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University of Tennessee at Chattanooga, blz328@mocs.utc.edu

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Calibration Study of a Bacterial Quantification Technique Using Transport Swabs and Line Inoculations on Agar Growth Medium

Seth LaRue

Departmental Honors Thesis

The University of Tennessee at Chattanooga

Department of Biology, Geology, and Environmental Sciences

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Henry G. Spratt., PhD	David K. Giles, PhD	David Levine, PT, DPT, PhD, OCS Emeritus, CCRP, Cert DN, FAPTA
Professor of Biology	Associate Professor of Biology	Professor & Walter M. Cline Chair of Excellence, Board Certified Specialist in Orthopedic Physical Therapy Emeritus
Thesis Director	Thesis Examiner	Thesis Examiner

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Table of Contents

Acknowledgements ……………………………………………………………………………………….2

List of Figures ………… ………………………………………………………………………………4

Abstract ……………………………………………………………………………………………5

1. Background and Introduction
    - 1.1 Literature Review ……………………………………………………………………...7
    - 1.2 Introduction ……………………………………………………………………....12
2. Materials and Methods ………………………………………………………………………..19
    - 2.1 Materials and Preparation ………………………………………………………20
    - 2.2 Experimental Design ……………………………………………………………21
    - 2.3 Calculations and Statistical Analysis ……………………………………………24
3. Results ……………………………………………………………………………………27
    - 3.1 *Escherichia coli* ……………………………………………………………………..28
    - 3.2 *Staphylococcus aureus* ………………………………………………………………30
    - 3.3 Comparison of *E. coli* and *S. aureus*: Relationship Between Bacterial CFUs on Swab ……………………………………………………………………………………32
4. Discussion ……………………………………………………………………………………..35
5. Limitations and Future Research …………………………………………………………...40
6. References …………………………………………………………………………………..42
7. Appendices
    - 7.1 Appendix I ………………………………………………………………..………48
    - 7.2 Appendix II
        - II.1 *E. coli* ……………………………………………………………………………50
        - II. 2 *S. aureus* …………………………………………………………………...52

List of Figures

Figure 1: Experimental design scheme used in this study ……………………………………………..22

Figure 2: Technique used to estimate the surface area of the swab and short line inoculation, providing a correction factor ………………………………………………………………………………..26

Figure 3: Swab and slides used to estimate surface area …………………………………………….26

Figure 4: Comparison of *E. coli* and *S. aureus* Survivability ………………………………………….28

Figure 5: *E. coli* - relationship between the Average % Agreement of colony counts (comparing the viable plate counts with the line inoculations) and the number of cells on the glass slides ……………………..29

Figure 6: *E. coli* – relationship between average viable plate count CFUs from dried glass slides and average short line inoculation CFUs…………………………………………………………………30

Figure 7: *S. aureus* - relationship between the Average % Agreement of colony counts (comparing the viable plate counts with the line inoculations) and the number of cells on the glass slides ……………...31

Figure 8: *S. aureus* - relationship between average viable plate count CFUs from dried glass slides and average short line inoculation CFUs…………………………………………………………………32

Figure 9: Comparison of *E. coli* and *S. aureus* - relationship between the Average % Agreement of colony counts (comparing the viable plate counts with the line inoculations) and the number of cells on the glass slides ………………………………………………………………………………………………33

Figure 10: Comparison of *E. coli* and *S. aureus* - relationship between average viable plate count CFUs from dried glass slides and average short line inoculation CFUs………………………………………34

ABSTRACT

Enumeration of bacteria has been the subject of research for over a century. Some of the techniques that have been developed include viable plate counts, the most probable number assessment, and more modern molecular techniques. Many of the newer molecular-based techniques provide little or no information about the viability of the cells being counted, and are generally not quantitative. Having quantitative data for potentially pathogenic bacteria on surfaces can be very useful in many settings, particularly in healthcare facilities. Knowing the numbers of bacterial cells present on surfaces within healthcare facilities may be important to attempt to study the cause of healthcare associated infections (HAIs). This study was envisioned to help develop a rapid technique to quantify viable bacteria utilizing a short (5 cm) line inoculation on agar-based growth media, providing a quick assessment of the density of bacterial cells present. To do this required determination of the relationship between colony counts from line inoculations compared with viable plate counts from the same source of bacterial cells. This study of *Escherichia coli* and *Staphylococcus aureus* has produced data that suggests that there is a close relationship between the number of cells placed on a microscope slide and the colony counts obtained from swabs used to remove those cells from the slide, whether counted by a viable plate count or via a short line inoculation on an agar surface. When the number of colony forming units (CFUs) on the microscope slide are < 5000 for *E. coli*, and < 10,000 for *S. aureus* there is good correlation between the viable plate count and the line inoculation count. This relationship between the numbers of colonies in the starting material surface and the line inoculation falls off when the numbers of colonies from the source material (the microscope slide) is greater. Factors that may contribute to this loss of correlation when the source material has higher numbers of CFUs may include surface area issues similar to limitations to statistically valid viable plate counts that have been known for a century. The results of this work should lead

to additional studies with different species of bacteria, as this study only included one Gram-positive, non-motile coccus (*S. aureus*) and one Gram-negative, motile rod (*E. coli*). Other species of bacteria should be studied.

Section 1

Background and Introduction

1.1 Literature Review

It is important to be able to enumerate bacteria efficiently, accurately, and cost-effectively from environmental samples. This is especially true when examining and enumerating samples from healthcare settings. Many enumeration techniques are either expensive, time-intensive, or labor intensive. Techniques such as real-time PCR (RTPCR) or Quantitative PCR (QPCR) are very specific and accurate for specific bacterial species. However, the issue lies with viability and the means through which this technique enumerates bacterial cells (as gene-count, which requires a separate calibration). This study aims to calibrate a short line (5 cm) inoculation technique developed by the University of Tennessee at Chattanooga's Clinical Infectious Disease Control research group (CIDC) to rapidly enumerate bacterial contamination on surfaces in healthcare facilities. With this technique, surfaces are swabbed, the swabs are transported to the CIDC lab on ice where they are then used for a short-line inoculation on an agar plate. Through the use of different types of agar-based media (e.g., selective and differential, or simply non-selective) different types of bacteria can be identified and enumerated. After the inoculated colonies have grown up and are counted, a density of contamination score can be given for the swabbed sites. This calibration study was necessary in order to provide data to link the density of contamination scores to the much more commonly utilized results of viable plate counts in Colony Forming Units (CFUs). This provides a fast and cost-effective way of enumerating viable bacteria. However, it is important to examine other

enumeration techniques available, which offer different types of data that might be important in differing ways.

One of the oldest techniques used to enumerate bacteria is the most probable number (MPN) estimate of bacterial numbers in aquatic samples. Developed to study water quality, the original MPN tests focused on counting coliform bacteria through the use of a lactose broth medium, and a multiple tube inoculation to determine fermentation of the lactose (Galvin 2010). To provide quantitative data, this MPN technique requires a complex statistical treatment of the results obtained to yield a "MPN" index per 100 ml for the initial aquatic sample. The MPN technique is still utilized for many studies. Recently, Galvin et al. (2010) sampled effluent wastes from hospitals and other clinical sites to determine the most probable number (MPN) of *E. coli* and the percentage of that bacteria that was antimicrobial resistant. They created a commercial MPN technique to enumerate how many *E. coli* isolates were resistant to specific antimicrobial agents. While this form of bacterial enumeration is based on an older technique, these researchers modified the technique to focus more on antimicrobial resistance. Although providing new data, the basic MPN technique used remains a very labor, materials, and time intensive procedure.

Another group of researchers at a hospital in Winnipeg, Canada developed a modified enumeration technique that combined two separate older techniques (Warner and Glassco 1963). One of the methods they used involved setting agar plates of various media types with the lid off on the floor to allow airborne bacteria to land on the plates. The plates were left out for several hours. The second method was using a Bourdillon "small" slit air sampler in 5-minute increments. The data between the two techniques varied, but was similar to previous published articles using the same methods. However, when numbers generated by their technique were

compared with the number of hospital infections in a unit of that hospital, little agreement between bacterial numbers from their work and the numbers of infections was found. While this was an attempt to enumerate bacteria in a clinical hospital setting, it used techniques that apparently did not generate data that might have been useful in a discussion of hospital infections. Today we know that studies of bacterial contamination of environmental surfaces in healthcare facilities suggest that enumeration of bacteria on high-touch surfaces or the floors may prove much more important (Han 2015).

Many new techniques to enumerate of bacteria have been developed. One field of study where innovative enumeration techniques have been developed is the food industry. Although the target of these enumerations is very different from a clinical setting, these techniques are useful in examining many species of bacteria that are potentially pathogenic that may be found in foods. According to Gracias and McKillip (2004), many laboratories depend on typical microbiology techniques such as viable plate counts when looking for bacterial contamination of foods. This is due to the complexity, cost, and accuracy of other techniques. One modification of the viable plate count technique used with foods is the addition of a selective layer of agar on top of nonselective media such as tryptic soy agar (TSA) for viable plate counts. This selective layer enriches isolation of targeted bacteria by increasing the sensitivity of the media. This technique is a lower cost method than modern molecular tests, and in addition provides data on viability of bacteria found in the foods. However, this still requires the use of serial dilutions which can be time, materials, and labor intensive. A similar technique is to use specific selective media for plating such as XGAL, CHROMagar, and Colilert. These different types of media are extremely useful due to the target species they will allow to grow, but again a serial dilution is still required in order to inoculate the media. As noted above, the CIDC has modified the

inoculation of media like these through the use of line inoculations. The short-line inoculation technique can allow the use of eight swab samples per plate, increasing the cost-effectiveness of the process.

Other techniques used to identify bacteria from many different types of samples include metabolic tests. Although not readily used for enumeration, specific utilization of sugars, amino acids, and other nutrients have been used with environmental samples to identify the presence or absence of specific species of bacteria (Talaiekhozani et al. 2015). These techniques most often require the use of pure cultures, which inserts another step into the process. Additionally, these technique are time and labor intensive. Based on a requirement for pure cultures, other methods that enable identification of bacterial species, but not necessarily their numbers include: immunomagnetic separation (IMS), enzyme-linked immunosorbent assay (ELISA), and ATP bioluminescence assay (Gracias 2004). In order to make many of these identification tests quantitative, it would be possible to set up tests following a MPN design. However, to do this would add a great deal of cost, time, materials, and labor.

Utilization of viable plate count technology in other, novel ways to enumerate bacteria has led to other new techniques. One of these is the drop plate technique. This technique, like the standard viable plate count method, uses a serial dilution and plating. The standard technique is to dilute and place a 100µl aliquot onto an agar plate and spread it. The drop plate technique places a "drop" of 10 µl onto an agar plate which is allowed to dry into the agar. One of the benefits of this technique is that the same plate can be used for multiple drops, which saves time and money. Some data using this drop test suggests that this technique is just as accurate as the standard viable plate count procedure, but it has not yet been fully adopted (Naghili et al. 2013).

An entirely different group of tests to enumerate bacteria from environmental samples has been developed using molecular techniques. Essentially, these tests require the extraction of DNA from the samples, with further work required to identify specific genes on this DNA. Much like the biochemical tests used to identify bacteria discussed earlier, determination of the presence or absence of genes from DNA~~,~~ does not enumerate the bacteria present. Through the use of the Polymerase Chain Reaction (PCR), specific genes can be amplified if the appropriate primers are used in the process. Modifications to the PCR process developed in the early 2000s resulted in a process that will count gene-copy as the amplification process goes through sequential cycles (Zhu 2020). This technique, quantitative real-time PCR (QPCR or RT-PCR), produces gene-copy numbers based on the original starting DNA sample. Brinkman *et al.* (2003) utilized QPCR to evaluate six species of the yeast *Candida* sp. for water samples collected from Lake Michigan. First, the researchers had to create a stock culture to calibrate the QPCR. This involved using a mini bead-beater and centrifuging the yeast to isolate the genomic DNA. Next, a primer and probe set had to be developed to target the specific yeast species. The QPCR method also has a minimum gene detection limit for each yeast species. These detection limits had to be determined prior to using the Lake Michigan water samples (Brinkman 2003). It is important to recognize that the QPCR method was used for water samples rather than in a healthcare setting. González *et al.* (2003) have used QPCR to enumerate acetic acid bacteria from environmental samples. However, these studies demonstrate the time intensive nature of the QPCR method. While it has high specificity and accuracy for quantification when properly calibrated, the method is very complex, costly, and requires a lot of time intensive preparation. Another problem that QPCR has when enumerating bacteria from environmental samples is the fact that QPCR data provides no indication whether the DNA from the sample came from viable

bacteria, from dead bacteria, or from unculturable bacteria that may not grow on media used in microbiology labs (Li et al 2014, Wade 2002). Questions of viability of bacteria, especially for samples from healthcare facilities are of great importance in any studies of HAIs.

Several of the techniques discussed here have been used for enumerating bacteria in a variety of settings. However, many of them have drawbacks such as time, cost, labor, or complexity. An additional issue, particularly with molecular techniques used to enumerate bacteria is the fact that the data provided does not necessarily yield evidence of viability of the bacteria being identified. Additionally, throughput of large numbers of samples, especially using molecular techniques poses problems, including high expenses. Therefore, the development of rapid identification techniques for viable bacterial cultures from environmental samples that also provides enumeration could be quite helpful, especially in the healthcare setting. The line inoculation technique presented here, with proper calibration can provide data on numbers of viable bacteria present in environmental samples. The calibration process discussed here will convert the density of contamination scores produced from the colonies found after the line inoculations into colony forming units (CFUs). Having such data in units of CFU allows for comparison of values from many different settings where viable plate counts have been used to enumerate bacteria. This line inoculation technique is much cheaper, systematic, and capable of processing larger amounts of samples than other techniques used previously.

1.2 Introduction

Bacteria are occupants of every habitat on Earth. From deep-sea hydrothermal vents to the lining of the human intestines, species of bacteria are found. This theme is especially true for hospitals and other healthcare facilities. A critical health issue in these facilities is healthcare-

associated infections (HAIs). According to Wang, et al. (2019), HAIs impact hundreds of millions of people each year globally. Primarily, an infected patient is noted to have an HAI when the patient has been admitted to the hospital for 48 hours, and the infection was not present at the time of admission (Wang 2019). While hospitals consistently sanitize essential equipment and high touch surfaces, bacteria are still found in high numbers on surfaces throughout the facilities. Additionally, this cleaning has not always been effective and may be related to the frequency of HAIs annually. Wang, et al. (2019) provided data on the occurrence of HAIs from the World Health Organization (2011) such that high-income countries reported 3.6 to 12% incidence and low-middle income countries reported 5.7 to 19.1% (from 1995-2010). Links between environmental bacterial contamination in healthcare clinics and HAIs have been observed for intensive care units (Shiomori et al. 2001, Adams and Dancer 2020), and may exist for other hospital units.

Pathogen transmission in a clinical setting can occur from numerous different surfaces. Surfaces that may play roles in the transfer of pathogens to patients have been the focus of research. Donskey (2019) found that portable and shared equipment contributed to the dissemination of bacteria throughout the hospital. This equipment comes into direct and indirect contact with patients and the staff which can lead to the transmission of potential pathogens. Specifically, it was found that electronic thermometers have the most likely chance as a vector for pathogen transmission. It was noted that other studies have suggested that portable equipment can lead to the dissemination of viral DNA markers throughout healthcare facilities, including into other wards (Donskey 2019).

Stationary equipment and high touch surfaces are often cleaned, but contamination can still be found on these surfaces. One study sought to identify hospital floors as a potential source

for the circulation of pathogenic bacteria onto high touch surfaces. In this study, the researchers transferred a specific amount of surface area on the floor near patient beds with a nonpathogenic virus (a bacterial virus that would not target human cells) as a marker; the objective of this study was to evaluate if the virus swabbed on the floor would be transmitted to other surfaces. The virus marker was not only found on the high touch surfaces in the patient's room but also in adjacent rooms and on nursing stations located outside of the patient's room (Koganti 2016).

Concern for contamination of clinic floors has resulted in studies focused on the frequency of the isolation of pathogens on surfaces. Deshpande et al. (2017) examined how frequently isolation room floors are contaminated with common healthcare-associated bacterial pathogens. Although it is fairly well-known that floors are highly contaminated, disinfection regimens for floors are poor (or even absent entirely) because there is little to no direct patient contact with the floors. However, the researchers found that high-touch objects like medical equipment, personal items, and room linens actually often do come into contact with the floors. Therefore, the hospital was focusing on cleaning high-touch objects, but these objects were still getting contaminated from the floor (Deshpande 2017). This result is partially due to the lack of attention paid to the floors of the hospitals in cleaning protocols used by their staff. Otter et al. (2013) noted that according to recent research, contaminated surfaces are significant factor in the transmission of bacteria that can lead to healthcare-associated infections.

A separate study sought to identify the mechanisms by which floor surfaces or ground contamination led to human infection. The study noted that shoe soles are consistently contaminated with infectious bacteria that cause HAIs. Rashid, et al. (2017) states that upwards of 40% of shoe soles found outside of clinical settings are contaminated with *Clostridium difficile*. Therefore, it is likely that individuals entering a healthcare setting may be introducing

potential pathogens via their shoes. The researchers came to the conclusion that there are direct and indirect pathways that lead to contaminated floor surfaces to contact humans. The direct pathways included direct human contact and aerosolization. Direct human contact is the result of patients touching clothing such as socks and shoes that have been in contact with the contaminated floor. Aerosolization is created by individuals walking on the contaminated floor, which allows for the microorganisms to become airborne, possibly on dust particles. The study also identified an indirect pathway through arthropod-borne transmission (Rashid 2016). Another study focused on the efficacy of a recently developed shoe sole UVC device to lower pathogen colonization on floors, surfaces, and patients. The researchers noted that other studies had identified the presence of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and multi-drug-resistant Gram-negative bacteria on shoe soles. The researchers added that shoe soles can have a high contamination of bacteria (Rashid 2017).

Methicillin resistant *S. aureus*, VRE, and multi-drug-resistant gram-negative bacteria are commonly found in clinical settings and are a major cause of HAIs. A subset of HAIs called surgical site infections (SSI) is the second most common HAI behind urinary tract infections. *Staphylococcus aureus* is the most frequently isolated pathogen from SSIs. The second most common was bacteria in the Enterobacteriaceae, with *Escherichia coli* as the most predominant (Cantlon 2006). This is important to note because, according to Cantlon et al., about 2 million patients acquire HAIs each year and about 77,000 of them will die with HAI being the direct cause or a contributing factor. With *S. aureus* being the predominant pathogen isolated from SSIs in this study (and others [Manion 2003, Olson 1990]), it is critical that our study focuses on such a widely found pathogen. *Escherichia coli* was the second most predominant species isolated from SSIs. In a separate study, *E. coli* was found to be the seventh most common agent

of SSIs (Weiss 1999). Due to its high association with SSIs and being a well-known human commensal with a high capacity for pathogenic activity in intestinal surgeries, this study also focuses on *E. coli*.

This information is important to recognize the hazard that the presence of these pathogenic microorganisms in hospital settings provides for healthcare workers and patients. It would seem that studies to enumerate the variety of species of microorganisms that are present in healthcare facilities should be routine. The studies mentioned previously mostly focused on bacterial transmission and community structure, rather than enumerating the specific pathogenic microorganisms present in/on the specific sites found in the healthcare facilities. A small number of studies actually provide data linked to viable bacterial cell numbers in healthcare facilities. This is due in part to limitations inherent in the laboratory techniques used to evaluate the presence or absence of bacterial groups and to enumerate specific species of bacteria in the environments.

Enumeration of viable bacteria requires that some sort of culture-based study be conducted. An example of a good way to enumerate viable bacteria is to utilize a viable plate count technique, which is practiced widely across the food industry. To use this technique a known quantity of food is extracted, serially diluted, and plated for viable plate counts. This allows the researchers to calculate an estimate of the CFUs in the original food matrix (Bajwa 2013). Through the use of a serial dilution with plating to an appropriate growth medium, colony counts can be obtained. Studies of this nature tend to be time and material consuming. However, trying to replace viable plate counts with gene-copy numbers using RT-PCR again brings up the question of viability. Work done by Keilman et al. (2021) using a modified agar medium

inoculation process and line inoculations, leading to a colony count may help provide a new technique to scan hospital environmental surfaces for viable bacterial contaminants.

Beginning in 2015, UTC's Clinical Infectious Disease Control research group (CIDC) has sought to survey numerous potentially pathogenic bacteria in outpatient physical therapy clinics, as well as in inpatient units in Erlanger Hospital (e.g., Keilman et al. 2021, Spratt et al. 2014). In order to provide a rapid assessment of a multitude of samples from the clinics, CIDC developed an assessment protocol where transport swabs collected from facility surfaces were used to inoculate different types of agar media (selective and differential) with a short line inoculation technique. The amount of bacteria present along the lines of growth after incubation is used to determine density of contamination estimates as either "low" (1-5 colonies), "medium" (6-15 colonies), or "high" (>15 colonies). This technique provides a good semi-quantitative assessment of pathogens that are present at the locations that they are collected. However, this technique is only minimally quantitative.

The objective of this study is to help provide data on the enumeration of two potential pathogens, *E. coli* and *S. aureus*, linking viable plate counts (CFU) to the density of contamination estimates provided by the CIDC's line inoculation enumeration technique. The two bacterial species tested were *S. aureus* and *E. coli*. These bacterial species have been chosen because they are known occupants of surface environments in Erlanger Hospital and due to their relevance as human commensal bacteria or as potential pathogens. Enumeration of these bacterial species was done *in vitro*, using microscope slides coated with known quantities of these bacteria as target surfaces. Through the use of swabs to remove the bacteria from the slides, enumeration of cells on the swabs and enumeration of bacteria on line inoculations has resulted

in calculation of correction factors for these species to convert density of contamination estimates into actual CFU values.

Section 2

Materials and Methods

This study was conducted in the Clinical Infectious Disease Control (CIDC) research lab in Holt Hall, on the UTC campus (Holt Hall, room #305). Standard microscope slides were placed in glass petri dishes and autoclaved to produce uniformly sterile surfaces having the same surface areas. Lab strains of *S. aureus* and *E. coli* were obtained from the UTC Microbiology Prep lab (Traceable to ATCC strain 25922 [*E. coli*], and ATCC strain 25923 [*S. aureus*]). These cultures were maintained in the CIDC lab using either tryptic soy agar (TSA) or tryptic soy broth (TSB), both obtained from Fisher Scientific (Suwanee, GA). Serial dilutions were conducted using sterile 0.85% saline, and in some cases in sterile TSB. As serial dilutions were made the tubes were stored in an ice bath to arrest their growth. A separate sterile transport swab (with liquid Stuart's medium, Fisherbrand, Fisher Scientific, Suwanee, GA) was used to remove bacterial cells from each glass slide onto which the cells had previously been placed and dried (see below).

As noted above, the purpose of this study is to calibrate the line inoculation technique with a viable plate count to provide a quicker and more cost-effective way to estimate the CFUs of viable bacterial cells present on the sample swabs. This was done by quantifying bacterial cells using viable plate counts of pure cultures of *E. coli* or *S. aureus*, which would be used to produce known quantities of these bacteria on standardized surfaces (sterile glass microscope slides). To do this a known aliquot (from the 10^{-3} dilution) of one of the pure cultures was placed onto replicated (triplicates) of sterile glass microscope slides. Two sets of triplicate slides were used for each experiment for one pure culture; one set was swabbed to determine the CFUs of bacteria placed on the slides. The second set of replicated slides was used to inoculate a TSA

plate using the short (5 cm) line inoculation technique. The data acquired was used to determine an estimate of the CFUs at the initial sample based the number of CFUs produced by the short line inoculation. Using glass microscope slides allowed for a controlled environment where the bacteria was dried and swabbed each time. This study converted the density of contamination scores into an estimate of the original CFUs.

2.1 Materials and Preparation

As noted above, glass microscope slides were placed inside glass petri dishes and sterilized in the autoclave. These petri dishes were then placed in a drying oven (60 ^{o}C) to ensure that all of the sterile slides were dry. For each experimental replicate, individual sterile slides were aseptically transferred into individual plastic petri dishes. Tryptic soy agar was the medium of choice to both grow up the cultures and plate them. TSA was made by adding TSA to distilled water according to the manufacturer's instructions in a 1L Erlenmeyer flask and mixing it thoroughly while on high heat until boiling. For the TSA slants, boiled aliquots of the liquid TSA were transferred into multiple test tubes prior to sterilization in the autoclave. For TSA plates, the 1L flask of boiled TSA was directly autoclaved. After sterilization, the TSA tubes were placed on a slanted surface and allowed to solidify to produce slants. The TSA in the 1L flask was poured into pre-sterilized plastic Petri dishes directly after sterilization at about 15 mL per dish. The plates were allowed to solidify, stacked and allowed to dehydrate for 2-3 days before use.

Sterile 0.85% NaCl saline was also used in the study. To make the solution, 8.5 g/l of NaCl was added to distilled water in a 1L flask and hand-stirred until all NaCl had dissolved. The flask of saline was then autoclaved. Additionally, sterile TSB was also used for dilutions in this study. To make the TSB solution, TSB was added to distilled water according to the

manufacturer's instructions and stirred at low heat until all of the TSB had dissolved. The TSB was then autoclaved. Prior to each experiment, the bacterial cultures were grown on TSA slants over 48-72 hours at room temperature. When a 24-hour culture was needed, they were grown at 37°C.

2.2 Experimental Design

To begin an experiment, the selected bacterial culture was grown on a TSA slant over 24-72 hours at room temperature. Bacterial cells were removed from the TSA slant by adding 1 milliliter (mL) of sterile 0.85% NaCl saline using a sterile pipet and teasing the cells off the slant to make a suspension. The 1 mL of solution was then removed from the slant and transferred to a sterile test tube. The general serial dilution scheme for cells present in the suspension from the slant is diagramed in Figure 1. From the bacterial suspension, 0.1 mL was transferred to 9.9 mL of sterile tryptic soy broth (TSB), which was kept in an ice bath until it was used later. Note that the diluent used here was TSB and not 0.85% saline. This was due to the need to have these cells suspended in a rich organic mixture (the TSB) to help support their survival after being dried down on the surface of sterile microscope slides (see below). When cells are suspended in a rich organic mixture their survival on drying is much greater (Spratt et al., 2019). This mixture was then mixed using a vortex mixer for 15 seconds. One mL of this mixture was then transferred to a test tube containing 9.0 mL of sterile TSB. This mixture was vortexed for 15 seconds and 1 mL was transferred to a test tube containing 9.0 mL of sterile 0.85% NaCl saline. Once this transfer was complete, the previous tube containing 9.0 mL TSB + bacteria was placed on ice to preserve bacteria cells and slow their growth. This tube (the 10^{-3} dilution tube) was later used to place 0.025 mL aliquots onto glass microscope slides. The remaining 1:10 dilutions needed to complete the serial dilution for the viable plate count were made as indicated in Figure

1. The serial dilution ran through the 10^{-7} dilution. For the viable plate counts 0.1 mL from each of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions were plated. Later in the study additional dilutions were added to the scheme to produce lower numbers of cells for the aliquot added to the microscope slides. For the plating to TSA plates, the desired dilution was vortexed for 15 seconds and 0.1 mL was removed. This aliquot was then placed onto the plate and spread using a spreading rod, previously sterilized by flaming with alcohol.

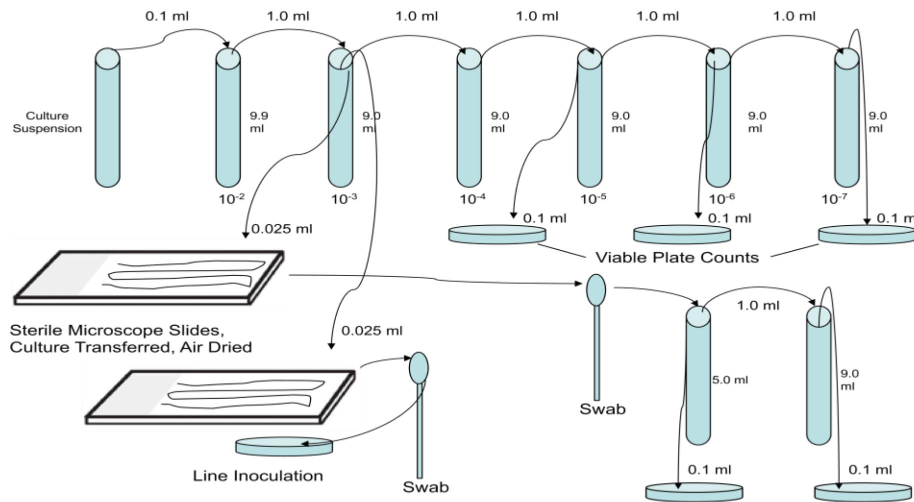

Figure 1: Experimental design scheme used in this study

For a majority of the experiments run for both cultures, the 10^{-3} dilution was used to transfer 0.025ml aliquots onto two triplicates of glass microscope slides. Use of this dilution of cells resulted in a final number of cells being placed on the sterile microscope slide surface from $2.53x10^5$ to $2.58x10^3$ cfu/ml for *E. coli* and $4.23x10^5$ to $2.80x10^3$ cfu/ml for *S. aureus*. The 10^{-3} dilution was removed from the ice bath and was vortexed for 15 seconds for each transfer of 0.025ml onto the glass microscope slides. The aliquot was placed on the slides and spread ("painted") on the surface to place cells over a uniform surface area, which were then air dried. For a typical experiment, the first set of triplicate slides was dried using a stream of filtered air for approximately 5 minutes per slide. This first set of slides, once dried, was immediately

swabbed using a sterile transport swab to remove as many of the bacteria on the surface of the slide as possible, moving and rotating the swab to bring the entire swab surface in contact with the surface of the slide. The used swabs were immediately placed back in their transport tubes and stored on ice to ensure high bacterial survival for subsequent steps in the process. Once swabs for three slides were collected, they were later removed from the ice bath and used to transfer cells picked up to either sterile saline (for viable plate counts) or for line inoculations on TSA (see Figure 1).

Bacteria picked up by the swabs for enumeration by viable plate counts, were first suspended in a tube of 5 ml of sterile saline (see Figure 1). To accomplish this, the desired swab was removed from its protective plastic tube and placed approximately 2 inches into the sterile test tube. Flamed scissors were then used to cut the swab from its shaft, allowing it to drop into the TSB. This process took approximately 10 seconds per swab to complete. The test tube was then vigorously mixed using a vortex mixer for 30 seconds to allow for suspension of bacterial cells that had been present on the swab surface. One ml of the mixed TSB + swab was transferred to another test tube containing 9.0 ml of sterile saline. Both tubes were then vortexed for 15 seconds and plated (0.1 ml) onto TSA plates and allowed to grow for 48 hours. *E. coli* was grown at room temperature and *S. aureus* at 37°C.

As previously mentioned, further dilution was occasionally required in the initial serial dilution to decrease the number of colonies present when the short line inoculation technique was used. One to two extra test tubes of sterile TSB was added between the first 9.9 mL TSB and 9.0 mL TSB tubes. The dilution for these extra tube(s) ranged from 1:2, 1:3, 1:5, 1:7, 1:9, 1:10, 1:2+1:10, 1:5+1:10, and 1:7+ 1:10. This was necessary due to the original serial dilution scheme producing high numbers for the short line inoculation. To produce lower values that fall within

the “low” and “medium” contamination scores, these dilutions were added. No other aspect of the protocol was changed.

In order to provide a comparison between viable plate counts and the line inoculation from glass microscope slides with known quantities of bacteria on them for swabbing and transfer to TSA agar using a line inoculation technique (as the CIDC samples are treated), another set of triplicated, dried bacterial slides was prepared. While the first triplicate of slides were being processed (and used to count the cells by way of a viable plate count), the second triplicate set of slides was being allowed to air dry, using filtered air to complete the process. After collecting cells from this second set of slides the swabs were immediately placed on ice. Once all three slides were swabbed, they were used to perform the short (5 cm) line inoculation onto TSA plates, replicating the technique used by UTC’s CIDC from previous studies at Erlanger Hospital. The inoculated plates were then incubated for 48 hours. *E. coli* was grown at room temperature and *S. aureus* at 37°C.

Once grown, colony counts were calculated for both species of bacteria for the two different treatment techniques used. For the line inoculations made on the plates density of contamination scores (as used by the CIDC) were generated. Low densities represented 1-5 colonies (“Low” contamination), medium densities had 6-15 colonies (“Medium” contamination, and high densities had >15 colonies (“High” contamination). These contamination scores were compared to the number of bacteria (via viable plate counts) taken from the first set of microscope slides to calibrate the estimation protocol. To statistically analyze the calibration experiments, a Student’s T-test was used on the means of the replicates.

2.3 Calculations and Statistical Analysis

To determine the percent agreement between the line inoculation and colony forming units on the swab, viable plate counts from the plated slides had to determined. This value was used to determine how many CFUs/ 5mL was in the TSB suspension after swabbing. Once this value was determined for each triplicated slide, an average was taken. Next, colonies had to be determined for the short line inoculations (see Appendix I).

Since swabs were used to transfer bacteria onto the microscope slides and to transfer bacteria onto the agar plates via line inoculations, it was important to have a good estimate of the surface area of the swabs. A brief search of the literature did not turn up any published work focused on transport swab surface area. So, a procedure was developed to calculate the surface are of the swabs used. Having knowledge of this surface area was important because in the line inoculation only one portion of the swab was brought into contact with the agar as the swab was moved approximately 5 cm across the agar surface, although the entire swab was used to pick up bacteria from the slides (or from some environmental site). To account for bacteria remaining on the swab after a line inoculation the total surface area of swab, and the surface area of the swab that comes in contact with the agar should be known. To estimate these surface areas the following procedure was used. First, to estimate the surface area of the swab placed in contact with the agar surface for a line inoculation one side of a sterile swab was placed onto a glass slide without moving it. The wet mark left after the swab was placed on the glass was outlined (on the back of the slide) with a Sharpie. Then, to estimate the total swab surface area, a fresh swab was first marked with a small black dot on the shaft of the swab. The swab was then placed at one end of a new glass slide (with the black dot facing up), and the swab was rolled down the slide until the black dot again was facing up. This motion resulted in an elliptical mark on the slide, which was then traced with a Sharpie on the back of the slide. Then, to account for the tip

of the swab, the tip of that same swab was placed on an unused portion of the slide to make a circular mark on the slide, which was also traced on the back of the slide. The areas of these tracings were then calculated, and a correction factor to account for the entire surface area of the swab (when used for a line inoculation) was made. The number of colonies counted on the agar petri dishes used for the line inoculations were then multiplied by this correction factor to produce as accurate an estimation of the number of CFUs that were present on the swabs (coming from the original glass slides). Figures 2 and 3 represents this swab area calculation procedure.

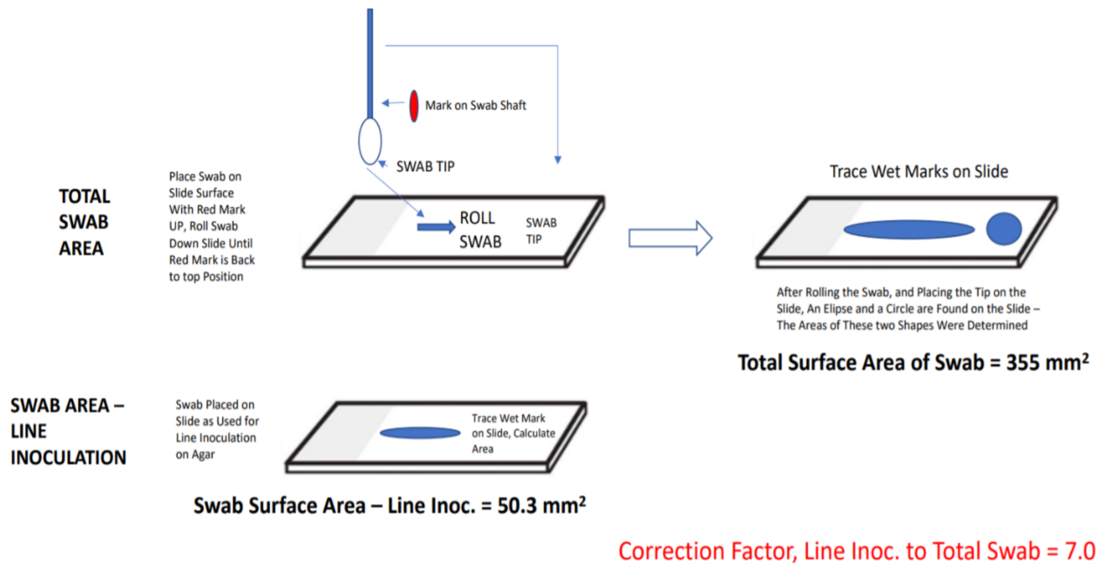

Figure 2: Technique used to estimate the surface area of the swab and short line inoculation, providing a correction factor

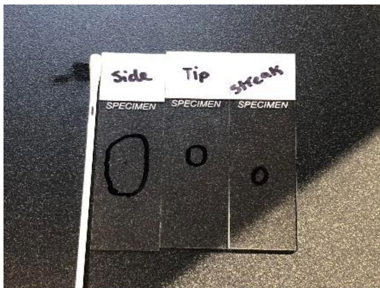

Figure 3: Swab and slides used to estimate surface area. Tracings of the swab contact patches are evident in the photo.

To determine the survivability of each bacterial species, the initial bacterial suspensions CFUs/ ml had to be calculated. This was done by dividing the highest viable plate count number within the statistically valid 30-300 CFUs/ ml range by the dilution factor that was plated. This allowed the CFUs/ml to be calculated for each dilution in the serial dilution scheme. The CFUs/ ml transferred to the slides were then determined (see Appendix I). Using the average CFUs/ 5 mL (per run), the percent survival of each bacterium per run was determined and averaged.

The statistical test run on the *E. coil* and *S. aureus* survival data was a two-tailed, independent means t-test. Statistical significance was observed when $p < 0.05$.

Section 3
Results

The process of transferring *E. coli* and *S. aureus* to sterile glass microscope slides required that these cells be air dried. The air-drying process is often associated with massive die off of bacterial cells (e.g., Spratt et al 2019). For all of the experiments run here the calculated survival of *E. coli* and *S. aureus* about 15 minutes after drying on the slides was about 12 % for *E. coli* and 30% for *S. aureus* (Figure 4). This result was significant (two-tailed, independent means t-test, $p < 0.00001$).

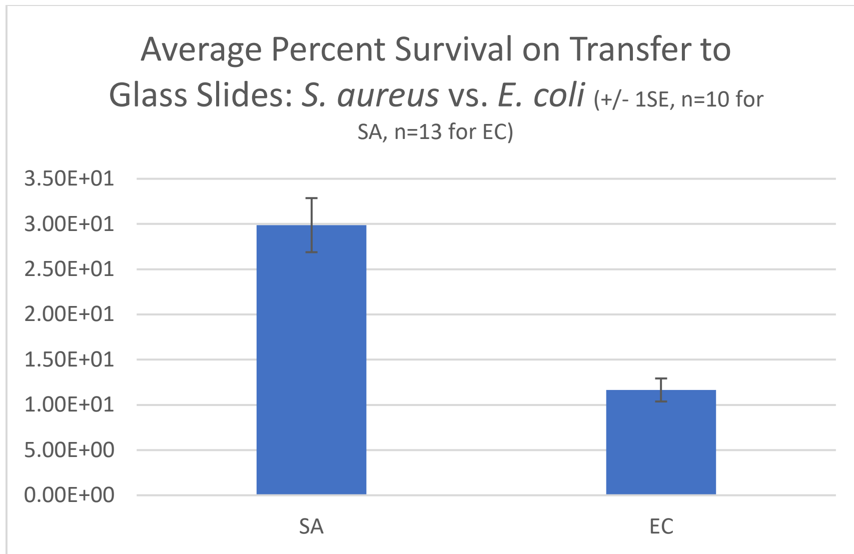

Figure 4: **Comparison of *E. coli* and *S. aureus* Survivability after transfer to sterile glass microscope slides and air drying (approximately 15 minutes after the transfer). This difference in the survival for the two cultures was highly significant (two -tailed independent means t-test, $p < 0.00001$).**

For the portion of the experiments focused on the transfer of *E. coli* or *S. aureus* from slides to serial dilution viable plate counts and for the line inoculations, each individual experiment was run in triplicate, and the values plotted in the following graphs are based on averages. For a complete summary of all data collected, and calculated values for each experiment, see Appendix II.

3.1 *Escherichia coli*

The agreement between line inoculation colony counts and the number of viable *E. coli* counted from the 5 ml mixed tube (CFUs/ 5ml) had an inverse relationship. A power curve was used to best fit the data and provide an equation to represent the relationship between viable plate

count of the cells dried on the glass slides and bacterial cells represented on the short line inoculation (Figure 5). Note that the agreement between this trend line and the data for *E. coli* is not perfect ($R^2 = 0.345$) .

<u>*E. coli* equation</u>: **Avg % Agreement = 69.233 x (Viable Cell Count of Slide)$^{-0.326}$**

At higher CFUs/ 5ml on the glass slides the percent agreement between viable plate count of colonies from cells dried on the glass slide and colonies from the line inoculation was lower, but more consistent. At lower CFUs/ 5ml, the agreement was typically higher, although variable in different experiments. Therefore, bacterial samples with lower CFUs had higher agreement, likely due to a higher chance of survival with less competition.

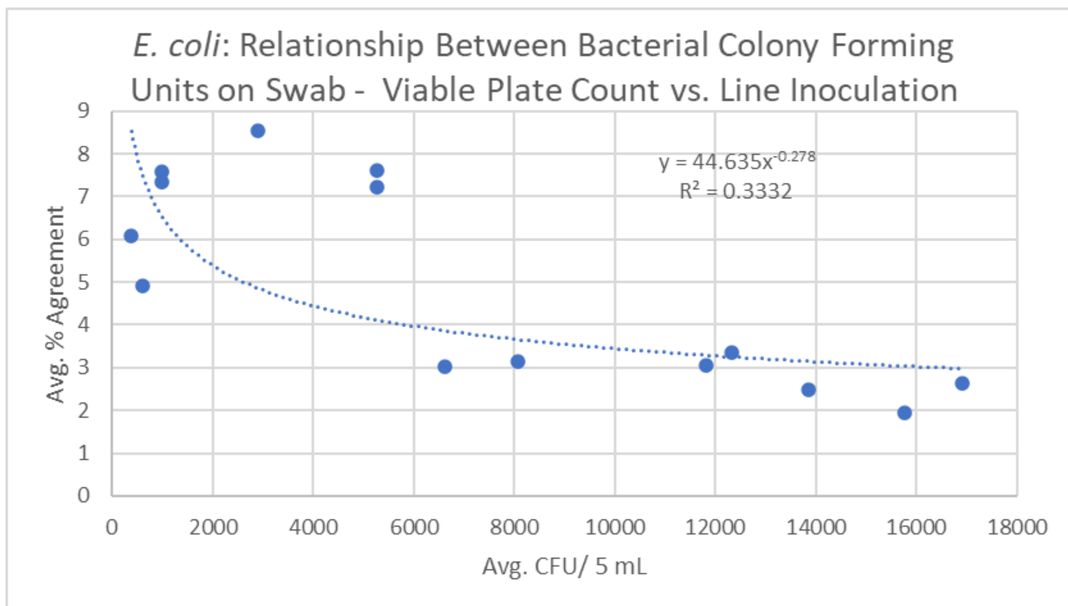

Figure 5: *E. coli* - relationship between the Average % Agreement of colony counts (comparing agreement between viable plate counts for cells dried on glass slides with colony counts from the line inoculations) and the number of dried cells on the glass slides.

The relationship between average line inoculation colony counts and the average number of viable *E. coli* counted on agar plates from the 5 ml mixed tube showed a potential maximum statistically valid limit of 35-40 CFUs from the short line inoculation (Figure 6). An exponential

curve was used to best fit the data and display this maximal statistical limit for *E. coli*. Note that the trendline and data for *E. coli* is not perfect ($R^2 = 0.411$)

E. coli equation: **Avg. Viable Plate Count CFUs = $12.745e^{0.0456x}$ (Avg. Line Inoculation CFUs)**

The trendline is linear in appearance from the 0-35 CFU short line inoculation range. At this point, the trendline begins to gradually increase in slope and exponentially increases after 40 CFUs. The 37 CFU point from the short line inoculation could prove to be the maximal statistical limit for the technique.

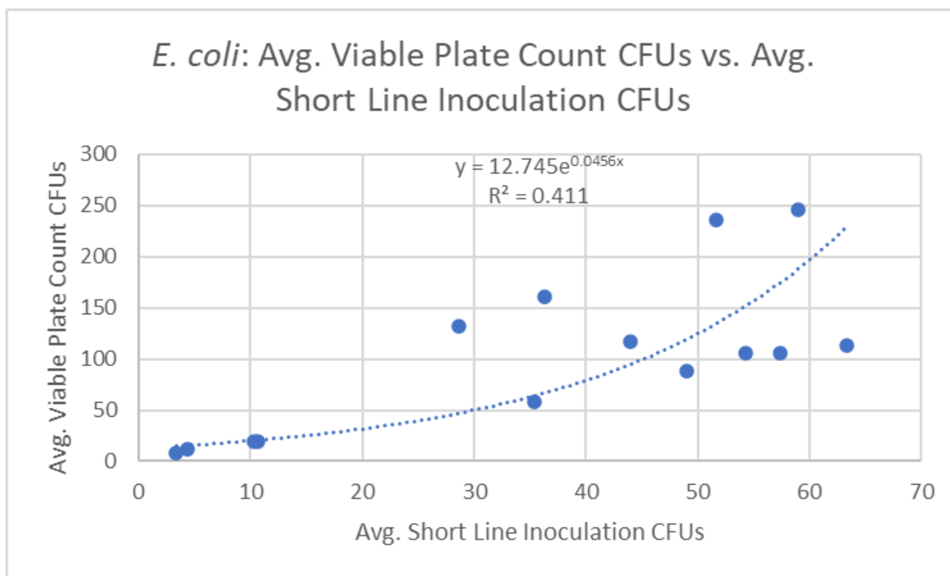

Figure 6: *E. coli* – relationship between average viable plate count CFUs from dried glass slides and average short line inoculation CFUs

3.2 *Staphylococcus aureus*

Similar to *E. coli,* the agreement between line inoculation colony counts and the number of viable *S. aureus* counted from the 5 ml mixed tube (CFUs/ 5ml) on the glass slide had an inverse relationship. A power curve was used to best fit the data and provide an equation to represent the relationship between viable plate count of the cells dried on the glass slides and

bacterial cells represented on a short line inoculation (Figure 7). Note that the agreement between this trend line and the data for *S. aureus* gave a better fit than the line for E. coli (R^2 = 0.798) .

<u>*S. aureus* equation</u>: **Avg % Agreement = 275.06 x (Viable Cell Count of Slide)$^{-0.516}$**

At higher numbers of *S. aureus* on the slide surface the percent agreement between viable plate counts of colonies from cells dried on the glass slide and colonies from the line inoculation was lower. At lower CFUs/ 5ml, the agreement was typically higher, although slightly variable in different experiments. Therefore, bacterial samples with lower CFUs had higher agreement, likely due to a higher chance of survival with less competition. This could also be due to the lower CFUs grown after line inoculations and more ease in counting. *Staphylococcus aureus* showed more consistency between the two variables at both high and low concentrations of CFUs/ 5ml than was observed for *E. coli* (Figure 7).

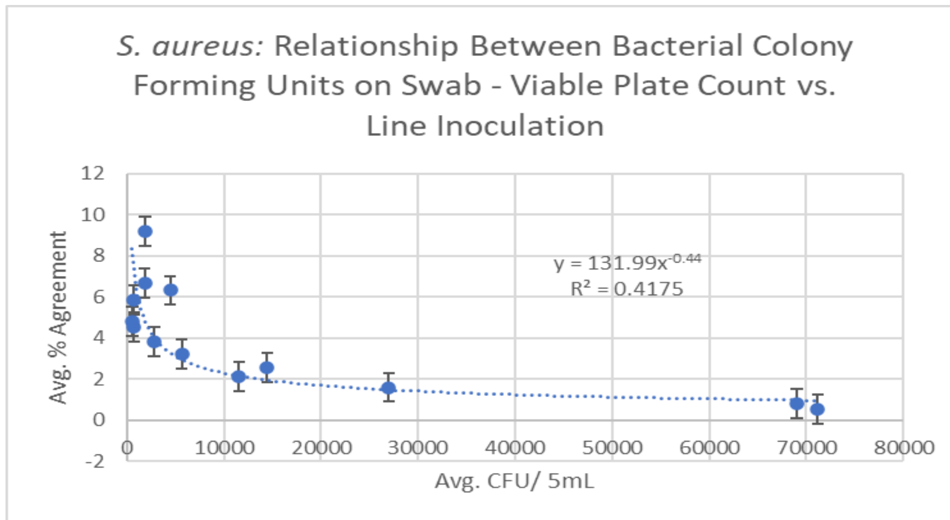

Figure 7: *S. aureus* - relationship between the Average % Agreement of colony counts (comparing agreement between viable plate counts for cells dried on glass slides with colony counts from the line inoculations) and the number of dried cells on the glass slides.

The relationship between average line inoculation colony counts and the average number of viable *S. aureus* counted on agar plates from the 5 ml mixed tube showed a potential maximum statistical limit of 35-40 CFUs from the short line inoculation (Figure 8). An exponential curve was used to best fit the data and display this maximal statistical limit for *S. aureus*. Note that the trendline and data for *S. aureus* is not perfect ($R^2 = 0.2057$)

S. aureus equation: **Avg. Viable Plate Count CFUs = $21.063e^{0.0307x}$ (Avg. Line Inoculation CFUs)**

The trendline is linear in appearance from the 0-35 CFU from short line inoculation range. At this point, the trendline begins to gradually increase in slope and exponentially increases after 40 CFUs. The 37 CFU point from the short line inoculation could prove to be the maximal statistical limit for the technique.

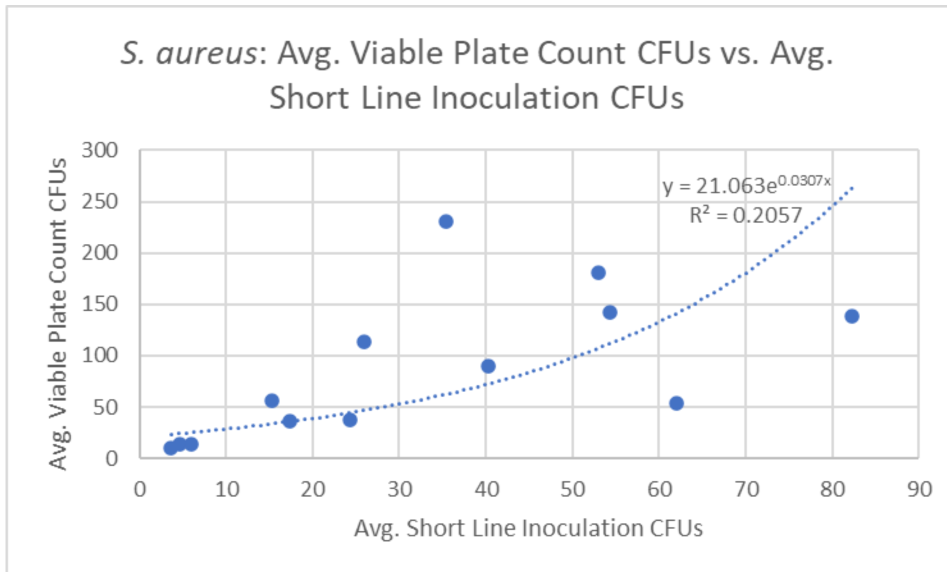

Figure 8: *S. aureus* - relationship between average viable plate count CFUs from dried glass slides and average short line inoculation CFUs

3.3 Comparison of *E. coli* and *S. aureus:* Relationship Between Bacterial CFUs on Swab

When examining the relationship between the average % agreement of the viable plate count of dried cells on the slide and colony count from line inoculations with the actual numbers of cells on the glass slides, both *E. coli* and *S. aureus* exhibited similar trends. Lower numbers of cells on the glass slide resulted in better agreement between the numbers of cells counted on the glass slide and numbers of colonies that grew on the line inoculation. The trend lines converge around 10 % agreement, suggesting that when numbers of cells picked up by the swab are very low, factors not measured in this study may also reduce agreement between the number of cells picked up by the swab and colonies that grow up in a line inoculation.

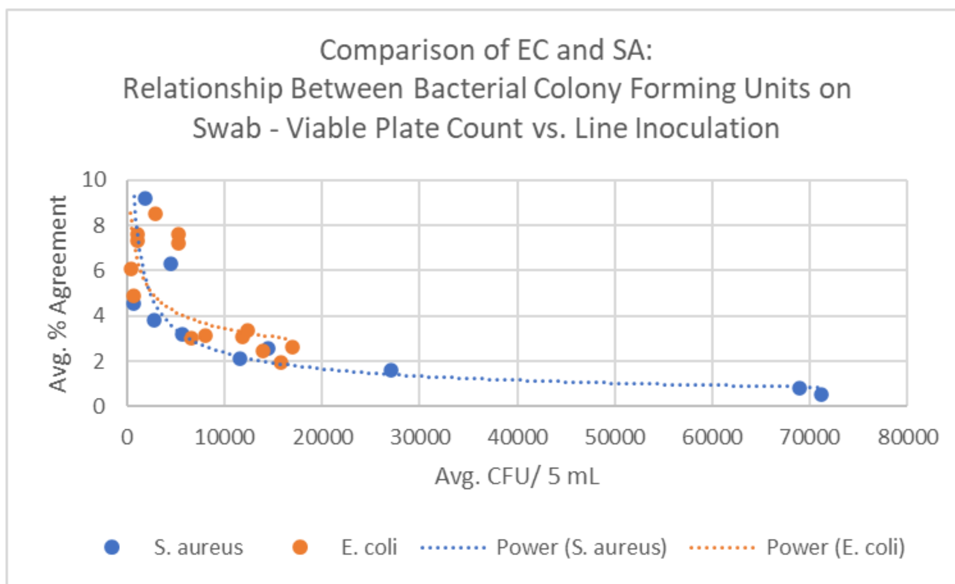

Figure 9: Comparison of *E. coli* and *S. aureus* - relationship between the Average % Agreement of colony counts (comparing the viable plate counts with the line inoculations) and the number of cells on the glass slides.

Comparing the relationship between average short line inoculation CFUs and viable plate count CFUs from the 5 mL mixed tube for *E. coli* and *S. aureus* together reaffirmed the maximal statistical limit for short line inoculations of 37 CFUs (Figure 10). Both trendlines are linear in nature prior to the 35 CFUs from short line inoculations point. At this point, the slopes for both

gradually increase. At the 35-40 CFU range, the trendlines converge, suggesting that there is a maximal statistical limit of 37 CFUs for the short line inoculation technique.

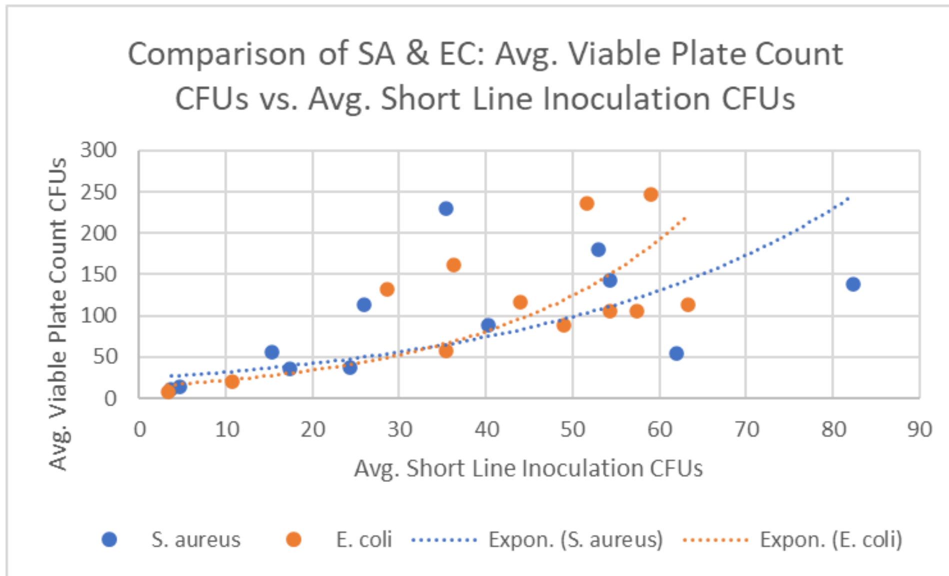

Figure 10: Comparison of *E. coli* and *S. aureus* - relationship between average viable plate count CFUs from dried glass slides and average short line inoculation CFUs

Section 4

Discussion

The results for percent agreement between the viable plate counts of cells removed from glass slides on swabs and colony counts from swabs used for line inoculations on agar surfaces suggests a relationship between the number of cells picked up by a swab and the number of colonies that grow up after a line inoculation exists, at least for *E. coli* and *S. aureus*. When higher numbers of cell CFUs/ 5ml were present on the glass slides, there was a much lower agreement between the numbers of colonies generated from the swabs for the viable plate counts and the colony counts from the short line inoculations. When lower numbers of cells were present on the glass slides, the relationship between number of colonies obtained from the glass slide and the number of colonies obtained from the line inoculations was much higher. This means that when lower numbers of cells on the surface were picked up by the swab, colony counts from the short line inoculation were more reproducible. This relationship could be similar to the reason viable plate counts must be within a statistically valid range to be useable, where viable counts for 10 cm diameter petri dishes require colony numbers in the 30-300 CFU range. The surface area utilized on the agar surface for the growth of colonies will limit the maximal number of colonies that can be observed as being separate colonies on the petri dish. This relationship appears to also be true for colonies on the line inoculations.

In an attempt to standardize a statistical limit for viable plate counts, Breed and Dotterrer (1916) evaluated several different limits to determine how many discrepancies per range of counts were present. To do so, they plated milk samples on triplicates of agar plates at three different dilutions, 1:100, 1:1000, and 1:10000. An average was taken of the CFUs counted and any value that was 20% or more above or below the average was listed as a discrepancy. In

total, 1,435 plates were inoculated. Of the resultant colony ranges tested, 40-200 and 30-400 had very similar results. After five days of incubation, the 40-200 range had the fewest discrepancies by 2.9%. However, after an additional two days of incubation (7 days total), the 30-400 range had the fewest discrepancies by 1.6 percent. They determined that the two ranges (40-200, 30-400) produced similar satisfactory results. While the researchers did not state a specific standardized range, they built the framework for the current standardized statistical limit for viable plate counts, which is 30-300 (Breed and Dotterrer 1916). According to Tomasiewicz *et al.* (1980), the aforementioned study is what led to the standardization of the 30-300 range. Tomasiewicz *et al.* also noted that other studies determined the counting errors to be much higher when the CFUs are greater than 300 (Tomasiewicz 1980). The statistical range for viable plate counts is important when considering ranges of colonies produced from the line inoculations used in this study. Similar to viable plate counts, the short line inoculation technique showed that at a certain point, the colonies became numerous and extremely difficult to differentiate and count, if at all possible. Therefore, a limit to colony counts from line inoculations used to estimate numbers of cells present on transport slides needs to be established.

Other limitations to the use of transport swabs to pick up bacterial cells from environmental surfaces exist. When a transport swab is used to remove bacterial cells from some environmental surface, space available on the swab for the cells is directly related to the surface area of the swab coming in contact with the surface (the estimated total surface area for the transport swabs used in this study was approximately 355 mm^2). Therefore, as cell numbers on the swab surface increase (or the density of cells on the environmental surface increase) a saturation limit for the swab will be reached. When the swab reaches maximum capacity for bacteria that swab cannot pick up any additional cells. When examining the graphs of %

agreement between numbers of dried cells on the slides with colonies generated from the line inoculations for *E. coli* or for *S. aureus*, there was an initial rapid drop-off of % agreement between the colony counts for the bacteria on the slide surface and the colony counts from the line inoculations until the trend line began to flatten out. The points where the trend lines change their slope (the inflection point) may be a good indicator of where this limit is. By using data generated here, those limits for *E. coli* and *S. aureus* are approximately 5,000 CFU, and closer to 10,000 CFU, respectively.

These limits to the use of line inoculation colony counts to relate back to CFU present on environmental surfaces may require further study for different species of bacteria. The two species studied here are very different types of bacterial cells with regard to cell wall composition, shape, size, and motility. *Staphylococcus aureus* is a Gram-positive coccus with a smaller colony size and no motility. *Escherichia coli* is a Gram-negative rod that is motile. Additionally, *E. coli* has a larger colony size than *S. aureus*. Another point worth mentioning is the differential survival exhibited by both species in this study, with *S. aureus* being able to survive the drying process on the slides compared with *E. coli*. All of these factors have the potential to play a huge role in how many CFUs result from the short line inoculation. Such precautions to the use of swabs to recover viable bacterial cells from surfaces may make enumeration of the cells present on the surfaces via a line inoculation more reliable. For example to limit over-crowding of bacteria on swab surfaces, a swabbing procedure that obtains two swabs for two immediately adjacent sites, with one swab from an area that is ½ the surface area of the other swab, and comparing colony counts from the two swabs might help determine if the numbers obtained are valid. This would increase the time and materials needed for these studies,

but may represent a good first sampling regimen, adjusting future swabbing to keep the CFUs obtained on the swabs in the valid range for this technique.

The displayed relationship between the percent agreement between the viable plate counts of cells removed from glass slides on swabs and colonies from the short line inoculation is possibly related to the surface area for the bacterial colonies to grow. The petri dishes used for TSA plates have a surface area of 7,854 mm^2 (for a 10 cm diameter petri dish). The short line inoculation plates were divided into 8 distinct sections, resulting in a surface area of no more than 980 mm^2 per swab used. If the same statistical limits exist between maximal colony counts for viable plate counts (300 colonies) and the short 5 cm line inoculations, which are made on approximately 1/8 of the petri dish surface = 12% of the total plate surface area, would then be = 300 x 0.12 = 37 colonies. So, a reasonable estimate for maximal number of colonies to count from the line inoculations would be 37, which is close to the number of colonies counted on the line inoculations at the inflection points in the graphs (Figures 6, 8, and 10).

Figures 7 and 8 examine this relationship and further demonstrate that there is a maximal statistically valid range for counting CFUs from the short line inoculation at 37 CFUs. Both graphs exhibit an increase in slope from linear to exponential at this point which indicates that after this point, the short line inoculation does not accurately reflect the bacterial CFUs swabbed. Figure 10 reiterates this such that the trendlines converge at around 37 CFUs. Therefore, this is the statistically valid maximum limit for this technique. The contamination score ranges should therefore be recalculated to accommodate for this finding.

The lower agreement between numbers of CFUs on the glass slides and the CFUs of the line inoculations may be related to crowding between colonies growing along the line. The bacterial cells that are inoculated have limited area to grow with very high competition among

other cells. It is very likely that this is another factor leading to the low percent agreement as the number of *E. coli* or *S. aureus* dried cells on the slides increases.

This study also provides a good look at the survival of *E. coli* and *S. aureus* as they dry on a glass surface. Data presented here suggests that *S. aureus* is much more likely to survive on a surface after drying in TSB (30% survival) compared with *E. coli* (about 11% survival). Others have observed strong survival of *S. aureus* on surfaces. According to Landers *et al.* (2010), *S. aureus* has been found to survive up to 4 weeks on the surfaces of a variety of healthcare equipment. Spratt et al. (2019) found that *S. aureus* can survive on the surface of therapeutic ultrasound heads for up to three days, depending on the level of organic matter present with the cells on the heads. Whether the differential survival of *E. coli* and *S. aureus* may be due to physical differences in these cells (e.g., cell wall structure) is not clear.

One difference between *E. coli* and *S. aureus* that might help explain the differences in survival of these cells as the dry on the glass slides is the tolerance of these cells to drying. According to Pettit and Lowbury (1968), dry conditions favor the survival of Gram-positive cocci, such as *S. aureus*. This could be an explanation as to why *S. aureus* had a higher survivability when being dried in this study. The aforementioned study examined the survival of *E. coli* and *S. aureus* on glass coverslips. The results of this study are similar to the results presented here; *S. aureus* had a higher survival when allowed to air dry on the glass coverslips when compared to *E. coli*. This may be due to the Gram-positive cocci's ability to survive in low humidity conditions. When being dried with a filtered stream of air, there is little to no humidity. Therefore, *S. aureus* would be expected to have a higher survival rate, as described in this study (Pettit 1968).

Section 5

Limitations and Future Research

This study has provided data that can help researchers who are trying to count bacterial cells present on surfaces in a relatively quick, inexpensive way. Overall, this study has succeeded in generating data that can help calibrate the use of a line inoculation with a swab that picked up bacterial cells present on a surface to determine numbers of those cells on the surface. There are limits to the use of this technique that appear to be related to the surface areas of both the swabs and the petri dish surface areas utilized for the line inoculations. When the bacteria are cocci, as for *S. aureus* in this study, the maximal number of cells on the surface that can be effectively counted using the line inoculation technique is about 10,000 CFU. For the larger *E. coli* bacilli (that are also motile) the reliable cut off for numbers of cells that can be effectively counted using this line inoculation technique is only about 5,000 CFU. Thus, if a swab has been saturated by high numbers of cells from a highly contaminated surface, a proposed technique to improve line inoculation efficiency may be to initially take two swabs from two different surface areas on the environmental surface being tested. If the colony counts from the line inoculations for the two swabs agree and the counts are < 5,000 CFU for *E. coli*, and < 10,000 CFU for *S. aureus*, then either surface area used for swabbing would give reliable data. If these numbers for the two initial swabs did not agree, then it may be necessary to repeat the process by swabbing smaller surface areas until the data from the two swabs agree.

This study was conducted *in vitro* and did not utilize swabs collected from a healthcare facility. For example, the bacterial species tested here were placed on glass slides and quickly dried using a stream of filtered air. This does not resemble a healthcare setting where the bacteria would be found on a variety of different surfaces, where they would be subject to a

variety of environmental conditions, including moisture content. The bacteria may exhibit different survivability in this kind of setting compared to this study. However, it would be difficult to expect to be able to replicate this study inside an actual healthcare facility (to say nothing about gaining Institution Review Board committee approval!), where patients and staff might be put at risk from the bacteria used. Therefore, it was necessary for this study to be conducted *in vitro* in a controlled environment.

Future research that could benefit this quick enumeration technique might involve the use of different bacterial species, such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus sp.,* and some additional Gram-positive cocci to help compare these different potential pathogens. Using different cell types (e.g., Gram-positive vs. Gram-negative) and both motile and non-motile cells will help to provide data that could improve the use of line inoculations to quickly count bacteria present on surfaces. Another variable that could be studied in this way might be the survivability and enumeration of bacteria found on dust particles. The ability of dust to move bacteria around in healthcare settings has been well documented.

REFERENCES

Adams, C. E., and S. J. Dancer. 2020. Dynamic transmission of Staphylococcus aureus in the intensive care unit. International Journal of Environmental Research and Public Health. 17, 2109; doi: 10.3390/ijerph17062109.

Bajwa, A., Tan, S., Mehta, R., & Bahreyni, B. (2013). Rapid Detection of Viable Microorganisms Based on a Plate Count Technique Using Arrayed Microelectrodes. *Sensors*, *13*(7), 8188–8198. https://doi.org/10.3390/s130708188

Breed, R. S., & Dotterrer, W. D. (1916). THE NUMBER OF COLONIES ALLOWABLE ON SATISFACTORY AGAR PLATES. *Journal of Bacteriology*, *1*(3), 321–331. https://doi.org/10.1128/jb.1.3.321-331.1916

Brinkman, N. E., Haugland, R. A., Wymer, L. J., Byappanahalli, M., Whitman, R. L., & Vesper, S. J. (2003). Evaluation of a Rapid, Quantitative Real-Time PCR Method for Enumeration of Pathogenic Candida Cells in Water. *Applied and Environmental Microbiology*, *69*(3), 1775–1782. https://doi.org/10.1128/aem.69.3.1775-1782.2003

Cantlon, C. A., Stemper, M. E., Schwan, W. R., Hoffman, M. A., & Qutaishat, S. S. (2006). Significant pathogens isolated from surgical site infections at a community hospital in the Midwest. *American Journal of Infection Control*, *34*(8), 526–529.

Deshpande, A., Cadnum, J. L., Fertelli, D., Sitzlar, B., Thota, P., Mana, T. S., Jencson, A., Alhmidi, H., Koganti, S., & Donskey, C. J. (2017). Are hospital floors an underappreciated reservoir for transmission of health care-associated pathogens? *American Journal of Infection Control*, *45*(3), 336–338.

Donskey, C. J. (2019). Beyond high-touch surfaces: Portable equipment and floors as potential sources of transmission of health care–associated pathogens. *American Journal of Infection Control*, *47*.

Galvin, S., Boyle, F., Hickey, P., Vellinga, A., Morris Dearbháile, & Cormican, M. (2010). Enumeration and Characterization of Antimicrobial-Resistant Escherichia coli Bacteria in Effluent from Municipal, Hospital, and Secondary Treatment Facility Sources. *Applied and Environmental Microbiology*, *76*(14), 4772–4779.

Gracias, K. S., & McKillip, J. L. (2004). A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology*, *50*(11), 883–890. https://doi.org/10.1139/w04-080

González, Ã., Hierro, N., Poblet, M., Mas, A., & Guillamón, J. M. (2006). Enumeration and detection of acetic acid bacteria by real-time PCR and nested PCR. *FEMS Microbiology Letters*, *254*(1), 123–128. https://doi.org/10.1111/j.1574-6968.2006.00586.x

Han, J. H., Sullivan, N., Leas, B. F., Pegues, D. A., Kaczmarek, J. L., & Umscheid, C. A. (2015). Cleaning Hospital Room Surfaces to Prevent Health Care–Associated Infections. *Annals of Internal Medicine*, *163*(8), 598–607. https://doi.org/10.7326/m15-1192

Keilman, R., S. Harding, M. Rowin, E. Reade., P. Klingborg. D. Levine, and H. Spratt, Jr. 2021. Investigations of Staphylococcal contamination on environmental surfaces of a neonatal intensive care unit of a children's hospital. *American Journal of Infection Control.* doi: 10.1016/j.ajic.2021.05.007.

Koganti, S., H. Alhmidi, M. E. Tomas, J. L. Cadnum, A. Jencson and C. J. Donskey (2016). Evaluation of Hospital Floors as a Potential Source of Pathogen Dissemination Using a Nonpathogenic Virus as a Surrogate Marker. Infection Control & Hospital Epidemiology 4.

Li, L., N. Mendis, H. Trigui, J. D. Oliver, S. P Faucher. 2014. The importance of the viable but non-culturable state in human bacterial pathogens. Frontiers in Microbiology, doi: 10.3389/fmicb.2014.00258.

Manian, F. A., Meyer, L., Setzer, J., & Senkel, D. (2003). Surgical Site Infections Associated with Methicillin-Resistant Staphylococcus Aureus: Do Postoperative Factors Play a Role? . *Clinical Infectious Diseases* , *36*(7), 863–868.

Naghili, H., Tajik, H., Mardani, K., Razavi Rouhani, S. M., Ehsani, A., & Zare, P. (2013). Validation of drop plate technique for bacterial enumeration by parametric and nonparametric tests. *Veterinary Research Forum* , *4*(3), 179–183.

Olson, M. M., & Lee, J. T. (1990). Continuous, 10-Year Wound Infection Surveillance. *Archives of Surgery*, *125*(6), 794–803. https://doi.org/10.1001/archsurg.1990.01410180120020

Otter, J. A., Yezli, S., Salkeld, J. A. G., & French, G. L. (2013). Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. *American Journal of Infection Control*, *41*(5), S6–S11.

Pettit, F., & Lowbury, E. J. (1968). Survival of wound pathogens under different environmental conditions. *Journal of Hygiene*, *66*(3), 393–406. https://doi.org/10.1017/s0022172400041267

Rashid, T., H. M. Vonville, I. Hasan and K. W. Garey (2016). Mechanisms for floor surfaces or environmental ground contamination to cause human infection: a systematic review. Epidemiology and Infection **145**(2): 11.

Rashid, T., K. Poblete, J. Amadio, I. Hasan, K. Begum, M. J. Alam and K. W. Garey (2017). Evaluation of a shoe sole UVC device to reduce pathogen colonization on floors, surfaces and patients. Journal of Hospital Infection **98**(1): 6.

Richards, M. J., Edwards, J. R., Culver, D. H., & Gaynes, R. P. (1999). Nosocomial infections in medical intensive care units in the United States. *Critical Care Medicine*, *27*(5), 887–892. https://doi.org/10.1097/00003246-199905000-00020.

Shiomori, T., H. Miyamoto, K. Makishima. 2001. Significance of airborne transmission of methicillin-resistant Staphylococcus aureus in an otolaryngologyhead and neck surgery unit. Arch Otolaryngol Head Neck Surg.127:644-648.

Spratt, Henry G., Jr., David Levine, Susan McDonald, Sarah Drake, Katherine Duke, Casey Kluttz, and Kate Noonan. 2019. Survival of *Staphylococcus aureus* on therapeutic ultrasound heads. *American Journal of Infection Control.* 47:1157-1159. https://doi.org/10.1016/j.ajic.2019.02.019.

Spratt, H. G., Jr., D. Levine, and L. Tillman. 2014. Physical therapy clinic therapeutic ultrasound equipment as a source for bacterial contamination. *Physiotherapy Theory and Practice*.

30:507-511. ISSN: 0959-3985 (print), 1532-5040 (electronic), DOI: 10.3109/09593985.2014.900836.

Talaiekhozani, A., Alaee, S., & Ponraj, M. (2015). Guidelines for quick application of biochemical tests to identify unknown bacteria. *Accounts of Biotechnology Research* , *2*(2), 65–82.

Tomasiewicz, D. I. A. N. E. M., Hotchkiss, D. O. N. A. L. D. K., Reinbold, G. E. O. R. G. E. W., Read, R. A. L. S. T. O. N. B., & Hartman, P. A. U. L. A. (1980). The Most Suitable Number of Colonies on Plates for Counting1. *Journal of Food Protection*, *43*(4), 282–286. https://doi.org/10.4315/0362-028x-43.4.282

Wade, W. 2002. Unculturable bacteria – the uncharacterized organisms that cause oral infections. J. Royal Soc of Medicine 95:81083

Wang, L., K. Zhou, W. Chen, Y. Yu, and S. Feng (2019). Epidemiology and Risk Factors for Nosocomial Infection in the Respiratory Intensive Care Unit of a Teaching Hospital in China: A during 2013 and 2015. BMC Infectious Diseases **19**(1)Prospective Surveillance

Warner, P., & Glassco, A. (1963). Enumeration of Air-Bore Bacteria in Hospital. *Canadian Medical Association Journal* , *88*, 1280–1283.

Weiss, C. A., Statz, C. L., Dahms, R. A., Remucal, M. J., Dunn, D. L., & Beilman, G. J. (1999). Six Years of Surgical Wound Infection Surveillance at a Tertiary Care Center. *Archives of Surgery*, *134*(10), 1041–1050. https://doi.org/10.1001/archsurg.134.10.1041

WHO. Report on the burden of endemic health care-associated infection worldwide. World Health Organization 2011.

Zhu, H., Zhang, H., Xu, Y., Laššáková, S., Korabečná, M., & Neužil, P. (2020). PCR past, present and future. *BioTechniques*, *69*(4), 317–325. https://doi.org/10.2144/btn-2020-0057

Appendix I

Sample Calculations

Experiment 9: ***E. coli***						
Serial Dilution	$5*10^{-7}$	= TMTC	$5*10^{-8}$	=TMTC	$5*10^{-9}$	=101
A	10^{-1}	= 212	10^{-2}	= 31		
B	10^{-1}	= 260	10^{-2}	= 29		
C	10^{-1}	= 267	10^{-2}	=31		
#25	65					
#26	52					
#27	60					

Viable Plate Count vs. Line Inoculation

CFUs/ 5 ml from plated swabs =

A. (212/0.1)*5 = 10,600 CFUs/ 5ml
B. (260/0.1)*5 = 13,000 CFUs/ 5ml
C. (267/0.1)*5 = 13,350 CFUs/ 5ml

Avg. CFUs/ 5ml = (10,600 + 13,000 + 13,350)/3 = 12316.67 CFUs/ 5ml

Line Inoculation CFUs with correction factor =

65*7 = 455 CFUs
52*7 = 364 CFUs
60*7 = 420 CFUs

Percent Agreement – Line to Swab CFU/ ml

(455/12316.67)*100 = 3.69%
(364/12326.67)*100 = 2.95%
(420/12316.67)*100 = 3.41%

Avg. Percent Agreement = (3.69% + 2.95% + 3.41%)/3 = 3.35%

Percent Survivability

CFUs/ ml in original bacterial suspension = 101/ $5*10^{-9}$ = $2.02*10^{10}$ CFUs/ ml

CFUs from Serial Dilution to Slide (CFU/0.025ml) = (($2.02*10^{10}$)*0.00005)*0.025 = $2.53*10^{5}$

Avg. CFUs/ 5ml (see above) = 12316.67

% Survivability = (12316.67/$2.53*10^{5}$)*100 = 4.88%

Appendix II

Data Collected and Calculated Values

II.1 *E. coli*

	E. Coli																								
	RUN 1																								
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	161																			
A 10^-1	167	A 10^-2	21																						
B 10^-1	TMTC	B 10^-2	33																						
C 10^-1	TMTC	C 10^-2	78																						
1	>15																								
2	>15																								
3	>15																								
	RUN 2						# in ONC	Avg. VPC	VPC Chart	Avg. Line	Line Chart	From SD to slide	From swab to TSB	% Survivability	AVG % Survival				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	34	3.40E+09	1.05E+02	1.05E+02	5.73E+01	5.73E+01	cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml		AVG % Surv +/- SD	AVG % Surv +/- SE		cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	59	A 10^-2	5						88		49	8.50E+04	5266.666667	6.20E+00	1.25E+01	4.59E+00	1.27E+00	A	2950	5266.6667	50	350	6.64556962	7.620253	0.948143
B 10^-1	157	B 10^-2	4						161.3333333		36.33333333							B	7850	5266.6667	67	469	8.905063291		
C 10^-1	100	C 10^-2	5						236.3333333		51.66666667							C	5000	5266.6667	55	385	7.310126582		
4	50								113.3333333		63.33333333														
5	67								246.3333333		59				% survival (EC)										
6	55								117.3333333		44				6.2										
									105.3333333		54.33333333				6.52										
	RUN 3								132.3333333		28.66666667	From SD to slide	From swab to TSB	% Survivability	14.6				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	85	8.50E+09	8.80E+01	5.80E+01	4.90E+01	35.33333333	cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml	8.15				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	36	A 10^-2	4						19.66666667		10.66666667	2.13E+05	13850	6.52E+00	10.18				1800	13850	45	315	2.274368231	2.476534	0.675569
B 10^-1	TMTC	B 10^-2	63						7.666666667		3.333333333				10.74				31500	13850	67	469	3.386281588		
C 10^-1	165	C 10^-2	11						12.33333333		4.333333333				4.88				8250	13850	35	245	1.768953069		
7	45								19.66666667		10.33333333				12.61										
8	67														11.27										
9	35														12.91										
															23.2										
	RUN 4											From SD to slide	From swab to TSB	% Survivability	15.25				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	221	10^-8	16	2.21E+09	1.61E+02		3.63E+01		cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml	14.89				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	144	A 10^-2	20									5.53E+04	8066.666667	1.46E+01	16.67				7200	8066.6667	36	252	3.123966942	3.152893	0.10823
B 10^-1	150	B 10^-2	19												19				7500	8066.6667	38	266	3.297520661		
C 10^-1	190	C 10^-2	16																9500	8066.6667	35	245	3.037190083		
10	36																								
11	38																								
12	35																								
	RUN 5											From SD to slide	From swab to TSB	% Survivability					From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	58	5.80E+09	2.36E+02		5.17E+01		cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml					cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	284	A 10^-2	36									1.45E+05	11816.66667	8.15E+00					14200	11816.667	49	343	2.902679831	3.060649	0.356526
B 10^-1	245	B 10^-2	30																12250	11816.667	60	420	3.554301834		
C 10^-1	180	C 10^-2	10																9000	11816.667	46	322	2.724964739		
13	49																								
14	60																								
15	46																								
	RUN 6											From SD to slide	From swab to TSB	% Survivability					From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	235	10^-8	38	2.35E+09	1.20E+02				cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml					cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	131	A 10^-2	18									5.88E+04	5983.333333	1.02E+01					6550	5983.3333	58	348	5.816155989	#VALUE!	#VALUE!
B 10^-1	98	B 10^-2	12																4900	5983.3333	48	288	4.813370474		
C 10^-1	130	C 10^-2	15																6500	5983.3333	TMTC	#VALUE!	#VALUE!		
16	58																								
17	48																								
18	TMTC																								
	RUN 7											From SD to slide	From swab to TSB	% Survivability					From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	63	6.30E+09	1.13E+02		6.33E+01		cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml					cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	TMTC	A 10^-2	42									1.58E+05	16916.66667	1.07E+01					21000	16916.667	53	371	2.193103448	2.62069	0.373689
B 10^-1	265	B 10^-2	22																13250	16916.667	62	434	2.565517241		
C 10^-1	TMTC	C 10^-2	33																16500	16916.667	75	525	3.103448276		
19	53																								
20	62																								
21	75																								
RUN 8												From SD to slide	From swab to TSB	% Survivability					From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	59	5.90E+09	1.28E+02				cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml					cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	TMTC	A 10^-2	110																						
B 10^-1	TMTC	B 10^-2	124																			INVALID			
C 10^-1	TMTC	C 10^-2	151																						
22	TMTC																								
23	TMTC																								
24	TMTC																								

RUN 9									
SD:	5*10^-7	TMTC	5*10^-8	TMTC	5.00E-09	101	2.02E+10	2.46E+02	5.90E+01
A 10^-1	212	A 10^-2	31						
B 10^-1	260	B 10^-2	29						
C 10^-1	267	C 10^-2	31						
25	65								
26	52								
27	60								
RUN 10									
SD:	3*10^-7	TMTC	3*10^-8	TMTC	3.00E-09	50	1.67E+10	1.17E+02	4.40E+01
A 10^-1	TMTC	A 10^-2	35						
B 10^-1	286	B 10^-2	23						
C 10^-1	TMTC	C 10^-2	31						
28	49								
29	45								
30	38								
RUN 11									
SD:	2*10^-7	TMTC	2.00E-08	187	2*10^-9	17	9.35E+09	1.05E+02	5.43E+01
A 10^-1	106	A 10^-2	17						
B 10^-1	170	B 10^-2	19						
C 10^-1	40	C 10^-2	3						
31	58								
32	39								
33	66								
RUN 12									
SD:	1.11*10^-7	TMTC	1.11E-08	205	1.11*10^-9	31	1.85E+10	1.32E+02	2.87E+01
A 10^-1	129	A 10^-2	13						
B 10^-1	118	B 10^-2	19						
C 10^-1	150	C 10^-2	14						
34	14								
35	35								
36	37								
RUN 13									
SD:	5.00E-08	TMTC	5.00E-09	50	5.00E-10	4	1.00E+10	5.80E+01	3.53E+01
A 10^-1	47	A 10^-2	8						
B 10^-1	68	B 10^-2	8						
C 10^-1	59	C 10^-2	10						
37	42								
38	24								
39	40								
RUN 14									
SD:	2.00E-08	258	2.00E-09	25	2.00E-10	3	1.29E+10	1.97E+01	1.07E+01
A 10^-1	16	A 10^-2	2						
B 10^-1	26	B 10^-2	1						
C 10^-1	17	C 10^-2	0						
40	15								
41	12								
42	5								
RUN 15									
SD:	1.43E-08	103	1.43E-09	11	1.43E-10	0	7.20E+09	7.67E+00	3.33E+00
A 10^-1	8	A 10^-2	3						
B 10^-1	8	B 10^-2	0						
C 10^-1	7	C 10^-2	1						
43	1								
44	7								
45	2								
RUN 16									
SD:	1.43E-08	148	1.43E-09	22	1.43E-10	1	1.03E+10	12.33333333	4.333333333
A 10^-1	14	A 10^-2	1						
B 10^-1	13	B 10^-2	0						
C 10^-1	10	C 10^-2	1						
46	6								
47	5								
48	2								
RUN 17									
SD:	2.00E-08	207	2.00E-09	22	2.00E-10	2.00E+00	1.04E+10	19.66666667	10.33333333
A 10^-1	19	A 10^-2	3						
B 10^-1	19	B 10^-2	1						
C 10^-1	21	C 10^-2	2						
49	12								
50	5								
51	14								

From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
2.53E+05	12316.66667	4.88E+00
From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
1.25E+05	15766.66667	1.26E+01
From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
4.68E+04	5266.666667	1.13E+01
From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
5.13E+04	6616.666667	1.29E+01
From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
1.25E+04	2900	2.32E+01
From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
6.45E+03	983.3333333	1.52E+01
From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
2.58E+03	383.3333333	1.49E+01
From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
3.70E+03	616.6666667	1.67E+01
From SD to slide	From swab to TSB	% Survivability
cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml
5.18E+03	983.3333333	1.90E+01

From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
10600	12316.667	65	455	3.694181326	3.35318	0.304294
13000	12316.667	52	364	2.955345061		
13350	12316.667	60	420	3.410013532		
From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
17500	15766.667	49	343	2.175475687	1.953488	0.201834
14300	15766.667	45	315	1.997885835		
15500	15766.667	38	266	1.687103594		
From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
5300	5266.6667	58	406	7.708860759	7.221519	1.505025
8500	5266.6667	39	273	5.183544304		
2000	5266.6667	66	462	8.772151899		
From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
6450	6616.6667	14	98	1.481108312	3.032746	1.100568
5900	6616.6667	35	245	3.702770781		
7500	6616.6667	37	259	3.914357683		
From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
2350	2900	42	294	10.13793103	8.528736	1.944398
3400	2900	24	168	5.793103448		
2950	2900	40	280	9.655172414		
From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
800	983.33333	15	105	10.6779661	7.59322	2.982666
1300		12	84	8.542372881		
850		5	35	3.559322034		
From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
400	383.33333	1	7	1.826086957	6.086957	4.792874
400		7	49	12.7826087		
350		2	14	3.652173913		
From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
700	616.66667	6	42	6.810810811	4.918919	1.929359
650		5	35	5.675675676		
500		2	14	2.27027027		
From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
950	983.33333	12	84	8.542372881	7.355932	2.746809
950		5	35	3.559322034		
1050		14	98	9.966101695		

II. 2 *S. aureus*

S. Aureus								# in ONC	Avg. Line	Line Chart	Avg. VPC	VPC Chart														
RUN 1														From SD to slide	From swab to TSB	% Survivability	AVG % Sur	AVG % Surv +/- SD	AVG % Surv +/- SE	From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	47	10^-8	3		4.70E+08	4.03E+01	4.03E+01	8.93E+01	89.33333333		cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml	3.07E+01	9.45E+00	2.99E+00	cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	135	A 10^-2	12							15.33333333		56.33333333		1.18E+04	4466.666667	3.80E+01				6750	4466.66667	30	210	4.701492537	6.320895522	1.229555
B 10^-1	37	B 10^-2	0							54.33333333		142.3333333								1850	4466.66667	42	294	6.582089552		
C 10^-1	96	C 10^-2	9							82.33333333		138								4800	4466.66667	49	343	7.679104478		
1	30									62		54														
2	42									53		180.6666667														
3	49									35.33333333		230.6666667														
										26		113														
RUN 2										24.33333333		37		From SD to slide	From swab to TSB	% Survivability		% surv. (SA)		From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	248	10^-7	31	10^-8	3		2.48E+08	1.53E+01	4.67E+00	5.63E+01	14.33333333		cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml		38.01		cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	45	A 10^-2	4							3.666666667		10.66666667		6.20E+03	2816.666667	4.54E+01		45.43		2250	2816.66667	13	91	3.230769231	3.810650888	0.422404
B 10^-1	87	B 10^-2	6							17.33333333		36.33333333						17.57		4350	2816.66667	16	112	3.976331361		
C 10^-1	37	C 10^-2	4							6		14.33333333						23.19		1850	2816.66667	17	119	4.224852071		
4	13																	25.12								
5	16																	35.2								
6	17																	43.98								
																		24.04								
RUN 3														From SD to slide	From swab to TSB	% Survivability		20.56		From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	169		1.69E+10						cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml		25.6		cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	TMTC	A 10^-2	145											4.23E+05				37.43								
B 10^-1	TMTC	B 10^-2	294															30.03						INVALID		
C 10^-1	TMTC	C 10^-2	157															32.95								
7	TMTC																									
8	TMTC																									
9	TMTC																									
RUN 4														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	162		1.62E+10	5.43E+01		1.42E+02			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	TMTC	A 10^-2	229											4.05E+05	71166.66667	1.76E+01				114500	71166.6667	52	364	0.51147541	0.53442623	0.047058
B 10^-1	TMTC	B 10^-2	78																	39000	71166.6667	61	427	0.6		
C 10^-1	TMTC	C 10^-2	120																	60000	71166.6667	50	350	0.491803279		
10	52																									
11	61																									
12	50																									
RUN 5														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-6	TMTC	10^-7	TMTC	10^-8	119		1.19E+10	8.23E+01		1.38E+02			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	TMTC	A 10^-2	149											2.98E+05	69000	2.32E+01				74500	69000	78	546	0.791304348	0.8352657	0.048536
B 10^-1	TMTC	B 10^-2	138																	69000	69000	80	560	0.811594203		
C 10^-1	TMTC	C 10^-2	127																	63500	69000	89	623	0.902898551		
13	78																									
14	80																									
15	89																									
RUN 6														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	3*10^-7	TMTC	3*10^-8	TMTC	3.00E-09	43		1.43E+10	6.20E+01		5.40E+01			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	TMTC	A 10^-2	38											1.08E+05	27000	2.51E+01				19000	27000	71	497	1.840740741	1.607407407	0.173271
B 10^-1	TMTC	B 10^-2	60																	30000	27000	55	385	1.425925926		
C 10^-1	TMTC	C 10^-2	64																	32000	27000	60	420	1.555555556		
16	71																									
17	55																									
18	60																									
RUN 7														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	2*10^-7	TMTC	2.00E-08	164	2*10^-9	22		8.20E+09	5.30E+01		1.81E+02			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	293	A 10^-2	28											4.10E+04	14433.33333	3.52E+01				14650	14433.3333	51	357	2.473441109	2.570438799	0.285553
B 10^-1	TMTC	B 10^-2	36																	18000	14433.3333	47	329	2.279445727		
C 10^-1	213	C 10^-2	19																	10650	14433.3333	61	427	2.958429561		
19	51																									
20	47																									
21	61																									
RUN 8														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	1.43*10^-7	TMTC	1.43E-08	105	1.43*10^-9	17		7.34E+09	3.53E+01		2.31E+02			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	261	A 10^-2	17											2.62E+04	11533.33333	4.40E+01				13050	11533.3333	28	196	1.699421965	2.144508671	0.348071
B 10^-1	214	B 10^-2	29																	10700	11533.3333	42	294	2.549132948		
C 10^-1	217	C 10^-2	13																	10850	11533.3333	36	252	2.184971098		
22	28																									
23	42																									
24	36																									
RUN 9														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	10^-7	TMTC	10^-8	94	10^-9	13		9.40E+09	2.60E+01		1.13E+02			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	134	A 10^-2	16											2.35E+04	5650	2.40E+01				6700	5650	27	189	3.345132743	3.221238938	1.065775
B 10^-1	91	B 10^-2	9																	4550	5650	15	105	1.85840708		
C 10^-1	114	C 10^-2	11																	5700	5650	36	252	4.460176991		
25	27																									
26	15																									
27	36																									
RUN 10														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	5.00E-08	TMTC	5.00E-09	36	5.00E-10	6		7.20E+09	2.43E+01		3.70E+01			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	42	A 10^-2	4											9.00E+03	1850	2.06E+01				2100	1850	15	105	5.675675676	9.207207207	2.936329
B 10^-1	22	B 10^-2	1																	1100	1850	24	168	9.081081081		
C 10^-1	47	C 10^-2	4																	2350	1850	34	238	12.86486486		
28	15																									
29	24																									
30	34																									
RUN 11														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	2.00E-08	112	2.00E-09	14	2.00E-10	0		5.60E+09	4.67E+00		1.43E+01			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	13	A 10^-2	0											2.80E+03	716.6666667	2.56E+01				650	716.666667	5	35	4.88372093	4.558139535	0.460442
B 10^-1	18	B 10^-2	1																	900	716.666667	5	35	4.88372093		
C 10^-1	12	C 10^-2	3																	600	716.666667	4	28	3.906976744		
31	5																									
32	5																									
33	4																									
RUN 12														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	1.43E-08	57	1.43E-09	5	1.43E-10	0		3.99E+09	3.666666667		10.66666667			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	10	A 10^-2	0											1.43E+03	533.3333333	3.74E+01				500	533.333333	2	14	2.625	4.8125	3.093592
B 10^-1	14	B 10^-2	0																	700	533.333333	2	14	2.625		
C 10^-1	8	C 10^-2	1																	400	533.333333	7	49	9.1875		
34	2																									
35	2																									
36	7																									
RUN 13														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	2.00E-08	242	2.00E-09	26	2.00E-10	0		1.21E+10	17.33333333		36.33333333			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	35	A 10^-2	4											6.05E+03	1816.666667	3.00E+01				1750	1816.66667	14	98	5.394495413	6.678899083	1.816421
B 10^-1	33	B 10^-2	3																	1650	1816.66667	14	98	5.394495413		
C 10^-1	41	C 10^-2	7																	2050	1816.66667	24	168	9.247706422		
37	14																									
38	14																									
39	24																									
RUN 14														From SD to slide	From swab to TSB	% Survivability				From Swab	From Swab	Line Inoc	Line Inoc	Percent Agreement	AVG % Agreement	
SD:	1.43E-08	87	1.43E-09	7	1.43E-10	0		6083916084	6		14.33333333			cfu/ 0.025ml	cfu/ 5 ml (AVG)	Swab to SD cfu/ ml				cfu/5ml	AVG	cfu	Correction	Line to Swab cfu/ml		+/- SD
A 10^-1	11	A 10^-2	0											2175	716.6666667	32.95019157				550	716.666667	3	21	2.930232558	5.860465116	2.392525
B 10^-1	16	B 10^-2	4																	800	716.666667	6	42	5.860465116		
C 10^-1	16	C 10^-2	1																	800	716.666667	9	63	8.790697674		
40	3																									
41	6																									
42	9																									