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Food Science and Technology Department

B. S Food Science and Technology

Special Graduation Project

Comparison of Three Chicken Sampling Methodologies: Assessing Microbial Indicators and *Salmonella spp.* Prevalence

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Abstract

This study evaluated the performance of three different sampling methodologies: MicroTally® Mitt, MicroTally® Swab, and Composite Rinse in detecting microbial indicators (Mesophilic Aerobes and Enterobacteriaceae) and pathogen loads and prevalence (*Salmonella*) in chicken tender samples from a poultry processing operation. Four chicken tender pallets were sampled, each pallet divided into fourteen-pound bags and divided into two equal parts. One part was analyzed using a composite rinse methodology, the other using MicroTally® Mitt, and the other part using MicroTally® Swab methods. Samples were analyzed for microbial indicators using the Tempo® System and for *Salmonella* enumeration and prevalence using GeneUp® Quant *Salmonella* and GeneUp® *Salmonella* detection protocols. Data analysis was conducted using Statistical Analysis System (SAS), including ANOVA with Duncan for mean separation, and Chi-square for *Salmonella* prevalence. Results indicated no significant differences in Mesophilic aerobes Count (AC) and Enterobacteriaceae (EB) counts between the sampling methods. *Salmonella* prevalence was slightly higher in the MicroTally Mitt (50%) compared to the Composite Rinse (25%) and in MicroTally Swab (38%) compared to Composite Rinse (25%), but these differences were not statistically significant. Overall, the MicroTally Mitt and MicroTally Swab methods demonstrated comparable performance to the Composite Rinse method for detecting microbial indicators and pathogens in chicken samples, also, MicroTally Mitt and MicroTally allow testing more tenders in less time, which can be particularly beneficial in poultry processing facilities.

Keywords: Poultry Parts, Sampling methods, *Salmonella* detection, Total Mesophilic Aerobes Count.

Resumen

En este estudio, se evaluó el rendimiento de tres diferentes métodos de muestreo: MicroTally® Mitt, MicroTally® Swab y Composite Rinse, en la detección de indicadores microbianos (recuentos totales y Enterobacteriaceae) y cargas junto con prevalencia de patógenos (*Salmonella*) en muestra de tenders de pollo de una planta de procesamiento de aves de corral. Se tomaron muestras de cuatro lotes de tenders de pollo, dividiendo cada una de las cuatro bolsas de 10 libra por lote en dos partes. Una parte se analizó utilizando la metodología de Composite Rinse, para la otra se utilizó los métodos de MicroTally® Mitt y en otro se utilizó MicroTally® Swab. Las muestras se analizaron para detectar indicadores microbianos mediante el método de enumeración Tempo y para la enumeración y prevalencia de *Salmonella* y GeneUp Quant *Salmonella* y GeneUp *Salmonella*. El análisis de datos se realizó haciendo uso del programa Statistical Analysis System (SAS), donde se realizó un ANDEVA y una separación de medias Duncan, un análisis de Chi-cuadrado para la prevalencia de *Salmonella*. Los resultados no indicaron diferencias significativas en los recuentos de Mesófilos Aerobios (AC) y Enterobacteriaceae (EB) entre los métodos. La prevalencia de *Salmonella* fue ligeramente superior en el MicroTally Mitt (50%) en comparación con el Composite Rinse (25%) y en MicroTally Swab (38%) en comparación con el Composite Rinse (25%), pero estas diferencias no fueron estadísticamente significativas. En general, los métodos MicroTally Mitt y MicroTally Swab demostraron un rendimiento comparable al método Composite Rinse para la detección de indicadores microbianos y patógenos en muestras de pollo, además, MicroTally Mitt y MicroTally Swab permiten analizar más muestras en menos tiempo, lo que puede ser particularmente beneficioso en las instalaciones de procesamiento de aves.

Palabras clave: Poultry Parts, Sampling methods, *Salmonella* detection, Total Mesophilic Aerobes Count.

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Introduction

Poultry products play a vital role in global diets due to their high-quality protein content, essential nutrient provision, and lower fat content compared to products from other animal sources. They are also economically accessible and deeply embedded in culinary traditions worldwide. In 2022, the most consumed type of meat in the United States was chicken, with an average consumption of 98.9 pounds per capita, and is expected to keep increasing (Shahbandeh M., 2024)

The poultry market has demonstrated consistent growth, with projections indicating that poultry meat will represent 41% of all protein derived from meat sources in 2030. In the year 2020, the total poultry meat production escalated to 134 million tons, which was an increase of 1.2% from 2019 (Food and Agriculture Organization [FAO], 2021). This steady growth underscores the integral role of poultry within the food security framework of many countries and its impact on sustainable agricultural practices.

Given this context, the critical importance of microbial safety in poultry production can not be overstated, particularly pathogens such as *Salmonella* are recognized as the primary etiological agent associated with salmonellosis for humans. The major sources of *Salmonella* infections in humans are contaminated poultry eggs and meat products (Shaji et al., 2023) infecting the gastrointestinal tract and causing diarrhea, fever, nausea, and cramps.

Salmonella bacteria is a Gram-negative, motile, hydrogen sulfide-producing, acid-labile facultative intracellular microorganism that commonly causes gastroenteritis worldwide and causes cross-infection between humans and animals. Many animals are known carriers of the *Salmonella* bacterium (Ajmera & Shabbir, 2023).

The *Salmonella* family includes over 2,300 serotypes of bacteria which are one-celled organisms. Two serotypes, *Salmonella enterica* sv. Enteritidis and *Salmonella enterica* sv. Typhimurium are the most common in the United States and accounts for half of all human infections, belonging to the non-typhoidal *Salmonella* group (NTS), Non-typhoidal *Salmonella* (NTS) serovars are

among the most frequently reported causes of gastroenteritis across the globe, in developing countries. It has been estimated that almost 94 million cases of gastroenteritis due to NTS occur annually, culminating in 155,000 deaths per year (Fardsanei et al., 2021).

Salmonella outbreaks linked to poultry products not only pose direct health risks but also have substantial economic repercussions. These outbreaks can lead to consumer mistrust, adversely affecting poultry producers and retailers. The poultry industry must invest in preventive measures, including effective microbial detection and control strategies to mitigate the risk.

CDC estimates that *Salmonella* causes more foodborne illnesses than any other bacteria. Chicken is a major source of these illnesses. In fact, about 1 in every 25 packages of chicken at the grocery store are contaminated with *Salmonella* (Center For Disease Control and Prevention [CDC], 2024). That is why is important to highlight the need for increased consumer awareness about the risk of *Salmonella* in chicken, educating the public to have more mindful handling and cooking practices, and reducing foodborne illness.

From May 2018 to February 2019, a significant outbreak of *Salmonella infantis* was traced to raw chicken products, leading to 129 reported illnesses and one death in New York. This outbreak, investigated by the Center for Disease Control and Prevention (CDC), and Food Safety Inspection Service (FSIS), underscores the critical need for stringent monitoring and safety measures in poultry processing (United States Department of Agriculture [USDA], 2024). The widespread *Salmonella* contamination underscores the need for the poultry industry and regulators to reevaluate and strengthen safety protocols to prevent future outbreaks.

Food is the leading source of *Salmonella* infections and poultry products are one of the leading sources of foodborne *Salmonella* illnesses (USDA, 2024). Ensuring the microbial safety of poultry products involves a comprehensive approach that includes monitoring and controlling all pathogens at various points in the production process. This includes farm interventions, preventative actions,

such as carcass washing and chilling, and finally, educating consumers about proper handling and cooking practices.

To combat these challenges there has been significant progress in the development of pathogen detection technologies. Food safety organizations are constantly researching to find the best way to detect pathogens such as *Salmonella* and reduce the likelihood of an outbreak, a clear example is the United States Department of Agriculture (USDA), which uses a sampling method for detecting *Salmonella* in chicken meat are the Composite Rinse, which consists of select a production line, pour 400 ml of Buffered Peptone Water into a sterile bag containing chicken part and shake, then pour the shaken BPW rinse into the sterile container, then cool samples and keep sample temperature approximately 4 °C, and then analyze it (USDA, 2012)

There are other sampling methods already investigated for the detection of pathogens in meat, such as the manual implementation of the MicroTally® Swab (MT-Swab) to scrub the surface of raw beef manufacturing trimmings for pathogen detection. As well as Microtally® Mitt (MT-Mitt), which provides a more user-friendly option for sample collection than the MT-Swab (Arthur et al., 2024).

Continued research and development in microbial detection and control strategies are crucial for reducing risks associated with poultry products. As the industry expands, is essential for regulatory agencies and poultry producers to work together to strengthen biosecurity protocols, and monitoring systems throughout the poultry production process and increase consumer education on safety practices. That is why, the objectives of this study were to determine the performance of three sampling methodologies (Composite Rinse, MicroTally® Mitt, and MicroTally® Swab) on microbial indicators (Mesophilic Aerobes and Enterobacteriaceae) and to determine the prevalence of three sampling methodologies (Composite Rinse, MicroTally® Mitt, and MicroTally® Swab) on microbial pathogens (*Salmonella spp*.)

Materials and Methods

Study Location

Chicken Tender Samples

Located in Lubbock Texas, the research study was carried out at the facilities of Texas Tech University's Department of Animal and Food Sciences. Microbiological studies were conducted at the Experimental Science Building in the ICFIE laboratories, Chicken tender pallets were collected from a poultry processing plant, in Georgia United States of America, and were stored in the ICFIE Food Safety laboratory.

Materials

A microbiological study was conducted to evaluate four chicken tender pallets. Four bags of chicken tenders were received, each representing one different combo. Although each bag contained twenty tenders, only fifteen were used for analysis. The tenders in each bag were divided into three equal parts: one part was analyzed using a Composite Rinse methodology using five chicken tenders, another using other five chicken tenders for MicroTally® Mitt, and the final part with the MicroTally® Swab method.

Composite Rinse

A pound of chicken tenders (5 tenders) was transferred to a sterile bag mixed with 100 ml of Buffered Peptone Water (BPW). The sample was then massaged for 1 minute to detach the bacterial cells. After this, the liquid solution was transferred to a Whirl Pak Sterile Bag. From the liquid solution, 40 mL of aliquot was transferred to two Falcon Tubes of 50 mL for analysis using GeneUp Quant methodology. An additional 5 mL of aliquot was transferred from the sterile bag to a 10 mL sterile tube for GeneUp Detection Protocol. The tubes were then incubated for 8 to 24 hours at 42 °C.

MicroTally

For MicroTally Mitt, soil towels were rubbed on both sides vigorously over meat, 1 minute per side (towels were pre-moistened with 25 mL of Buffered Peptone Water). After rubbing the towels, they were folded, and returned into bags, ensuring the towel was completely submerged in media. Buffered Peptone Water was added to get to a final volume of 200 mL and mixed. The bags were incubated from 8-24 hours at 42 °C. For MicroTally Cloth followed the same protocol as MicroTally Mitt.

Microbial Analysis

Samples were analyzed for microbial indicators using the Tempo® System, which consists of inoculating a vial of culture medium specific for each microorganism with the addition of the sample being tested. The inoculated medium is transferred into the cards by the TEMPO filler instrument, which is designed to replicate the Most Probable Number (MPN) method with 3 sets of sixteen wells. Once filled, the card is hermetically sealed to prevent contamination. Tempo Cards for Mesophilic Aerobes Counts (AC) and Enterobacteriaceae (EB) were incubated from 18 to 24 hours at 37 °C, and then cards were read using a TEMPO reader to obtain results.

The initial dilution used for tempo is described in Table 1:

AC (Mesophilic Aerobes Count) → (1/4) 3 mL in Vial and 1 mL of sample in vial

EB (Enterobacteria) → (1/40) 3.9 mL in Vial and 100 µL of sample in vial

Table 1

Range of detection for TEMPO

Dilution	Range of detection
1/ 4	1 to 4.9x10^3 CFU/g or mL
1/40	10 to 4.9x10^4 CFU/g or mL

For *Salmonella* enumeration and prevalence were performed using BioMérieux GeneUp® Quant which is a PCR-based molecular diagnostic system to detect *Salmonella* for 0 (Non- Incubation) and 4 hours and GeneUp® *Salmonella* detection protocols to confirm the presence of *Salmonella*.

Experimental Design

A Randomized Blocks Design (RBD) was used evaluating three treatments (MicroTally Mitt, MicroTally Swab, and Composite Rinse) each having four repetitions per treatment with two replicates per repetition, for a total of 24 experimental units. Data analysis was conducted using the Statistical Analysis System (SAS), including an ANOVA with a DUNCAN mean separation to identify differences between methods, and a Chi-square analysis to assess the prevalence of *Salmonella spp*. across sampling methodologies.

Results and Discussion

Researchers have been striving to identify optimal methods for preventing food poisoning and mitigating outbreaks in the food industry. A significant part of this work has focused on sampling techniques. Composite Rinse is the official sampling method approved by the United States Department of Agriculture (USDA). The Food Safety and Inspection Service (FSIS) employed Composite Rinse to conduct additional microbial testing of chicken parts, also the FSIS has conducted additional microbial testing of comminuted chicken samples. Furthermore, the FSIS has expanded microbial testing to include chicken and turkey samples, enumerating positive *Salmonella* samples and performing Mesophilic Aerobes Count indicator testing (USDA, 2023).

To evaluate the hygiene of the entire meat production process, Aerobic Colony Count (ACC) is commonly employed (Belluco et al., 2016). Providing a reliable measure of microbial load and overall cleanliness throughout the production chain.

In that way, in this study, a real industry case was examined. The results (Table 2) demonstrated that Microtally Mitt appears to be the most similar method for prevalence of Mesophilic aerobes compared with the reference method (Composite Rinse), reporting statistical differences in 1 of 4 (25%) combos evaluated (p-value <0.05), while the method Microtally Swab compared with Composite Rinse reports statistical differences in 2 of 4 (50%) evaluated combos, (p-value of <0.05) being Microtally Mitt the method that recovers the higher counts of Mesophilic Aerobic Bacteria when comparing it with Microtally Swab (Table 2). Other study reports Mesophilic

Aerobic counts of 1.75±1.6cfu/g in freshly slaughtered breast meat samples (Sayed et al., 2020). In this study, a slightly higher count was found showing as highest value of 2.86 Log CFU/mL.

However, as an overall view in most of the combos there were no statistical differences (p-value > 0.05) between sampling methods. This indicates that the choice of sampling methods does not significantly affect the detection of Mesophilic aerobes bacteria in the real poultry industry.

Table 2

Mean separation for Mesophilic aerobes across four combos.

Combo	Rinse Mean ± SD Log CFU/mL	Swab Mean ± SD Log CFU/mL	Mitt Mean ± SD Log CFU/mL	CV (%)
1	1.95±0.13[a]	1.70±0.07[a]	2.06±0.03[b]	9.56
2	2.27±0.13[a]	2.21±0.19[a]	2.26±0.12[a]	1.39
3	2.42±0.01[a]	2.15±0.09[b]	2.34±0.03[a]	6.09
4	2.86±0.07[a]	2.19±0.06[b]	2.85±0.02[a]	14.61

Note. Means with different letters [ab] on rows indicate significant differences ($p \leq 0.05$) between sampling methods, C.V (%) Coefficient of Variation, SD Standard Deviation, LogCFU/mL logarithm colony form unit per milliliter.

Elevated counts of Enterobacteriaceae on poultry carcasses have been routinely linked with inadequate or unhygienic processing, inappropriate handling and/or storage conditions (Whyte et al., 2004). This underscores that if proper hygiene and safety protocols are not strictly adhered to during the processing, handling, and storage of chicken products, there is a higher likelihood that chicken tenders could harbor enterobacteria, increasing the risk of contamination. For this reason, monitoring Enterobacteriaceae counts is crucial in a poultry processing line to ensure safety of products such as chicken tenders and minimize contamination risks.

Therefore, the detectable levels of Enterobacteriaceae on chicken fillet or neck skin samples are relatively low, ranging from 1 to 3.18 log10 CFU/g (Projahn et al., 2018). This aligns with the results obtained (Table 3) having as highest value 2.52±0.13 Log CFU/mL, in Composite Rinse. The methods Microtally Mitt and Microtally Swab presented a similar behavior for prevalence of Enterobacteria

compared with the reference method (Composite Rinse), reporting statistical differences in 1 of 4 (25%) combos evaluated ($p < 0.05$). These findings align with previous research, which suggests that different sampling methods often produce comparable results in terms of microbial prevalence. However, some methods may exhibit greater consistency or variability depending on the context and type of microbe (Hannah et al., 2008).

Table 3

Mean separation for Enterobacteriaceae across four combos.

Combo	Rinse Mean±SD Log CFU/mL	Swab Mean±SD Log CFU/mL	Mitt Mean±SD Log CFU/mL	CV (%)
1	1.52±0.31[a]	1.01±0.50[a]	1.20±0.50[a]	20.67
2	1.97±0.25[a]	1.57±0.38[a]	1.84±0.12[a]	11.43
3	2.19±0.06[a]	1.80±0.05[b]	1.91±0.01[b]	10.12
4	2.52±0.13[a]	2.00±0.20[a]	2.35±0.25[a]	11.51

Note. Means with different letters [ab] on rows indicate significant differences ($p \leq 0.05$) between sampling methods, C.V (%) Coefficient of Variation, SD Standard Deviation, LogCFU/mL logarithm colony form unit per milliliter.

Previous studies found that the increase in prevalence was relatively small at 0.6 Log10 CFU/mL of Composite Rinse (Hannah et al., 2008). However, for this study, higher prevalence was obtained in Composite Rinse, ranging from 1.52 to 2.52 Log CFU/mL, with a difference of 1.0 Log CFU/mL. This indicates that while certain sampling methods may offer enhanced prevalence, the overall difference can be minimal in some cases.

In addition to Composite Rinse, alternative sampling methods, such as MicroTally Mitt and MicroTally swab, have recently begun to be studied. These methods have previously been used primarily for sanitation procedures (Sanchez, 2023). These alternatives methodologies offer distinct advantages in detecting microbial presence across various surfaces. In poultry plants, contamination can spread rapidly through contact with equipment and carcasses, implementing improved detection methos can be crucial in reducing bacterial loads.

Prevalence in Composite Rinse, Microtally Mitt, and Microtally Swab

A comparison of the percentage of *Salmonella spp*. prevalence across four combos with the value obtained according to previous studies is shown in Tables 4 and 5. According to previous studies, the prevalence of *Salmonella* post-pick and pre-chill averaged 32% and 34% (Thames et al., 2022). In this study, a higher prevalence is shown in the analysis with an average of *Salmonella spp*. prevalence of 50% (Table 4) where there is a noticeable difference from the average reported in previous studies. However, by using Chi-square analysis with a P value$\geq$ 0.05, there was no statistical difference (p-value 0.4141) between Composite Rinse, and Microtally Mitt (Table 4), and between Composite Rinse, and Microtally Swab the same behavior also occurs (Table 5) where no statistical difference (p-value 0.6547) was observed, indicating that all methodologies are similar.

In this study MicroTally Mitt (Table 4) demonstrated a higher prevalence result of *Salmonella*, with a 50% prevalence, exceeding the 16% rate reported by Fremonta (2024) for the MicroTally Method. Previous research supports these findings, showing that the MicroTally-Mitt consistently yielded numerically higher prevalence results for pathogens (Arthur et al., 2023).

Previous studies have focused on testing Microtally Mitt in beef production, particularly with pathogens such as *E. coli* STEC. These studies have demonstrated the efficacy of Microtally-based sampling in commercial beef processing scenarios in the industry (Arthur & Wheeler, 2020), showing results that meet with the findings of this research. An advantage of Microtally-based sampling methods is that allow testing more chicken tenders, time and labor savings, lowered risk of employee injury, no product loss due to sample collection, and increases finished product surface area sampled (Arthur & Wheeler, 2021).

Table 4

Chi-square analysis for Salmonella prevalence in Mitt compared with Rinse.

Sampling method	Total samples	Positives for Salmonella	Negatives for Salmonella	Prevalence	X^2 P value
Mitt	8	4	4	50%	0.4141
Rinse	8	2	6	25%	

Note. A significance level of 0.05 was used for statistical analysis. P-value less than 0.05 indicates statistically significant differences.

Table 5

Chi-square analysis for Salmonella spp. prevalence in MicroTally Swab and Composite Rinse

Sampling method	Total samples	Positives for Salmonella	Negatives for Salmonella	Prevalence	X^2 P value
Swab	8	3	5	38%	0.6547
Rinse	8	2	6	25%	

Note. A significance level of 0.05 was used for statistical analysis. P-value less than 0.05 indicates statistically significant differences.

Moreover, MicroTally Swab has been demonstrated to be an effective prevalence method for *Salmonella* (Flach et al., 2024). Table 5 shows the prevalence of *Salmonella spp.* (38%), which is slightly higher than the standard method (Composite Rinse) that reached 25%, however, this can be due to microbial distribution on the food matrix.

Overall, for *Salmonella,* there were no statistically significant differences among the sampling methods (p-value > 0.05), as they all showed similar concentrations. However, the MicroTally Mitt method demonstrated a higher and more consistent prevalence percentage (50%). While the Composite Rinse method was effective at capturing *Salmonella*, it exhibited greater variability in prevalence due to the type of samples (25%). This suggests that although all methods are comparably effective in terms of concentration, the MicroTally Mitt offered more accurate results in terms of consistent detection rates.

Conclusions

The study revealed that MicroTally Mitt, MicroTally Swab, and Composite Rinse methods have similar performance for recovering microbial indicators (Mesophilic Aerobes and Enterobacteriaceae) in chicken tender samples from poultry processing operations.

The study showed that MicroTally Mitt, MicroTally Swab, and Composite Rinse methods have similar performance for recovering *Salmonella* in chicken tender samples from poultry processing operations.

MicroTally Mitt and MicroTally Swab are comparable to the rinse technique. However, the MicroTally Mitt demonstrated no statistically significant differences, but it was able to recover a higher number of microbial indicator cells and showed a greater prevalence overall (50%).

Recommendations

Future studies should evaluate the accuracy, precision, and sensitivity of each method under inoculating samples and assessing microbial loads.

It is recommended that a cost analysis of different sampling methods should be conducted. This analysis should consider the cost of materials, labor, and time associated with each method. By comparing these costs against the benefits, such as time savings and accuracy, researchers can identify the most economical method for the food industry, helping to save money and improve efficiency.

Lastly, it is important to test the effectiveness of the sampling methods against a range of pathogens including *Campylobacter* to evaluate the prevalence rates and detection limits of each method.

References

Ajmera, A., & Shabbir, N. (2023). StatPearls: Salmonella. In A. Ajmera & N. Shabbir (Eds.), *StatPearls [Internet].* StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK555892/

Arthur, T. M., Brown, T., & Wheeler, T. L. (2023). Determination of Verification Parameters for Using the Manual Sampling Device for Fresh Raw Beef Trim. *Journal of Food Protection*, *86*(2), 100041. https://doi.org/10.1016/j.jfp.2023.100041

Arthur, T. M., Reno, F. J., & Wheeler, T. L. (2024). Validation of a New Method of Sampling Beef Manufacturing Trimmings for Pathogen Testing Using a Manual Sampling Mitt Approach. *Journal of Food Protection*, *87*(3), 100233. https://doi.org/10.1016/j.jfp.2024.100233

Arthur, T. M., & Wheeler, T. L. (2021). Validation of Additional Approaches and Applications for Using the Continuous and Manual Sampling Devices for Raw Beef Trim. *Journal of Food Protection*, *84*(4), 536–544. https://doi.org/10.4315/JFP-20-345

Belluco, S., Barco, L., Roccato, A., & Ricci, A. (2016). Escherichia coli and E nterobacteriaceae counts on poultry carcasses along the slaughterline: A systematic review and meta-analysis. *Food Control*, *60*, 269–280. https://doi.org/10.1016/j.foodcont.2015.07.033

Center For Disease Control and Prevention. (2024). *Chicken and Food Poisoning.* Center For Disease Control and Prevention. https://www.cdc.gov/food-safety/foods/chicken.html?CDC_AAref_Val=https://www.cdc.gov/foodsafety/chicken.html

Fardsanei, F., Soltan Dallal, M. M., Zahraei Salehi, T., Douraghi, M., Memariani, M., & Memariani, H. (2021). Antimicrobial resistance patterns, virulence gene profiles, and genetic diversity of Salmonella enterica serotype Enteritidis isolated from patients with gastroenteritis in various Iranian cities. *Iranian Journal of Basic Medical Sciences*, *24*(7), 914–921. https://doi.org/10.22038/ijbms.2021.54019.12142

Flach, M. G., Dogan, O. B., Miller, M. F., Sanchez, M. X., & Brashears, M. M. (2024). Comparison of Three Preharvest Sampling Strategies to Monitor Pathogens in Cattle Lairage Areas. *Journal of Food Protection*, *87*(4), 100258. https://doi.org/10.1016/j.jfp.2024.100258

Food and Agriculture Organization. (2021). *Agricultural Outlook 2021-2030*. https://openknowledge.fao.org/server/api/core/bitstreams/09e88a46-b005-4d65-8753-9714506afc38/content

Hannah, J. F., Fletcher, D. L., Cox, N. A., Smith, D. P., Cason, J. A., Northcutt, J. K., Richardson, L. J., & Buhr, R. J. (2008). Effect of Sand and Shaking Duration on the Recovery of Aerobic Bacteria, Coliforms, and Escherichia coli from Prechill Broiler Whole Carcass Rinsates. *Journal of Applied Poultry Research*, *17*(2), 272–277. https://doi.org/10.3382/japr.2007-00108

Projahn, M., Pacholewicz, E., Becker, E., Correia-Carreira, G., Bandick, N., & Kaesbohrer, A. (2018). Reviewing Interventions against Enterobacteriaceae in Broiler Processing: Using Old Techniques for Meeting the New Challenges of ESBL E. Coli? *BioMed Research International*, *2018*, 7309346. https://doi.org/10.1155/2018/7309346

Sanchez, A. (2023). *Enviro-mapping baseline of microbial indicators in a beef fabrication floor as a tool for sanitary design, sanitizing interventions, and pathogen monitoring: Enviro-mapping baseline of microbial indicators in a beef fabrication floor as a tool for sanitary design,*

sanitizing interventions, and pathogen monitoring. ttu-ir.tdl.org. https://ttu-ir.tdl.org/items/2b41dc10-6bb1-49ec-a150-0f71b0264456

Sayed, E., Shaltout, F., & Zakaria, M. (2020). *Aerobic Plate Count of Contaminants and Molecular Characterization of Eschereichia Coli in Raw Chicken Meat in Ismailia, Egypt*. https://www.researchgate.net/publication/344312386_Aerobic_Plate_Count_of_Contaminants_and_Molecular_Characterization_of_Eschereichia_Coli_in_Raw_Chicken_Meat_in_Ismailia_Egypt

Shahbandeh M. (2024). *Per capita meat consumption in the United States in 2022 and 2033, by type*. https://www.statista.com/statistics/189222/average-meat-consumption-in-the-us-by-sort/

Shaji, S., Selvaraj, R. K., & Shanmugasundaram, R. (2023). Salmonella Infection in Poultry: A Review on the Pathogen and Control Strategies. *Microorganisms*, *11*(11). https://doi.org/10.3390/microorganisms11112814

United States Department of Agriculture (2012). Nationwide Raw Chicken Parts Microbiological Baseline Data Collection Program. https://www.fsis.usda.gov/sites/default/files/media_file/2020-07/Baseline_Data_Chicken_Parts_Study_Design.pdf

United States Department of Agriculture. (2023). *New Sampling Instructions and Testing for Chicken Parts and NRTE Comminuted Poultry*.

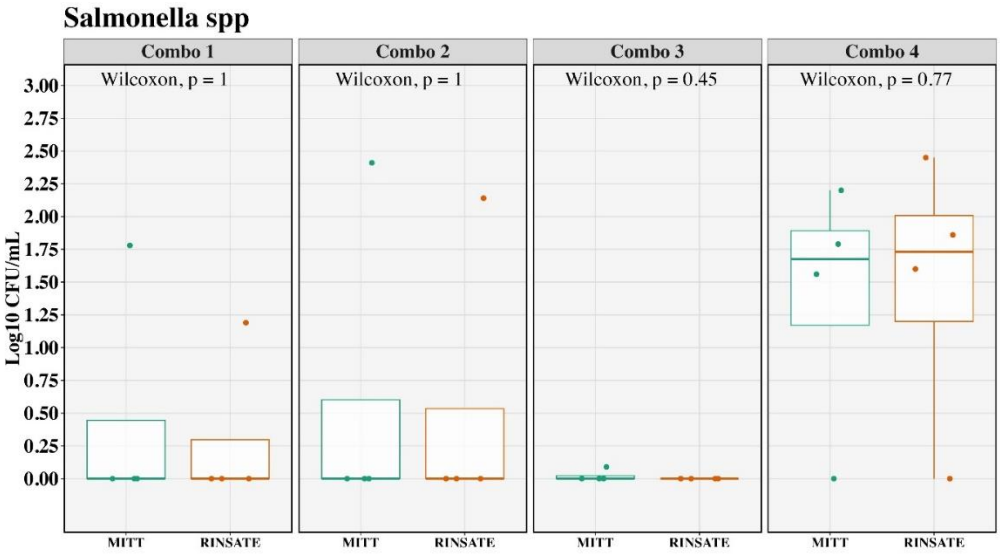
United States Department of Agriculture. (2024). *Finalizes Policy to Protect Consumers from Salmonella in Raw Breaded Stuffed Chicken Products.* U.S DEPARTMENT OF AGRICULTURE. https://www.usda.gov/media/press-releases/2024/04/26/usda-finalizes-policy-protect-consumers-salmonella-raw-breaded

Whyte, P., McGill, K., Monahan, C., & Collins, J. (2004). The effect of sampling time on the levels of micro-organisms recovered from broiler carcasses in a commercial slaughter plant. *Food Microbiology*, *21*(1), 59–65. https://doi.org/10.1016/S0740-0020(03)00040-6

Appendix

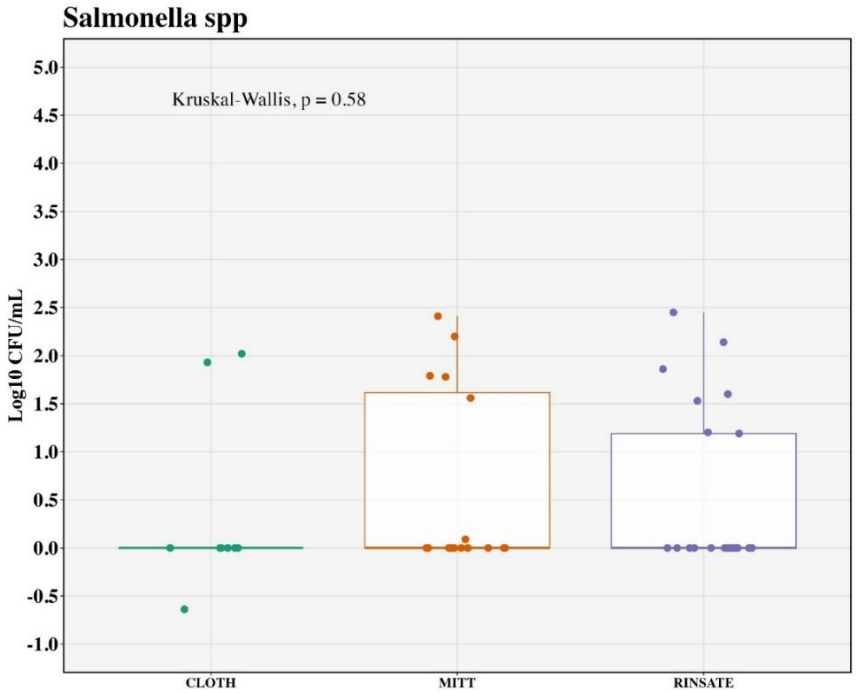
Appendix A

Comparison of MicroTally Mitt with Rinsate across four combos



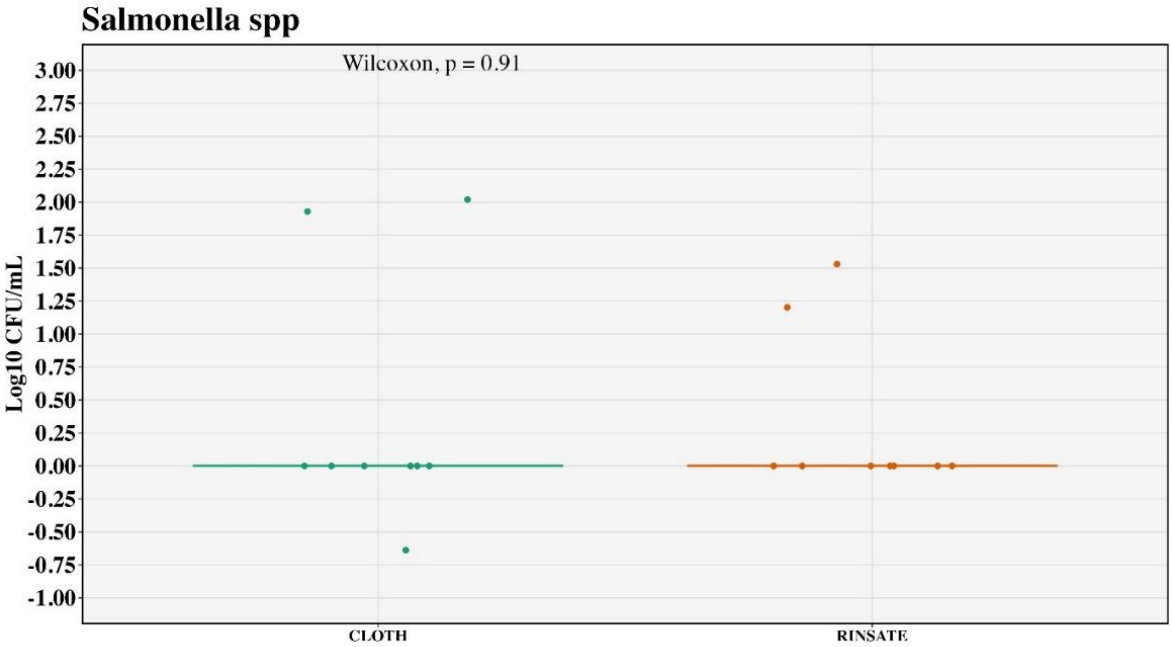
Appendix B

Comparison of Salmonella loads in three different sampling methods.



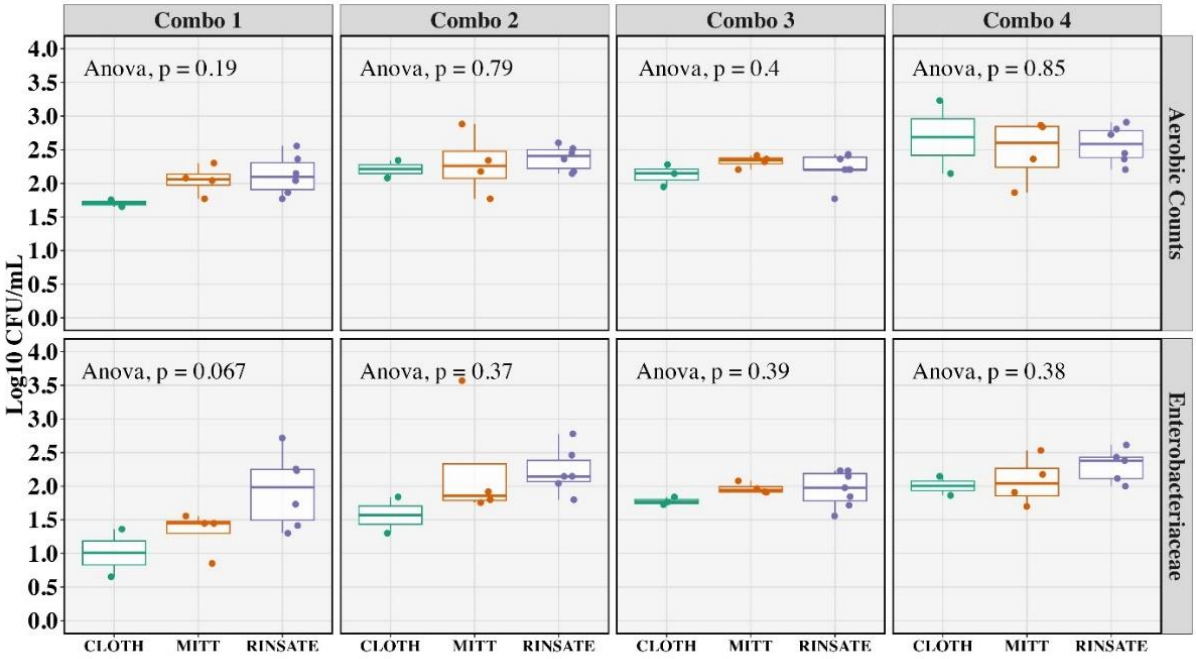
Appendix C

Comparison of Salmonella load in MicroTally Swab with rinsate



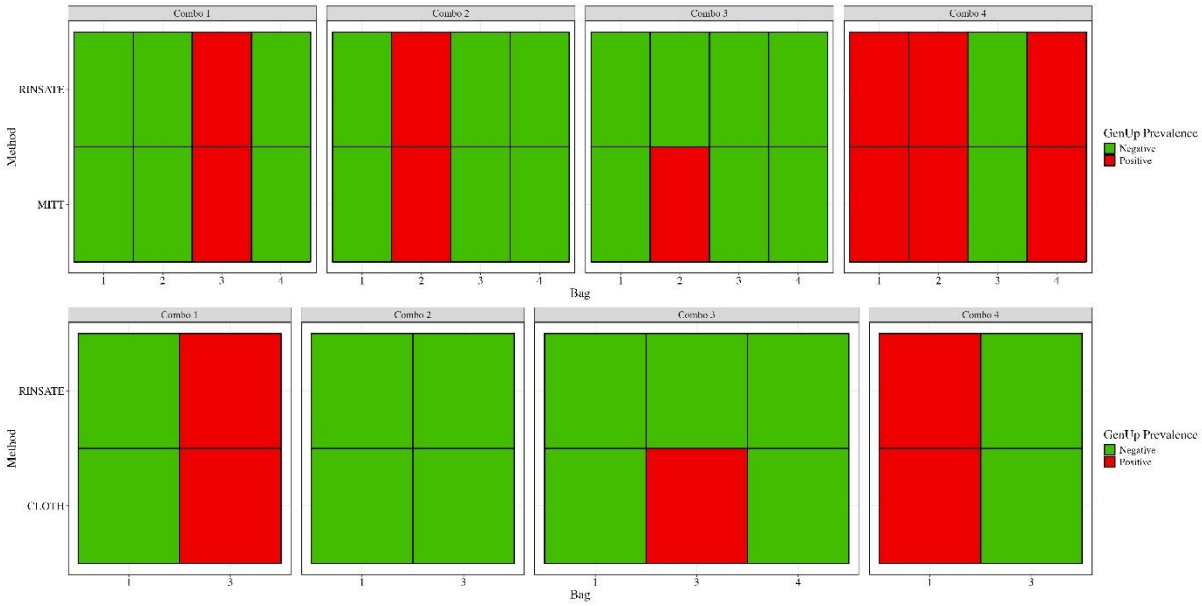
Appendix D

ANOVA of Mesophilic aerobes Counts and Enterobacteriaceae across four combos and three different sampling methods.



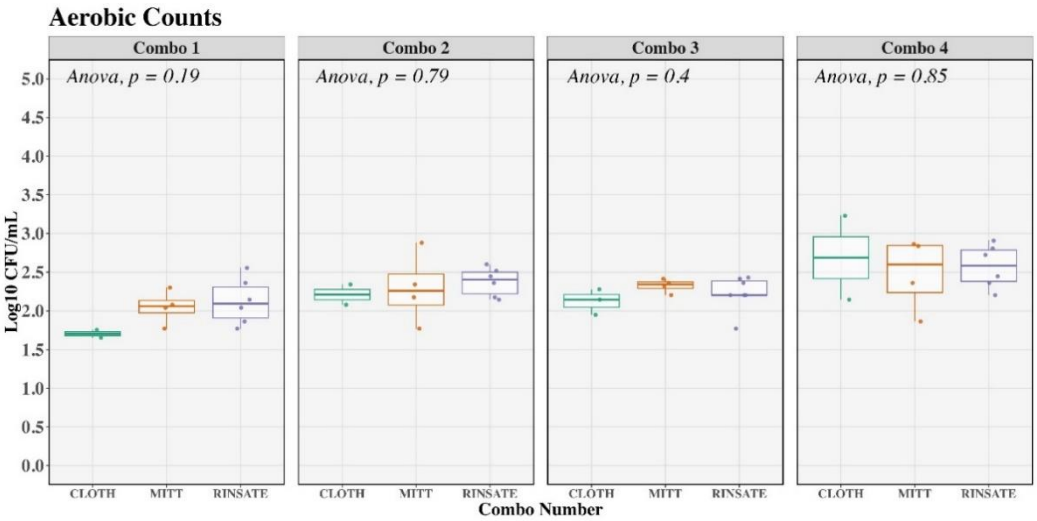
Appendix E

Prevalence of sampling methods across four combos



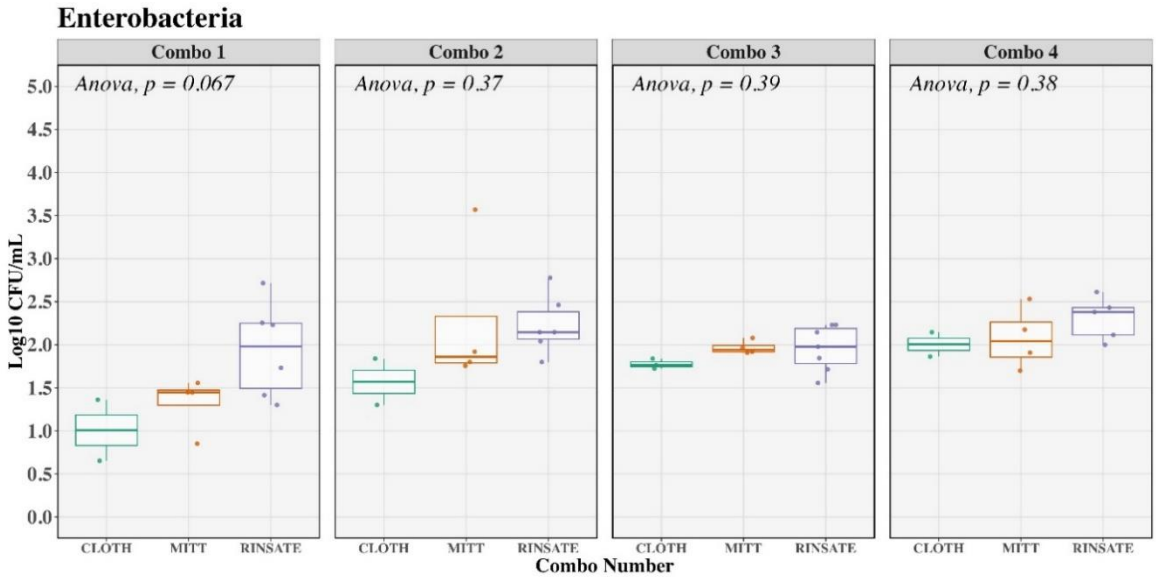
Appendix F

ANOVA for Mesophilic aerobes Count across four combos



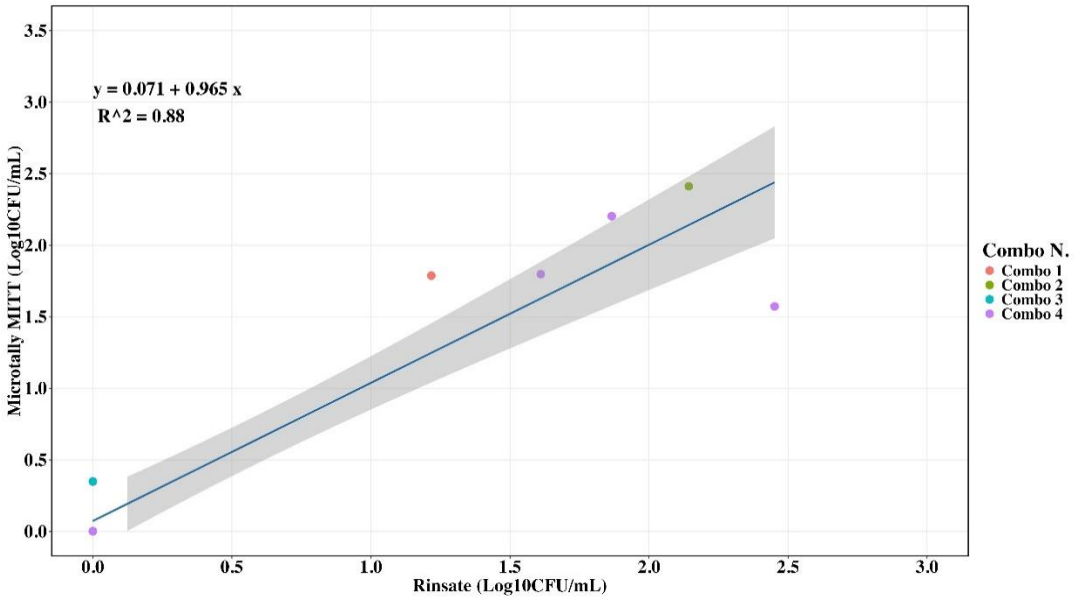
Appendix G

ANOVA for Enterobacteria across four combo



Appendix H

Linear Regression Model for Microtally Mitt and Composite Rinse



Appendix I

Linear Regression Model for Microtally Swab and Composite Rinse