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Environmental Sampling for *Listeria* spp. Using a MicroTally® Mitt and StickSponge™ for Environmental Sampling of Three Different Food Contact Surfaces

ABSTRACT

Listeria spp. threatens the food business. It can cause meningitis, septicemia, and miscarriage in a variety of foods. Pregnant, elderly, infants, and those with compromised immune systems are at risk. *Listeria* outbreaks can harm the food industry through public distrust, product recalls, and legal action. This study compares Dry Mitt, MicroTally®, and EZ Reach™ sampling methods for food contact surface sampling. Samples were examined using PCR and selective media. These procedures are used to test *Listeria* in food processing facilities. Statistical variations in *Listeria* recovery were seen between the two sampling strategies for three surfaces (p <0.01). Anova analysis showed substantial recovery rate variations between swabs and mitt sampling methods (p < 0.05), indicating separate performance. Swab and Dry Mitt (-0.056) differed from 1 (97.5% = 6.50) slope interval confidence. The linear model's intercept (1.1370) fits the dependent variable's measurement when the independent variable is zero (p = 0.659). Swab and Pre-Moist Mitt (0.7576) differed from 1 via slope interval confidence (97.5% = 2.754). The intercept (1.5365) was statistically insignificant (p = 0.0156), and the R^2 (0.547) indicated poor data matching for the linear model sampling and testing to minimize *Listeria's* impact on public health and the food business.

INTRODUCTION

Listeria monocytogenes is the pathogen responsible for causing listeriosis, a severe infection that affects both humans and animals. It is characterized by high rates of hospitalization and death, making it one of the most devastating foodborne diseases. (World Organization for Animal Health., 2018.) Among the 21 identified species of gram-positive bacteria in the *Listeria* genus, only *L. monocytogenes* and *Listeria ivanovii* have been identified as harmful to mammals.(Quereda, JJ., et al., 2020) The rise of pathogenic *Listeria* species as a prominent foodborne pathogen in the second half of the 20th century has had substantial economic consequences for society and the food business, especially due to outbreaks of listeriosis in humans and animals. Listeriosis is classified as a sporadic

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illness, indicating that it occurs seldom but has significant repercussions when it does manifest. Its elevated fatality rate, particularly among susceptible demographics including the elderly, expectant mothers, and immunocompromised individuals, presents a noteworthy public health issue (EFSA and ECDC, 2018).

The capacity of *L. monocytogenes* to induce illness in a wide range of hosts highlights its versatility and ability to cause disease. Although originally a saprophytic bacterium, the *Listeria* genus has successfully adjusted to several environmental habitats linked to human activities, such as farms (containing the excrement of mammals and birds), food, and food-processing facilities. The flexibility of the genus is ascribed to its exceptional capacity to perceive and react to environmental stressors, enabling it to thrive in such varied settings. This stress tolerance is also thought to enhance the transmission of *Listeria* from contaminated food into the gastrointestinal tracts of animal hosts. In addition, the capacity of *L. monocytogenes* to create biofilms, which are organized groups of bacterial cells surrounded by a matrix they make themselves, increases its potential to survive in food-processing settings, making it challenging to eliminate. The pathogen's capacity to endure and multiply in different surroundings emphasizes the significance of rigorous food safety procedures and monitoring initiatives to avert listeriosis outbreaks. (NicAogáin K, O'Byrne CP., 2016).

Food business operators (FBOs) who produce ready-to-eat foods must conduct tests on their processing facilities and equipment to determine if *L. monocytogenes* is present or absent. This is a necessary aspect of their sampling procedure. Guidelines for screening food processing areas and equipment to detect *L. monocytogenes* have been previously published by Carpentier and Barre in 2012. Major food corporations have the necessary resources to construct a highly efficient self-monitoring sampling plan and employ a knowledgeable quality management board. Nevertheless, smaller firms face greater challenges in implementing this (Luber et al., 2011; Vitas, Díez-Leturia, Tabar, & Gonzalez, 2013; Winkler & Freund, 2011).

Sampling methods play a crucial role in microbiological testing, especially when there is a concern about microbial contamination. Swabbing is a widely used technique that entails using a sterile swab to gather samples from various surfaces. Dry swabs are efficient for maintaining genetic material, however pre-moistened swabs are optimal for capturing live microbes. Swabbing is a frequently employed technique in microbiology for environmental monitoring and diagnostic applications. Mitts, however, are tactile instruments that enable direct sampling from surfaces. They are valuable for monitoring the cleanliness and contamination levels in food processing plants, offering a realistic approach to evaluating surface hygiene.

Dry swabs are advantageous for conserving genetic material and are appropriate for scenarios where moisture may impede subsequent analysis. The items are packaged in a sterile manner and can be stored at ambient temperature. Pre-moist swabs, on the other hand, are already moistened with a solution or buffer, which makes them perfect for gathering living bacteria. Microbiologists frequently employ them for diagnostic applications and environmental surveillance. Every sampling method possesses unique benefits, selected according to the individual application, and intended result. Swabs and mitts are useful for collecting samples from surfaces during hygiene inspections. Dry swabs are good for conserving genetic information, while pre-moistened swabs are perfect for collecting living microbes. Comprehending the advantages of each sample method is essential for efficient microbiological testing and contamination management in different settings.

The study's goal was to see how well three different sample methods—Dry Mitt, Pre-Moist Mitt, and Pre-Moist Swab—could get *Listeria* off Teflon and stainless-steel surfaces that had been inoculated with different amounts of a *Listeria* cocktail. The MicroTally® Swab is used to take samples by both the continuous sampling device (CSD) and the manual sampling device (MSD). The CSD and MSD methods have been shown to be as good as or better than other methods for sampling raw beef trimmings (Arthur & Wheeler, 2021; Wheeler & Arthur, 2018). However, some users found the MicroTally Swab to be difficult when using the MSD method. Since the MicroTally Mitt is a brand-new sampling tool, it is important to come up with rules for how it should be used in food processing areas so that it can successfully look for indicator bacteria or pathogens.

MATERIALS AND METHODS

Study design: This study aimed to assess the efficacy of MicroTally® Mitt (dry) and pre-moistened EZ StickSponge™ in detecting *Listeria* spp. in environmental samples. The evaluation involved three different surfaces (Stainless Steel sheet, Teflon Cutting Board, Stainless steel drains) and three different *Listeria* strains. (*L. monocytogenes* 311 in Auburn University, *L. innocua* 33091 isolated from feces, human (feces of healthy pregnant woman), and *L. welshimeri* 43550 isolated from soil (cornfield soil)).

Culture preparation and sample collection: Strains were removed from the -80°C freezer. Using a 10 µL inoculation loop, the frozen culture was transferred to a 9ml BHI (Brain Heart Infusion) broth twice for each strain and incubated at 37°C for 24 hours. After incubation, the culture was streaked onto MOX (Modified Oxford Agar) plates using a 10µL inoculation loop and incubate at 35°C for 24 hours. Isolated colonies were transferred into a 9ml tube of BHI and incubated at 37°C for 18 hours. BHI cultures were mixed for the 3 strains to create a cocktail. Dilutions of the cocktail were made in BHI broth transferring 1ml from the cocktail to the broth 10^8 to 10^2 to be able to use the right tube to get the inoculation level wanted.

Environmental swab EZ StickSponge™ samples (n=108) and MicroTally® Mitt samples (n=108) were used to collect samples from three different inoculated surfaces (Stainless Steel sheet, Teflon Cutting Board, Stainless steel drains). The stainless-steel sheets and drainLab andere provided from the ICFIE Lab the Teflon cutting boards from the Meat Lab at Texas Tech. Samples were inoculated with 1 mL of the cocktail of *L. monocytogenes*, *L. innocua*, and *L. welshimeri* at 4 different concentrations 1, 2, 3, and 4 log CFU/cm^2. Tested surface area for stainless steel sheet was 100 cm^2, drains 4-1/4 in, and Teflon 20 x 10 cm. Samples were inoculated and properly labeled with their corresponding inoculation level. Each surface was inoculated by adding 1 mL onto the different surfaces and spread with a flamed-glass spreader over the entire surface for 30 seconds until all the surface is wet. Inoculated surfaces were dried for 45 to 75 minutes at room temperature in the biohazard hood. One sponge of 1.5" x 3" biocide-free cellulose was used per sample, half of the surface was swabbed and firmly sampled by applying sponge-sticks horizontally and vertically, 5 times each on both sides, based on the stick sponge protocol to ensure that the designated area was covered, and the swab was placed inside a stomacher bag and stomached for 30 sec at 230 rpm and incubated at 37°C for 24 hrs. A 10" x 10" in size and packaged in a disposable sterile wrap mitt was aseptically taken out of its individual package and with a clean pair of gloves you grabbed the surface that was being sampled and it was used to scrub the other half of the surface for 45 seconds on each side to ensure a minimum of 90 seconds of scrubbing the surfaces. Once the sample was taken, the mitt was placed inside in its empty sterile bag where a 200 mL aliquot of Buffered Peptone Water (BPW) was added to the mitt and stomached for 30 sec at 230 rpm. From the primary bag, 30 mL were transferred into a 24 oz filtered Whirl-Pak bag using a 50 mL disposable serological pipette to a 30 mL aliquot of *Listeria* media (Hygiena) was added to each sample in the bag and incubated at 36°C for 26-30 hrs.

Processing sample collection: The presence of *L. monocytogenes* was determined using the BAX® System PCR Assay kits for *Listeria monocytogenes* and Genus *Listeria*. There were 72 lysates that were created for PCR platform and analyzed according to the manufacturer's instructions. Each plate was labeled with the kit name, inoculation level and surface. The sample was plated on MOX for confirmation of PCR results. 100 µL were added on the MOX plate and it was incubated at 35°C for 24 hrs. *Listeria* forms brown colored colonies with black zones due to aesculin hydrolysis.

Statistical analysis: To assess the effectiveness of each sampling method, the data was analyzed using R (Version 4.1.2) statistical software. A Fisher test was employed to compare prevalence between surfaces. To detect statistically significant differences, a *p*-value of 0.05 or less was employed.

Pre-moist samples: Environmental swab EZ StickSponge™ samples (n=81) were collected and pre-moist and dry MicroTally® Mitt samples (n=81) were collected from three different inoculated surfaces (Stainless Steel sheet, Teflon Cutting Board, Stainless steel drains). Samples were inoculated with 1 mL of a cocktail of *L. monocytogenes*, *L. innocua*, and *L. welshimeri* at a concentration of 5 log CFU/cm^2. Tested surface area for stainless steel sheet 100 cm^2, drains 10.975 cm, Teflon 200 cm^2. To inoculate add 1 mL onto the different surfaces and spread with a glass flamed spreader all over the surface for 30 seconds until all the surface is wet. Let it dry for 45 to 75 minutes at room temperature and biohazard hood for the attachment time. One pre-moist sponge of 1.5" x 3" biocide-free cellulose was used per sample, aseptically half of the surface was swabbed and firmly sampled by applying sponge-sticks horizontally and vertically, 5 times each on both sides, based on the stick sponge protocol to ensure that the designated area was covered, and the swab was placed inside a stomacher bag and stomached for 30 sec at 230 rpm and incubated at 37°C for 24 hrs. A 10" x 10"in size and packaged in a disposable sterile wrap mitt was aseptically taken out of its individual package and placed inside its bag, a 25 mL aliquot of BPW was added to pre-moisture the mitt. and with a clean pair of gloves, grab the surface that was being sampled and scrub the surface for 45 seconds on each side to ensure a minimum of 90 seconds of scrubbing the surfaces. Once the sample was taken you put the rest of the aliquot to ensure the mitt has 200 mL of media. A 175 mL aliquot of BPW was added and stomached for 30 sec at 230 rpm.

Processing sample collection: Labeled plates from 10^1, 10^2 and, 10^3 with their designated surface and inoculation level were used for identification. Once the sample was stomached and ready to use, they were plated on MOX for count and cell recovery results, 100 µL were pipette on the plate and incubated at 35°C for 24 hrs. After incubation time, colonies were examined and counted.

Statistical analysis: To assess the effectiveness of each sampling method, the data was analyzed using R (Version 4.1.2) statistical software. An ANOVA statistical test was performed to evaluate the difference between surfaces, followed by a Tukey-Kramer analysis for mean separation evaluation and a *t*-test for comparison. A linear regression model was performed to compare the load of dry mitt and pre-moist mitt with swabs to evaluate performance of both sampling methods. All data was evaluated at 95% confidence level. To detect statistically significant differences, a *p*-value of 0.05 or less was employed.

RESULTS

Statistical analysis based on the amount of *Listeria* positives and negative samples that as detected on BAX® System PCR Assay for Genus *Listeria* on the different surfaces is illustrated in *Figure 1*. It depicts the three surfaces with their respective sampling method. The surfaces had statistical differences (p<0.01) according to the Fisher's

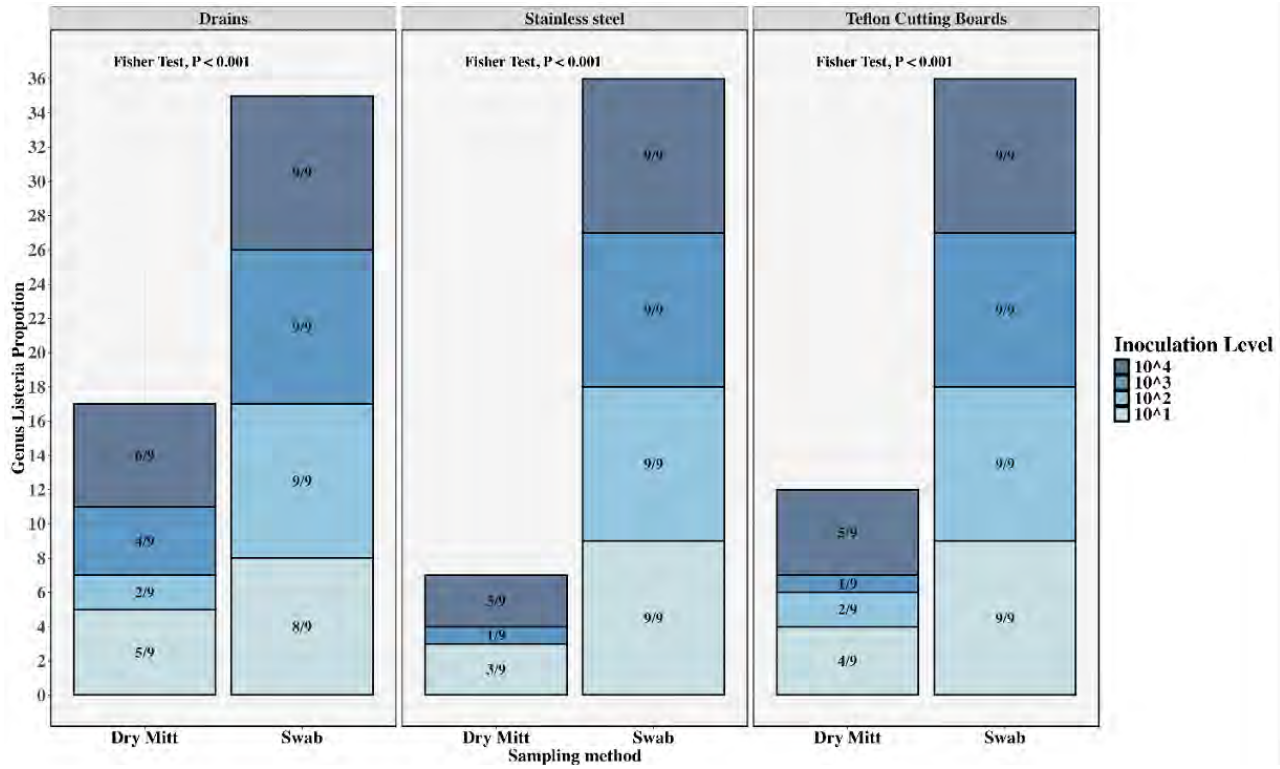

Figure 1. Comparison of genus *Listeria* proportion in percentage on two sampling methods (Dry Mitt and Swab) and 3 types of surfaces at 4 different inoculation levels (1, 2, 3, 4 logCFU/cm^2), two repetitions and two independent replications (n=24/treatment) using a Fisher's Exact test with 95% of Confidence Level.

TABLE 1. Percentage of recovery from three different surfaces on Dry Mitt for Genus *Listeria*

Surface	Inoculation Level (log CFU/cm^2)	%
Drain	1	56
	2	22
	3	44
	4	67
Stainless steel	1	33
	2	0
	3	11
	4	33
Drain	1	44
	2	22
	3	11
	4	56

TABLE 2. Percentage of recovery from three different surfaces on Pre-moist Swab for Genus *Listeria*

Surface	Inoculation Level (log CFU/cm^2)	%
Drain	1	89
	2	100
	3	100
	4	100
Stainless steel	1	100
	2	100
	3	100
	4	100
Drain	1	100
	2	100
	3	100
	4	100

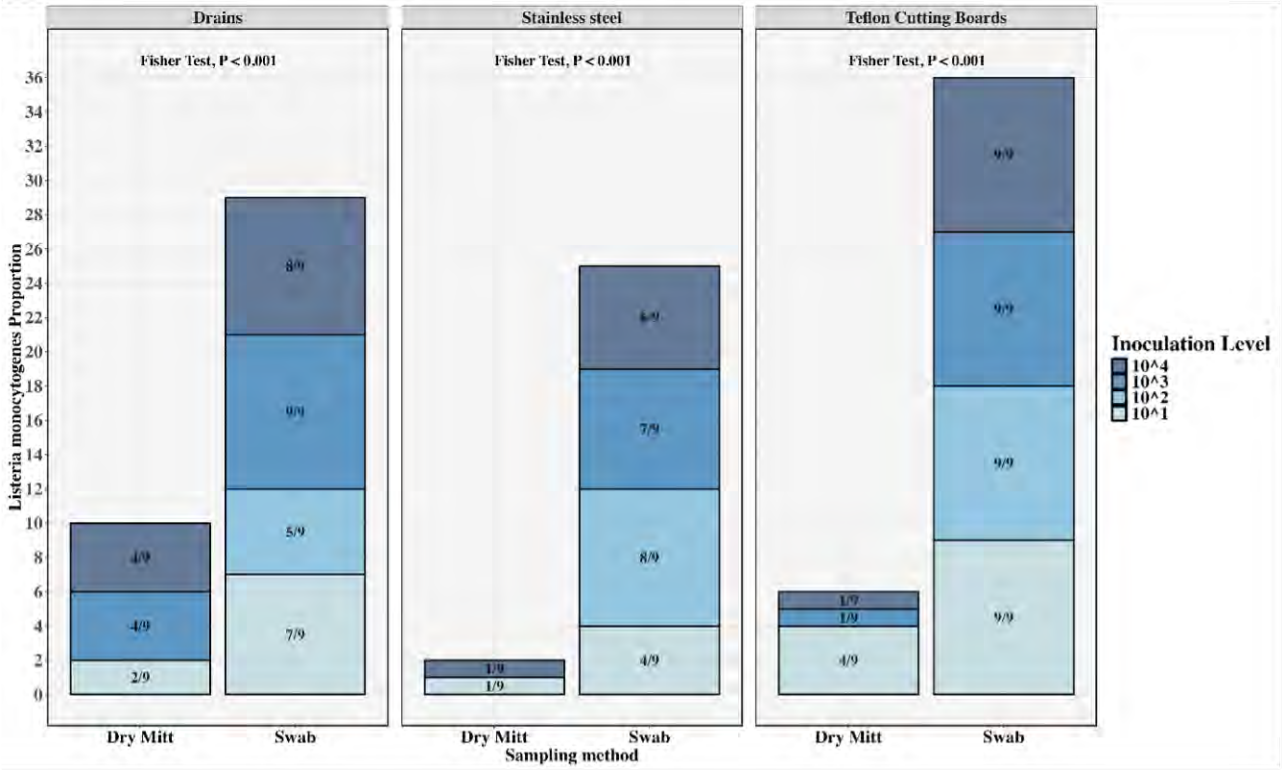

Figure 2. Comparison of *Listeria monocytogenes* proportion in percentage on two sampling methods (Dry Mitt and Swab) and 3 types of surfaces at 4 different inoculation levels (1, 2, 3, 4 log CFU/cm^2), two repetitions and two independent replications (n=24/treatment) using a Fisher's Exact test with 95% of Confidence.

TABLE 3. Percentage of recovery from three different surfaces on Dry Mitt for *Listeria monocytogenes*

Surface	Inoculation Level (log CFU/cm²)	%
Drain	1	22
	2	0
	3	44
	4	44
Stainless steel	1	11
	2	0
	3	0
	4	11
Drain	1	44
	2	0
	3	11
	4	11

TABLE 4. Percentage of recovery from three different surfaces on Pre-moist Swab for *Listeria monocytogenes*

Surface	Inoculation Level (log CFU/cm²)	%
Drain	1	78
	2	56
	3	100
	4	89
Stainless steel	1	44
	2	89
	3	78
	4	67
Drain	1	100
	2	100
	3	100
	4	100

exact test. *(Fig. 1)* The pre-moist swab approach indicated clear efficacy, obtaining a 100% recovery rate at all levels of inoculation (1, 2, 3, and 4 log CFU/cm²) on various surfaces, such as drains and stainless steel. However, the dry mitt approach showed irregular rates of recovery, with lower percentages in general. On drains, the recovery rates varied between 22% and 67%, whereas on stainless steel, the rates were significantly lower, ranging from 0% to 33% *(Table 1, 2)*.

Statistical analysis based on the number of positives and negatives that as detected on BAX® System PCR Assay for Genus *Listeria* on the different surfaces is portrayed in *Figure 2*. The three surfaces with their respective sampling method are illustrated. The three surfaces showed statistical differences between the two sampling methods in terms of *Listeria* recovery ($p<0.01$) according to the Fisher's exact test. The data indicates that the Pre-moist Swab method consistently produced higher rates of recovery compared to the Dry Mitt method. According to *Table 3*, the Dry Mitt technique achieved recovery rates ranging from 22% to 44% on Drain, 11% on Stainless Steel, and up to 44% on Teflon. *Table 4* shows that the Pre-moist Swab technique has recovery rates between 56% and 100% on Drain, 44% and 89% on Stainless Steel, and consistently 100% on Teflon.

For the comparison of the two sampling methods in *Figure 3*, counts were log10 transformed and analyzed, the slope in the linear regression represent the estimated increase in the dependent variable (Dry Mitt) when the independent variable (Swab) increases by 1 log CFU/cm^2. Slopes close to one represent equal performance of both sampling methods to display same recovery results, the slope for the comparison of Swab and Dry Mitt (-0.0056) was significantly different from 1, when the interval confidence of the slope was calculated (97.5% = 6.50). In the linear model the intercept represents the measurement obtained with the dependent variable when the independent variable is equal to zero, in this case, the intercept (1.137) was statistically equal to zero and (p = 0.659).

For the comparison of the two sampling methods in *Figure 4*, counts were log10 transformed and analyzed, the slope in the linear regression represent the estimated increase in the dependent variable (Pre-Moist Mitt) when the independent variable (Swab) increases by 1 log CFU/cm^2. Slopes close to one represent equal performance of both sampling methods to display same recovery results, the slope for the comparison of Swab and Pre-Moist Mitt (0.7576) was significant different from 1, when the interval confidence of the slope was calculated (97.5% = 2.75). In the linear model the intercept represents the measurement obtained with the dependent variable when the independent variable is equal to zero, in this case, the intercept (1.5365) was statistically equal to zero (p= 0.0156), the R^2 (0.547) represent a low fit of the data to the linear model.

DISCUSSION

This study's findings provide evidence that *Listeria* recovery on stainless steel, Teflon and drains varies by sampling method. Surfaces that are hydrophobic (using Teflon) and hydrophilic (using stainless steel) were included in this study. Stainless steel is a prevalent material utilized in food processing equipment and surfaces, and *Listeria* can easily attach to its sleek surface. Teflon is known for its non-adhesive characteristics but can still allow *Listeria* to attach., Møretrø and Langsrud (2004) reported that environmental factors, including temperature, sugar, salt, pH, and nutrients, which are common in foods and food-processing environments, have impacts on *L. monocytogenes* adhesion and biofilm formation.

Listeria detection in the drain was best in this study with both the Dry Mitt and Swab sample procedures demonstrating substantial findings. This suggests that the Drain surface may be more favorable for contamination or easier to sample efficiently. The Stainless Steel and Teflon surfaces exhibited the presence of *Listeria*, but with a lower prevalence in comparison to the Drain surface. Both Dry Mitt and Swab sample procedures were successful in detecting *Listeria* on all three surfaces. However, Swabbing generally exhibited a higher recovery of *Listeria* compared to Dry Mitt sampling. The statistical analysis, employing Fisher's exact test, verified the presence of significant differences in the prevalence of *Listeria* among the three surfaces. This suggests that the kind of surface material has an impact on both *Listeria* contamination and detection.

The rate of rise in *Listeria* recovery varied between the dry mitt and moistened swab techniques. The slope of (-0.056) indicates that for every 1 log CFU/cm^2 rise observed using the Swab method, there is a corresponding increase log CFU/cm^2 observed using the Dry Mitt method. This suggests that the Swab approach may have a higher level of sensitivity or efficiency in detecting *Listeria* when compared to the Dry Mitt method. The linear regression study showed that the Pre-Moist Mitt increased *Listeria* recovery results by 0.7576, compared to the expected slope of one for identical performance. This shows that the Pre-Moist Mitt detects *Listeria* like the Swab. The intercept of the linear model was statistically equal to zero, showing that the Pre-Moist Mitt initial measurement was not significantly different from the Swab technique when *Listeria* was not detected. Although low, R^2 = 0.547 indicates a moderate fit to the linear model. These findings emphasize the importance of sampling approach in *Listeria* detection monitoring protocols, and more research may be needed to completely grasp their consequences.

The boxplot analysis comparing the detection of *Listeria* counts on MOX agar using various sampling methods and surfaces showed statistically significant variations. The t-tests conducted for statistical analysis revealed significant differences between the Dry Mitt and Swab sample methods, as well as between the Pre-Moist Mitt and Swab procedures. Although the surfaces showed similar patterns, the amount of *Listeria* present was measurable. The significance of choosing a suitable sample approach for identifying *Listeria* is emphasized by these findings, as various strategies can produce markedly distinct outcomes. Additional investigation is necessary to comprehend the fundamental elements that contribute to these differences and to improve sampling techniques for more precise identification of pathogens in different settings.

The findings suggest that the presence of surface roughness and porosity has a substantial impact on the ability to detect and recover *Listeria*. The pre-moistened swab method showed superior recovery rates in comparison to the dry mitt method on all surfaces. More precisely, the pre-moistened swab technique achieved a 100% retrieval rate on smoother surfaces such as stainless steel and Teflon, but the dry mitt technique exhibited much lower retrieval rates. The diminished recovery rates seen with the dry mitt method (22% to 44%) compared to the pre-moist swab method (56% to 100%) can be attributed to the roughness and porosity of the drain surface. Rough and porous surfaces tend to capture microorganisms, therefore inhibiting their recovery. The pre-moist swab method's exceptional effectiveness is due

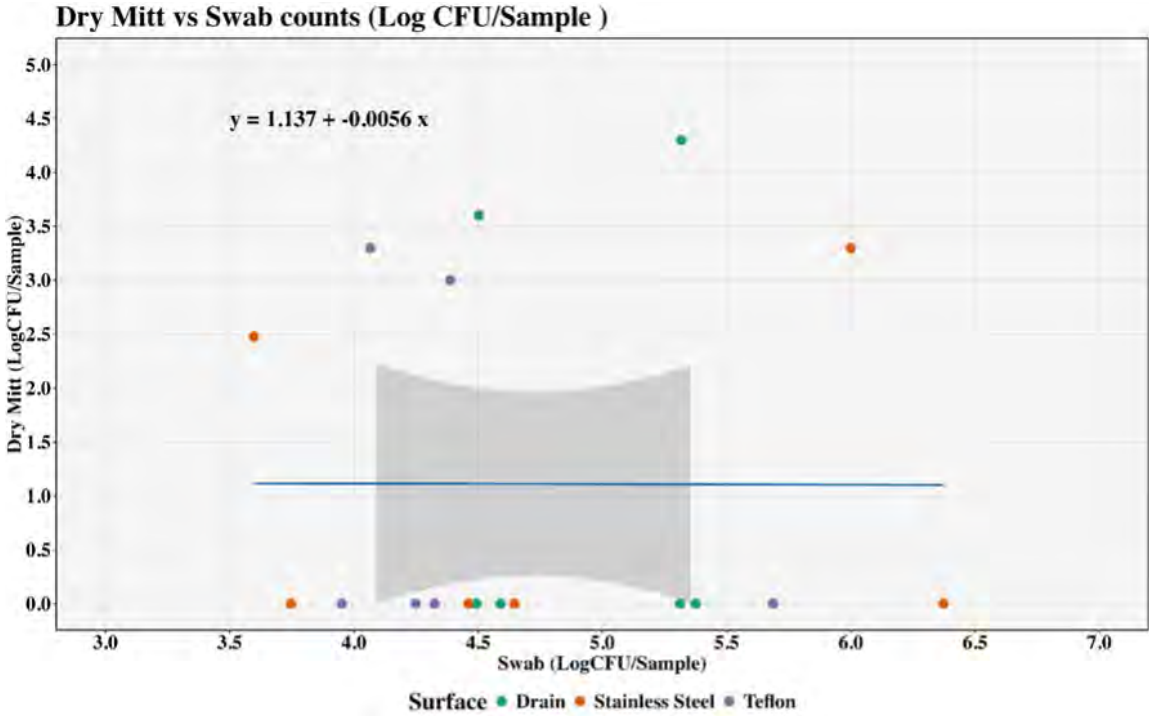

Figure 3. Graphical representation of the linear regression between Dry Mitt and ` (Dry Mitt and Swab) and 3 types of surfaces at 1 inoculation level (5 log CFU/cm^2), three repetitions and independent three replications. The dots represent the actual data points and surfaces.

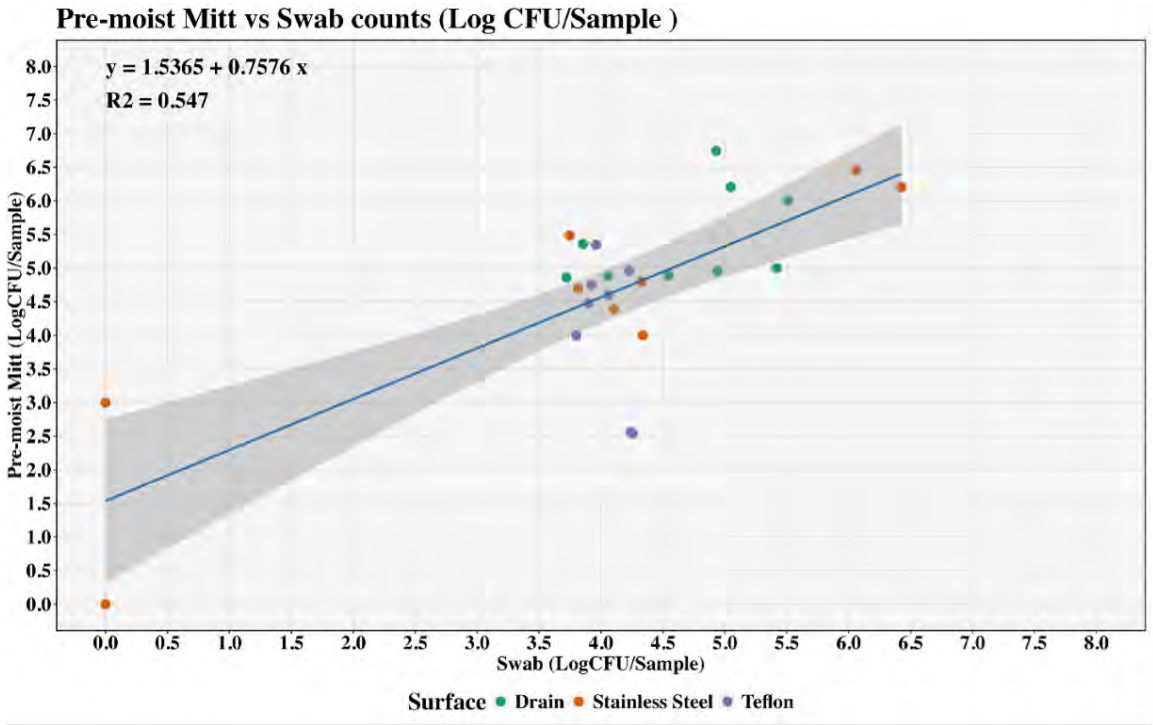

Figure 4. Graphical representation of the linear regression between (Pre-moist Mitt and Swab). The Dots represent the actual data points and surfaces. Two sampling methods and 3 types of surfaces at 1 inoculation level (5 log CFU/cm^2), three repetitions and independent three replications.

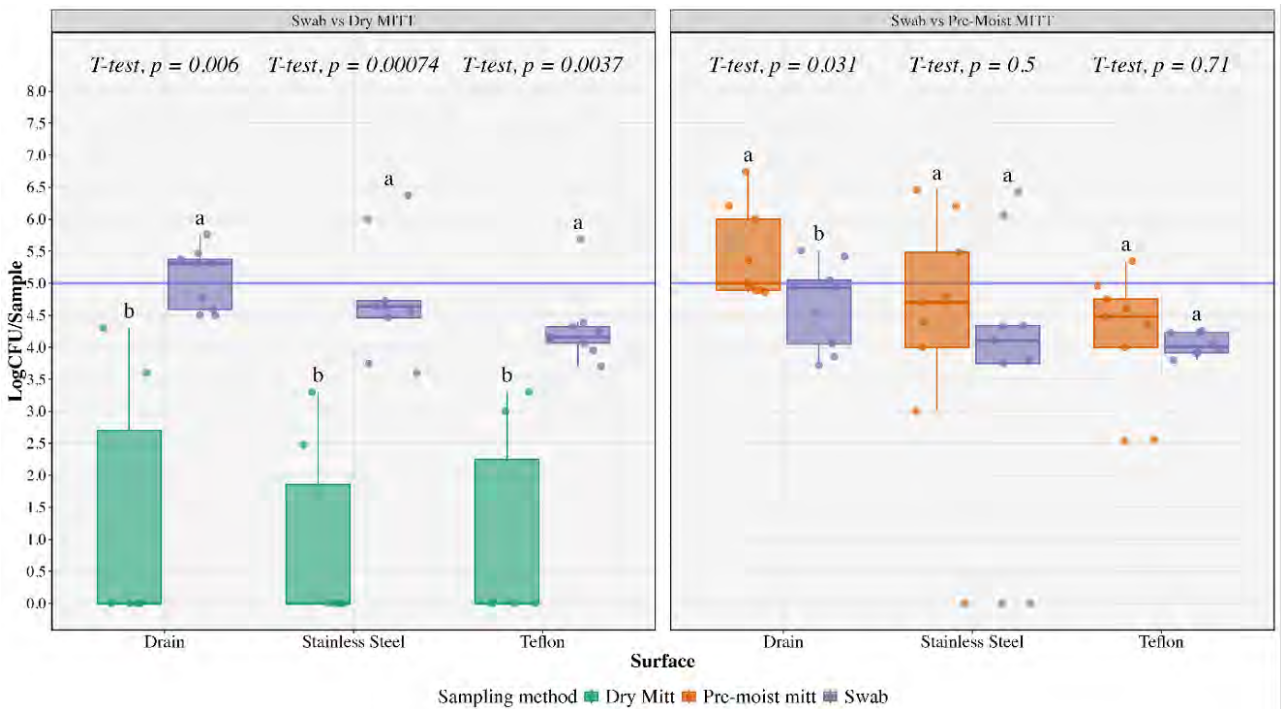

Figure 5. ***Listeria*** **spp. counts (log CFU/cm²) from three surfaces and one inoculation level (5 log CFU/sample) represented in the horizontal purple line. The horizontal line across each boxplot indicates the median, the bottom and top boxes are the lower and upper quartiles, and the vertical top and bottom lines represent the I interquartile range. Distinct letters show significant differences (*T*-test, $p < 0.05$). Actual data points are shown representing surface.**

to its capacity to penetrate surface imperfections and more efficiently dislodge and gather *Listeria* cells, leading to more precise and consistent recovery rates.

In conclusion, the pre-moistened swab showed an advantage over the dry mitt. The dry mitt showed significantly lower plate counts and lower positives results in PCR, indicating that the pre-moistened swab with is more efficient in recovering *Listeria* spp. However, this challenge can be mitigated by moistening the mitt as this resulted in comparable recovery on drains and stainless steel, but not Teflon. The result emphasizes the significance of the sample technique in recovering *Listeria*. Nevertheless, further research is necessary to fully understand the implications of these findings and determine the most effective method for detecting it in various situations. These studies may deepen the knowledge of the dynamics of *Listeria* spp. contamination and advance food safety procedures.

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