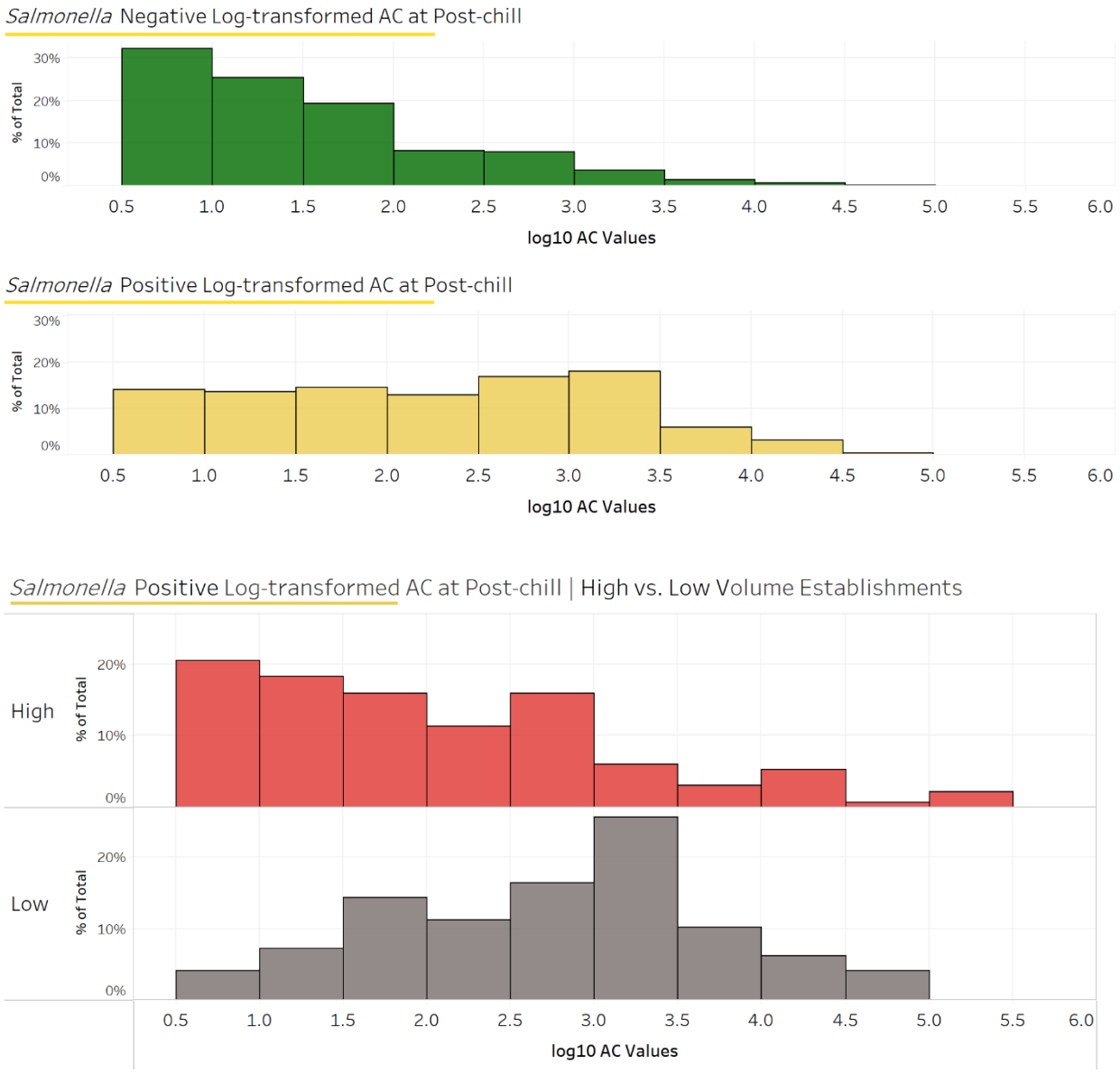


Figure 6. AC data as an indicator for *Salmonella* in post-chill samples. Top Panel: Comparison of log-transformed AC values in post-chill samples between *Salmonella* negative and *Salmonella* positive samples. Mean values are 1.5 and 2.4 respectively, indicating a 0.9 log difference separating *Salmonella* incidence (*P* <0.001). Bottom Panel: Comparison of log-transformed AC values in *Salmonella* positive samples between high and low volume producing establishments. Higher values at low volume establishments suggest correlation may be related to HACCP

effectiveness. Values below the detection limit (10 CFU/mL) were replaced with 5 CFU/mL for data analysis.

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