Efficacy of a Sonicating Swab for Removal and Capture of *Listeria monocytogenes* in Biofilm on Stainless Steel

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Running Head: Efficacy of a Sonicating Swab

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ABSTRACT

Listeria monocytogenes is of great concern in food processing facilities because it persists in biofilms, potentiating biotransfer. Stainless steel is commonly used for food contact surfaces and transport containers. L. monocytogenes biofilm on stainless steel served as a model system for surface sampling to test the performance of a sonicating swab in comparison to a standard cotton swab. Swab performance and consistency were determined using total viable counts. Stainless steel coupons sampled with both swabs were examined with SEM to visualize biofilms and surface structures (i.e. polishing grooves and scratches). LSCM was used to image and quantitate the percent area of remaining biofilm after sampling with each swab type. The total viable count was significantly higher (P ≤ 0.05) for the sonicating swab in comparison to the standard swab within each trial. The sonicating swab was more consistent in cell recovery than standard swab having lower CVs ranging from 8.9% to 12.3% and 7.1% to 37.6% respectively. SEM imaging showed biofilm remaining in the polished grooves of the coupons sampled with the standard swab but were noticeably absent for the sonicating swab. Percent area measurement of biofilm remaining on stainless steel coupons showed significantly (P ≤ 0.05) less biofilm remained when using the sonicating swab (median 1.1%) in comparison to the standard swab (median 70.4%).

The sonicating swab provided a higher recovery of cells with more consistency than the standard swab and is the first swabbing device to employ: sonication, suction, and scrubbing.

IMPORTANCE

Inadequate surface sampling can result in food borne illness outbreaks from biotransfer since verification of sanitization protocols relies on surface sampling and recovery of

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microorganisms for detection and enumeration. Swabbing is a standard method for microbiological sampling of surfaces. Although swabbing offers portability and ease of use, there are limitations, such as high user variability and low recovery, which can be attributed to many different causes. This study demonstrates some benefits that a sonicating swab has over the performance of a standard swab for removal and collection of a microbiological sample from a surface to provide better verification of surface cleanliness and to help in lowering the potential for biotransfer of pathogens into foods.

INTRODUCTION:

In 2011 to 2014 the Center for Disease Control has reported a total of 87 food borne disease outbreaks where cross contamination of ingredients were determined to be a contributing contamination factor (1-4). Many of these reports likely involve cross contamination from a food contact surface to the food item responsible for the outbreak. Employing insufficient cleaning protocols will leave behind microorganisms and organic materials that may be utilized for formation of new biofilms on these surfaces (5-7). Surfaces containing topographical features including imperfections from heavy use, damage, joints or abutments of materials, fasteners, and even porous areas provide an environment in which microorganisms are able to colonize and persist in complex biofilm formations. Examples of these surfaces are found in many industries including butcher houses, floors and machinery within food processing plants, and within medical devices in a hospital setting (8-10). Biofilms are composed of bacterial cells and extracellular polymeric substances (EPS); the formation of such matrix supports desiccation tolerance, adherence and protection from cleansers and sanitizers (11-13). Further, surface adherence especially inside and around complex topographical features enables colonization
solidity, reducing the overall efficacy of surface sampling techniques allowing for the microorganisms to essentially hide from detection.

Inadequate surface sampling can result in food borne illness outbreaks since verification of sanitization protocols relies on surface sampling and microorganism recovery for pathogen detection and/or enumeration of total viable count (TVC). Swabbing is the standard method for microbiological sampling of surfaces and most commonly the cotton tipped swab is used for this purpose (10, 14, 15). Although cotton swabbing offers portability and ease of use, there are limitations, such as high user variability and low recovery, which deem this approach unfavorable (8, 15).

Additionally, swab head material also has a significant effect on efficiency for surface sampling. Moore et al. (2002) showed that cotton significantly removed more bacteria from a stainless steel surface than Dacron or alginate while a foam tipped swab was found to be superior to all materials tested. Comparing the release of bacteria from different swab head materials Moore et al. 2002 showed no significant differences between cotton, Dacron, foam and alginate while, Hedin et al. 2010 demonstrated nylon will release significantly more bacterial cells than rayon.

Another factor influencing the TVC collected from a surface is whether or not a swab is moistened prior to sampling. Swabs that are moistened with a solution prior to swabbing promote a higher release of bacteria (15). Cotton swabs wetted with Ringer’s solution recovered ~46% of inoculum compared to ~3% using the dry swab and different wetting agents also had significant impact on bacteria release (15). Further, significantly more bacteria were recovered from a surface sampled using a cotton swab moistened with 3% Tween in comparison to cotton.
swabs moistened with 1/4 strength Ringer’s solution, TRIS buffer, MES buffer, and SprayCult (15).

Mechanical motion can also have an effect on the amount of bacteria recovered from a surface. Sonication is well established as a method to clean surfaces and aid in the removal of biofilms from many types of material surfaces (16-18). Sonication can be applied to remove adhered biofilms while maintaining cell viability if employed properly (14, 19, 20). Biofilm-associated bacteria grown on polyvinyl chloride and stainless steel slides had higher recovery rates when placed in a sonication bath in comparison to sampling with a cotton swab (20). Direct and indirect contact of stainless steel coupons inoculated with *Listeria monocytogenes* with a sonicating toothbrush yielded a significantly higher percent recovery (59% and 61%) respectively) than the Dacron tipped swab tested (21%) (19). *Listeria monocytogenes* has been linked to food borne illness outbreaks resulting in multiple deaths from consumption of contaminated dairy, fruits, sprouts, frozen vegetables, and packaged salads (1-4). *L. monocytogenes* is a bacteria of great concern in ready to eat (RTE) food and facilities because it is known to persist in biofilms and is found commonly in food processing environments (9, 19, 21). Stainless steel is a common surface material used in food processing and transport containers and has served as a representative food contact surface for biofilm growth in previous studies (19, 22, 23). We herein present a novel device for the sampling and enumeration of biofilm-associated *L. monocytogenes* on stainless steel surfaces. This device offers feasible and consistent sampling, and is the first swabbing device to employ three simultaneous functions: sonication, suction, and the mechanical scrubbing action of swabbing.

MATERIALS AND METHODS
Sonicating swab apparatus design and 3D printing

The toothbrush head of a commercially available ultrasonic toothbrush (Smilex AU-300D; Asahi Irica Co. Ltd, Saitama, Japan) was removed and replaced with a sonic swab adaptor designed in SolidWorks (Dassault Systèmes SolidWorks, Waltham, MA) and 3D printed on an Objet Connex3 Polyjet System (Stratasys, Eden Prairie, MN) at 30 micron layer thickness using the “Digital ABS” mixture of proprietary photo cured polymers, RGD 515 and RGD 525 (Stratasys).

The sonic swab adaptor (Figure 1) is comprised of a tightly fitted tube with a locking collar (to attach to the ultrasonic tooth brush base), a 3/16” barbed tube port (for fluid suction) and an adjacent socket to tightly hold a 3.5 in long piece of rigid 0.125 OD x 0.075 ID pultruded carbon fiber tube (TAP Plastics, Stockton, CA). A swab tip suction head was designed and 3D printed to be fitted on the distal end of the carbon fiber tube to support a piece of 0.0125 in thick 100 PPI reticulated polyurethane foam (US Plastics 88640; Lima, OH). The reticulated foam was slightly stretched around a dowel to form a small sock and glued to itself using Loctite Adhesive Super Bonder 414 (Henkel Corp, Westlake, OH). Sample collection was done in a 50 ml fluid trap, made from modification of a preassembled closed systems solution centrifuge tube (Corning 11705; Corning Life Sciences, Tewksbury, MA), located between the barbed suction tube port on the sonic swab adaptor and a diaphragm vacuum pump (Gast DOA-P704; Carlstadt, NJ) via 1/8” ID tubing (C-flex 374; Saint-Gobain, Malvern, PA). Repeated trials of suctioning 1ml of reverse osmosis (RO) water off of the test coupon surface, through the complete apparatus, were conducted to determine the appropriate vacuum setting.

Bacterial culture and growth condition
A *Listeria monocytogenes* serotype 4b culture was activated from a frozen glycerol stock (-80°C) in 10 ml of sterile tryptic soy broth (TSB) (BD 211825; Franklin Lakes, NJ) and incubated at 37°C for 24 h on an orbital shaker (150 rpm) then 100 µl was transferred to a fresh tube of TSB and grown with the same conditions (24). The culture was streaked for purity on Oxford agar (BD 222530) containing the modified antimicrobial supplement (BD 211763) incubated at 37°C for 24 h. The pure culture was maintained on Tryptic Soy agar (TSA) (BD 236950) held at 4°C.

**Growth of biofilm on stainless steel coupons in *Listeria* minimal media**

*Listeria* minimal media, Hsiang-Ning Tsai medium (HTM) was prepared as previously described (Tsai and Hodgson 2003) without agarose. Biofilm inoculum was prepared from 100 µl of a *L. monocytogenes* culture (10 ml TSB; 150 rpm; 37°C; 24hrs) combined with 30 ml of sterile HTM and vortexed. The biofilm HTM inoculum was poured over 8 sterile Type 304 stainless steel coupons (SSC) (22 mm x 50 mm) that had been placed side by side (2 rows of 4 coupons) in a sterile 120 mm x 120 mm square petri dish (Gosselin BP124-04; Corning Life Sciences, Tewksbury, MA). The inoculated SSC were incubated statically at 30°C for 72 h then each coupon was removed with sterile forceps and rinsed by immersion in 99 ml of sterile Butterfield’s buffer (BB) (3M FTBFD99; St. Paul, MN) with agitation. Agitation, to remove non-adherent cells, was accomplished by holding the SSC with forceps and paddling the BB solution for 3-5 s. Rinsed SSC were placed into a new sterile square petri dish, covered with 30 ml of HTM (non-inoculated) and then re-incubated at 30°C statically for 72 h before use in the swab comparison trials.

**Comparison studies for standard cotton swab to sonicating swab**
Biofilm latent SSC were removed from the HTM growth media and rinsed in BB, drained and blotted on the edge to remove excess BB then allowed to dry completely at room temp (RT) in the biological safety cabinet (BSC) (~30 min). Coupons were divided randomly for sampling to mitigate variations in biofilm and bacteria cell load per SSC across the 2 sampling methods. A total of 16 SSC were used in each of 3 swab comparison trials where SSC were sampled with a standard cotton swab (8 SSC) or the sonicating swab (8 SSC). Sterile cotton tipped wood swabs (Puritan 25-806 1WC; Guilford, ME) were wetted by dipping into a 9 ml tube of BB (3M BPPFV9BFD) then pressed against the side of the tube to release excess BB before sampling. The swabbing pattern used for the standard swab consisted of a back and forth motion, wiping horizontally over entire coupon, rotating the swab 180° (clean side down) and wiping the entire coupon vertically with the same motion. Following sampling, the standard swab was placed back into the same 9 ml tube of BB and the cotton end was snapped off to remain in the tube and stored at 4°C until enumeration. Enumeration was carried out after vortexing the cotton swab tip in the 9 ml tube of BB for 30 s followed by serial dilution and spread plating (100ul) on TSA. Sonicating swab was applied to a biofilm covered SSC after 1ml of BB had been placed onto its surface. The sonication action was turned on and the swab was moved over the surface with back and forth motion combined with a small circular pattern, for 30 s. Vacuum (-15 mmHg) was then applied while the sonication was still turned on and the 1 ml of BB was pulled into the sample trap tube. The SSC surface was rinsed via addition of 1 ml of BB and then removed with the sonicating swab (both sonication and vacuum turned on) using the same sampling pattern until the SSC appeared mostly dry. Flushing of the sonicating swab tubing and sample head to remove any lingering *L. monocytogenes* was carried out by immersion of the swab sampling head into the remainder of the 7 ml of BB and allowing the vacuum to collect the
BB into the sample trap tube. A total of 9 ml of BB was collected for each biofilm sample.

Enumeration was carried out after vortexing for 30s followed by serial dilution and spread plating (100ul) on TSA.

**SEM and LSCM sample preparation and imaging**

Biofilm samples for SEM imaging were grown on sterile 8 mm x 8 mm SSC and sampled using a standard swab or the sonicating swab using the same procedures as the comparison studies. Swabbed SSC and a non-swabbed control SSC were rinsed in BB at RT then fixed with 12.5% glutaraldehyde (Sigma-Aldrich 340855; St. Louis, MO) in 0.1M PIPES buffer (pH 7.2) (Electron Microscopy Sciences 19240; Hatfield, PA) at 4°C for 2 h. Samples were washed 3 times in a 0.1M PIPES buffer solution for 5 minutes, dehydrated for 10 min at each step of an ascending ethanol (Sigma-Aldrich 493511) series [25%, 50%, 70%, 90%, (100% x 3)] and critical point dried (Tousimis 931 CPD; Rockville, MD). Dried biofilm SSC’s were mounted via carbon tape to 12.7 mm aluminum stubs and coated with gold palladium (50 nm) by sputter deposition (Balzers Union MED 010; Balzers, Liechtenstein). SSC’s were observed under a Zeiss Evo 60 SEM (Carl Zeiss; Oberkochen, Germany) operating at 7.0 kV and with a 6-9 mm working distance and a 15° stage tilt. Each SSC had 5 unique regions of interest (ROI) (114.2 um x 85.2 um) imaged.

LSCM biofilm samples were grown on sterile 22 mm x 50 mm SSC and sampled using a standard swab or the sonicating swab with the same procedures as the comparison studies. Following drying for 30 min in the BSC, each swabbed coupon and a non-swabbed biofilm control was stained for 3 min in 0.1% acridine orange (AO) solution (Sigma-Aldrich 318337) prepared in sterile reverse osmosis (RO) water. The SSC were rinsed in RO to remove excess dye and then dried in the dark at RT in the BSC before imaging under a Zeiss LSM 710 confocal.
microscope (Carl Zeiss) using a Plan-Apochromat 20x/0.8 (WD=0.55 M27) objective. AO stained SSC samples were excited under a 405nm diode laser (7.5% power) using the 405 nm major beam splitter and the spectral detector set to capture AO fluorescence emissions between 450 nm to 650 nm. Each AO stained SSC (control, standard swab and sonicating swab) had 6 unique regions of interest (ROI) (708.49 um x 708.49 um) imaged. ImageJ version 1.49 (National Institutes of Health, USA) was used to determine the percent area of biofilm in each ROI for control and swabbed SSC using the area measurement tool (limited to threshold) with threshold adjustments to select all biofilm material.

**Statistical analysis**

Statistical analysis for the comparison study plate count data and the LSCM data was generated using SAS software, Version 9.4 of the SAS System for Windows Copyright© 2002-2012 (SAS Institute Inc. Cary, NC, USA). Analysis of variance was conducted on the plate count data using the MIXED model procedure with significant differences (P ≤ 0.05) between LSMEANS of the observed Log CFU/mm² determined by the PDIF statement. The model statement included swab type and experiment replicate as the fixed effects as well as the interaction and the denominator degrees of freedom determined using Residual method. Analysis of variance was conducted on rank transformed (PROC RANK) LSCM data using the MIXED model procedure with significant differences (P ≤ 0.05) between LSMEANS of the ranks determined by the PDIF statement. The model statement included the treatment and image as the fixed effects as well as the interaction and the denominator degrees of freedom determined using Kenward-Roger method. The REPEATED statement using Compound Symmetry as the covariance structure was included in the model.

**RESULTS**
Three commercial ultrasonic tooth brushes (Philips Sonicare Essence HX5611/01ST2, Emi-dent 6, and Smilex AU-300D) were evaluated for modification into a sonicating swab. The Sonicare and Emi-dent sonic tooth brushes proved too complex for incorporation of an adaptor. The Smilex sonic toothbrush proved to be easier to modify because of the sealed housing and sleeve style brush head design. All of the sonic generating components are housed within the case of the Smilex sonic toothbrush and the sealed design allows the unit to be wiped down with sanitizer, preventing cross contamination. A 3D printed adaptor sleeve incorporating a rigid tube holder and a suction port was successful in modifying the Smilex sonic toothbrush into a swab. The best suction performance for the complete system design was seen at -15 mmHg and was determined using repeated trials of suctioning 1 ml of RO water from stainless steel coupons. The system with the foam swab covering in place removed a majority of the RO water leaving behind a thin, wet film but no standing droplets.

SSC biofilm growth model

Defined minimal media has been used previously to assay biofilm formation of L. monocytogenes on stainless steel and Hsiang-Ning Tsai medium (HTM) is the simplest minimal media available for growth of L. monocytogenes (25-27). In our model system, HTM provided good L. monocytogenes cell growth and biofilm formation coverage over the SSC surface during incubation. We visually examined the biofilm coverage under LSCM after staining with acridine orange on day 3 and 6 of incubation and found mature biofilm formation by day 6 with fairly even distribution. L. monocytogenes biofilm formation is enhanced in nutrient-poor medium and significant enhancement was observed early in biofilm maturation (21). Bacterial biofilm formation was optimized for our model system to control between sample and between group
variations of the biofilm coverage which could influence swab cell recovery comparison data.

Production of biofilm SSC replicates in separate culture dishes increases overall variation and is
directly caused by variation of bacteria cell concentration in the inoculum. Maximizing the
number of SSC replicates that could be placed into a single culture dish (by cutting them into 22
mm x 50 mm sizes) and using a large format square petri dish (120 mm x 120 mm) controlled
between replicate variations related to the inoculum cell concentration. Randomly dividing SSC
from separate culture dishes to create 2 sets of 8 replicates used for each sampling method in
each comparison trial was aimed at controlling the between group variation.

Comparison studies for standard cotton swab to sonicating swab

The mean colony forming units (CFU) per mm² and the coefficient of variation (CV) of
*L. monocytogenes* recovered from biofilm latent stainless steel coupons with the standard swab
and sonicating swab are presented in Table 1. Within each trial, the mean Log CFU/mm² cells
recovered from the stainless steel coupons with the sonicating swab was significantly (P ≤ 0.05)
more than the mean Log CFU/mm² recovered with the standard swab. Box plot analysis of the
data distribution for each experimental trial shows an overlap in the range of Log CFU/mm²
recovered using the standard swab and the sonicating swab (Figure 2). Greater than 50% of the
sonicating swab cell recovery data is higher than the maximum value of the standard swab in
trial 1 and 2. At least 25% of the sonicating swab cell recovery data is greater than the maximum
value of the standard swab in trial 3. The sonicating swab’s recovery of *L. monocytogenes* had
lower variability than the standard swab in 2 of the 3 trials. The CVs calculated for each of the
experimental trials ranged from 7.1% to 37.6% for the standard swab and 8.9% to 12.3% for the
sonicating swab (Table 1).

SEM and LSCM imaging and analysis
Control unswabbed, standard swabbed, and sonicating swabbed SSC samples were examined with a scanning electron microscope (Figure 4). Micrographs of the SSC unswabbed control coupon surfaces at 1000x magnification showed contiguous *L. monocytogenes* biofilm coverage over the surface including within the polished grooves and surface imperfections of the coupon. Standard swab SSC sample micrographs clearly showed *L. monocytogenes* left on the SSC and were found more commonly within the polished grooves. Few visible *L. monocytogenes* cells were found on sonicating swab treated SSC sample micrographs even in or around complex microscopic surface structures and the polished grooves.

LSCM image analysis of the percent biofilm remaining on the stainless steel coupons for unswabbed control coupons, the standard swab, and the sonicating swab are presented in Figure 3. The unswabbed control samples had a median area biofilm coverage of 97.9%. The standard swab samples had a median area of 70.4% biofilm coverage remaining after swabbing and the sonicating swabbed samples have a median area of 1.1%. Significantly less biofilm was left behind by the sonicating swab than the standard swab from the SSCs (P ≤ 0.05). Further, no significant difference (P ≥ 0.05) was found between the standard swab and the unswabbed control SSCs but, the sonicating swab is significantly different from the control (P ≤ 0.05).

**DISCUSSION**

*L. monocytogenes* is a microorganism continuously associated with foodborne illness outbreaks (14). It can be found in food including ready-to-eat sandwiches, salads, sprouts and in food processing environments on equipment such as deli slicers and in milk storage tanks (19, 28). The occurrence of foodborne outbreaks as well as sporadic cases caused by this bacterium, can be attributed to its increased ability of surviving in food processing environments through biofilm formation (29, 30). *L. monocytogenes* is commonly known to survive in a biofilm.
among these environments as a result of insufficient cleaning protocols compounded by the microorganism’s ability to resist chemical disinfection (5, 7, 13, 31). In conjunction with the resilient structure of *Listeria* biofilms, the nature of the surface material, i.e. smooth or rigid, porous or nonporous, can determine the organism's ability to remain adhered to a surface (9, 14, 32). The location of bacteria biofilm and cell attachment can directly be responsible for the organism’s ability to evade detection. Mechanical release through swabbing hinges on the ability to make direct contact with the biofilm structure but, this may not possible in pores, grooves, scratches and other surface features. This creates biotransfer potential of pathogenic bacteria, especially *L. monocytogenes*, and even spoilage microorganisms in food products that come into contact with the contaminated surface during processing (29). Sonication does not need direct physical contact with the biofilm as does swabbing to facilitate release of bacteria. The shear forces in the acoustic boundary layer are considered responsible for the detachment of the bacteria (18). Sonication in this regard will minimize the effect that surface structure plays in collecting and hiding organisms from detection during surface sampling. Retaining the viability of bacteria recovered from a surface during sampling is also an important consideration that could affect test results and follow on actions, i.e. re-cleaning, re-sanitizing or product recalls. Improving the dislodgement, disaggregation, and dispersal of bacteria within a fluid mechanism, without loss of viability are ideal for sampling and enumeration processes (19). Sonication can provide an effective means of removal and declumping for bacteria cells from surfaces without a significant decrease in cell viability if low intensity ultrasound (higher frequencies) are used (33). Sonicating toothbrushes did not cause significant morphology changes to *Actinomyces viscosus* indicating loss of cell integrity and therefore no resulting changes to cell viability were seen (34). Kang et al 2007, stated recovery of viable bacteria may increase with exposure to
sonic energy. This is most likely explained by the increase in cells removed from the surface in combination with the declumping of cell aggregates.

In this study we present a novel sampling device that incorporates the sonicating action of a sonic toothbrush with the mechanical scrubbing action of a swab with suction in a convenient system design. Swabs are inherently a very practical tool for environmental sampling, they are low cost, easy to transport, readily available, and come in many different formats, i.e. material combinations, shapes, sizes. These conveniences are not always enough to overcome the shortfalls of highly variable sample recovery efficiencies seen with swabs and the lack of removing materials from within surface structures such as pores, scratches and grooves as seen in our SEM data (Figure 4) (14, 15, 35-37). This device has the potential to improve upon current swabbing technology by increasing cell recovery and lowering the variability in cell recovery. In turn this should increase the overall test method effectiveness and confidence. Here we report increases in the amount of L. monocytogenes recovered from stainless steel with the sonicating swab over the amount collected with a standard swab while at the same time having lower CVs for each experimental trial (8.9%, 10.4%, and 12.3% for the sonicating swab; 7.1%, 18.2%, and 37.6% for the standard swab). The poor reproducibility (i.e. higher variability) for recovery of bacteria cells from a surface as we found with the standard swab is consistent with previously reported findings by others using cotton or similar swabs (14, 15, 35-37). Inherent limitations with the standardization of swabbing technique can lead to extreme variability in results (i.e. swabbing pattern, angle of contact, and amount of pressure applied to the swab) (15, 35). In contrast the sonicating swab recovery data was less variable and it is potentially attributed to a few factors working together in our system design. Sonication will act to break up clumps of bacteria cells released from the surface and disperse the cells into solution lessening TVC.
variation from sample to sample. Further, we used suction through a foam swab sock for sample collection rather than absorption of the sample into the swab head material. Absorption will allow cells to become trapped in the swab head fibers affecting the variability of the TVC between samples and is problematic when quantification is necessary.

We hypothesize that the increased amount of *L. monocytogenes* collected with the sonicating swab, in comparison to the standard swab, from the SSC is attributed to sonication dislodging cells from microscopic surface features (i.e. scratches and polished grooves) and the disruption of cell clumps. The dislodging and disrupting of cells by sonication worked in tandem to increase the TVC of *L. monocytogenes*. Examination of the SSC by SEM post swabbing shows a remaining population of cells located in the polished grooves of the stainless for the standard swab that is nearly absent for the sonicating swab, supporting part of our hypothesis. LSCM imaging (Figure 4) and quantification (Figure 3) of biofilm remaining (area%) on the stainless steel showed a significant difference (P ≤ 0.05) between the sonicating swab (median 1.1%) and standard swab (median 70.4%) and further supports our hypothesis for the increase in the TVC.

In conclusion, this study demonstrates some benefits that a sonicating swab has over the performance of a standard swab for removal and collection of a microbiological sample from a representative surface. The sonicating swab offers feasible and consistent sampling of a surface to provide better verification of surface cleanliness and in turn would help towards lowering the potential for biotransfer. Our system design for the sonicating swab is the first swabbing device to employ three simultaneous functions: sonication, suction, and the mechanical scrubbing action of swabbing. A viable commercial system design would maintain these functions while incorporating a sterile disposable swab head, a sterile disposable sample head.
collection trap, and a reusable sonicating handle. In comparison to the cost of standard format swabs, a sonicating swab would require a greater investment, but could be justified by the potential increase in performance. Further work is needed to test the sonicating swab on other surfaces such as wood, ceramic, stone, and plastic to measure its performance.

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FUNDING INFORMATION

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REFERENCES


TABLES AND FIGURES:

**TABLE 1**: Recovery of *L. monocytogenes* from biofilm covered stainless steel coupons with a standard cotton swab and sonicating swab.

*Sonic swab is significantly different from standard swab within the same trial (P ≤ 0.05)*

**FIGURE 1**: 3D rendered drawing of the sonicating swab adaptor in front and isometric view.

Figure legend: Components of the sonicating swab adaptor comprised of A) tightly fitted tube with a locking collar B) barbed tube suction port and an adjacent socket C) rigid pultruded carbon fiber tube D) swab tip suction head

**FIGURE 2**: Box plots of *L. monocytogenes* recovery from biofilm covered stainless steel coupons with a standard cotton swab and sonicating swab from three separate trials.

**FIGURE 3**: Box plots of percent area *L. monocytogenes* biofilm remaining on SSC\(^a\) after swabbing with a standard cotton swab and a sonicating swab in comparison to an unswabbed control.

Box plots followed by different letters are significantly different (P ≤ 0.05)

\(^a\)SSC, stainless steel coupons
FIGURE 4: Representative images of *L. monocytogenes* biofilm on SSC\textsuperscript{a} surfaces examined under SEM and LSCM after swabbing with a standard cotton swab and a sonicating swab in comparison to an unswabbed control.

Figure legend: SEM images (1000x magnification) of stainless steel coupons A) unswabbed control, B) standard cotton swab, and C) sonicating swab. LSCM images (20x magnification) of acridine orange stained *L. monocytogenes* biofilm on stainless steel coupons D) unswabbed control, E) standard cotton swab, and F) sonicating swab.

\textsuperscript{a}SSC, stainless steel coupons; SEM, Scanning Electron Micrograph; LSCM, Laser Scanning Confocal Micrograph
**TABLE 1**: Recovery of *L. monocytogenes* from biofilm covered stainless steel coupons with a standard cotton swab and sonicating swab.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Standard Swab Log CFU/mm² (Mean ± SEM)</th>
<th>CV</th>
<th>Sonicating Swab Log CFU/mm² (Mean ± SEM)</th>
<th>CV</th>
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<tr>
<td>1</td>
<td>2.90 ± 0.07 7.1%</td>
<td></td>
<td>3.44 ± 0.15* 12.3%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.78 ± 0.23 37.6%</td>
<td></td>
<td>2.93 ± 0.09* 8.9%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.16 ± 0.20 18.2%</td>
<td></td>
<td>3.87 ± 0.14* 10.4%</td>
<td></td>
</tr>
</tbody>
</table>

*Sonic swab is significantly different from standard swab within the same trial (P ≤ 0.05)*