ABSTRACT

Testing the Monitoring Capability of ATP Bioluminescence

for Rapid Detection

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Hospital acquired infections (HAIs) have been one of the most serious complications of healthcare in recent years, causing upwards of 98,000 deaths per year. Hospitals have started using immediate feedback instruments like ATP bioluminescence assays (ATP) to determine the cleanliness of different surfaces and combat infections. The purpose of this study was to evaluate colony forming units (CFUs) and relative light units (RLUs) to determine if there was a correlation and assess a realistic benchmark for RLU counts to determine cleanliness for hospital surfaces. Five common and novel surfaces were sterilized and inoculated with MRSA to observe the cumulative bioburden on each surface type through contact plates and ATP. The surfaces were then cleaned with a disinfectant without the addition of mechanical action and tested. These results showed that hospitals cannot reasonably expect their surfaces to register at zero RLUs as nearly every surface showed no MRSA colonies after disinfection, but still had RLU values in the 10-20s. The copper sheet and solid surface with cupric oxide regularly produced the lowest RLU values, indicating that they are beneficial in retarding the viability of microbiological colonies. A test was also run in which the surfaces were only sterilized and tested for RLUs. These values were higher on average for almost every surface type, demonstrating that sterilization alone will not significantly alter the ATP bioburden on the different surfaces and that regular cleaning practices contribute to the reduction of bioburden by limiting resources for the pathogen to propagate.

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TESTING THE MONITORING CAPABILITY OF ATP BIOLUMINESCENCE FOR RAPID DETECTION

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Dedication

To Dr. Harris, for helping me through a process that was completely foreign to me and for allowing me to participate in her research.

To my parents, for supporting me and helping me believe that I had the ability to write a thesis.

CHAPTER ONE

Introduction

Over the past ten years, hospital acquired infections (HAIs) have become one of the most common complications within hospital care (Sharpe and Schmidt (2011). Each year, almost two million patients in the United States contract dangerous infections during hospital stays, resulting in over 98,000 deaths (Harris, Pacheco, & Lindner, 2010). Unfortunately, many of these infections arise from the subpar disinfection of high touch surfaces and objects. Visual assessments of the surfaces and objects in question have proven to be insufficient measurements of both cleanliness and biological burden (Lewis, Griffith, Gallo, & Weinbren, 2008). Many hospitals have started monitoring and instructing their cleaning staff, encouraging the use of immediate feedback items, such as adenosine triphosphate (ATP) bioluminescence assays, and have begun calculating the number of microbial colonies on surfaces and objects by using and incubating contact plates before and after disinfection to gain a better understanding of the soil on the surfaces. ATP has been used to monitor environmental cleaning, ensuring that the staff have met the necessary cleanliness standards, and used as a teaching tool because staff can read results seconds after testing a surface (Chan et al., 2015). However, no standard relative light unit (RLU) reading has been determined since each ATP monitoring system has a different sensitivity and scale for RLUs. In addition, studies have struggled to find a correlation between RLUs and colony forming units (CFU).

The purpose of this study was to analyze the data comparing RLU values produced by the 3M Clean-Trace Surface ATP bioluminescence assay (St. Paul, MN) and

CFUs on five different new and worn surfaces to evaluate for a potential correlation and determine a RLU standard to be used in hospital monitoring for cleaning and future research studies. An important aspect of this research was to determine if zero RLUs were a possible or feasible standard for hospitals to meet. An additional study was performed on sterilized surfaces to determine if the specimens produced an RLU value directly after sterilization or after disinfection with one novel and two common disinfectants. Presently studies use a wide range of RLU values, with some having a standard of 100 RLUs (Anderson, Young, Stewart, Robertson, & Dancer, 2011), while other studies provide no reasoning for the chosen "clean" RLU value of less than 250 (Zambrano, Jones, Otero, Ajenjo, & Labarca, 2014) or 500(Cooper, Griffith, Malik, Obee, & Looker, 2007). Studies have used values from 3 RLUs (Zambrano et al., 2014), to 25 RLUs (Mulvey et al., 2011), to 250 RLUs (Casini et al., 2017), to 500 RLUs (Knape, Hambraeus, & Lytsy, 2015), further demonstrating the need for a standard "clean" RLU value.

CHAPTER TWO

Review of Literature

Recent studies have found that HAIs are the most common complications after a hospital stay. According to Han et al. (2015), over 700,000 people contracted an HAI in the United States, resulting in approximately 75,000 deaths. Several common bacteria that cause HAIs are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp. (VRE), *Clostridium difficile* (CDF), *Acinetobacter* spp., and norovirus (Han et al., 2015). These microbes can survive for hours or even months on different hospital surfaces due to the provided food source on them (Weber & Rutala, 2013). For the purpose of this study, MRSA will be the bacterium of focus. HAIs can be transferred from room to room in a hospital by patients themselves and by the nurses and doctors attending to patients. Much of the transmission in hospitals is caused by the contamination of the gloves or hands of hospital personnel (Weber & Rutala, 2013). This contamination allows the bacteria to spread to new surfaces throughout the hospital, compromising more patients.

Recently, studies have been conducted to find different surfaces that may help reduce or destroy microbial populations. While stainless steel and laminate are some of the most widely used surfaces in hospitals, copper and silver have also become more common for the purported antimicrobial properties. Studies have shown that surfaces containing 99% copper have a significant reduction in bioburden when compared to

stainless steel (Eser, Ergin, & Hascelik, 2015). However, much of this literature is limited in its scope and many do not consider whether the disinfecting products used on the surfaces are appropriate to decrease the amount of wear that the surfaces are subjected to. In this report, we considered copper, stainless steel, high pressure laminate, a homogenous solid surface, and a solid surface with cupron oxide. Appropriate disinfectants were chosen in this experiment to maintain the integrity of the surfaces.

Regular disinfection of high touch objects (HTOs) is necessary to reduce the number of HAI incidences. One of the main chemicals used for disinfection is bleach, which is recommended by the Centers for Disease Control and Prevention (CDC). Bleach is a common product because it has a wide range of antimicrobial effects, including: 1) it does not leave toxic chemicals on the surface; 2) it is not affected by hard water; 3) it is inexpensive while producing quick results; 4) it can kill dried or fixed organisms in addition to removing biofilms; and 5) it is rarely the cause of toxic incidences (Rutala, Weber, & HICPAC, 2008). However, bleach also has several drawbacks as it can corrode metals, discolor fabrics, and produce chlorine gas when combined with other chemicals (Rutala et al., 2008). For this study, bleach was chosen as the disinfectant of interest.

Other chemicals commonly used in the disinfection process of healthcare environments include alcohols, aldehydes, peroxygens, and phenols. Alcohols have been proven to inactivate viruses, fungi, and bacteria and provide germicidal activity quickly when used at high concentrations (Jeong, Bae, & Kim, 2010). Aldehydes, while effective in eliminating bacteria, can cause respiratory damages through the fumes produced,

making them a less favored disinfectant (Jang et al., 2017). Peroxygens are substances with a highly active –O-OH group (Dagher, Ungar, Robison, & Dagher, 2017). This allows peroxygen compounds to react quickly, mainly oxidizing thiol moieties within cysteine and methionine units in enzymes and other proteins (Dagher et al., 2017). Phenols function by invading the cell wall and destroying cellular proteins, resulting in cell death (Rutala et al., 2008). However, phenols are less commonly used as they are readily absorbed by porous material and can discolor and inflame tissues (Rutala et al., 2008). Quaternary ammonium disinfectants and a novel disinfectant were used in the secondary study to test for ATP generated by the residues from the disinfectants on sterilized surfaces that could possibly disrupt the reading on a surface that should have a zero RLU reading.

Quaternary ammonium disinfectants are also commonly used in hospitals. These products work best against fungi, bacteria, and enveloped viruses (Rutala et al., 2008). The CDC suggests that quaternary ammonium disinfectants be used on non-critical (objects or surfaces that do not come in contact with mucous membranes or internal body tissues) surfaces and non-invasive medical equipment (Rutala et al., 2008). However, quaternary ammonium disinfectants become less effective when used with hard water, as the hard water creates insoluble precipitates, and with cotton or gauze pads, as they can absorb the active ingredients within the disinfecting solution (Rutala et al., 2008). Gram-negative bacteria can also survive in quaternary ammonium solutions, increasing risk of contaminating new surfaces (Rutala et al., 2008). Studies have shown that the use of quaternary ammonium disinfectants, in addition to regular cleaning practices, can

significantly reduce the contamination rates of MRSA in hospital rooms (Yuen, Chung, & Loke, 2015).

A novel disinfectant was also chosen for this experiment to investigate its efficiency and remaining residues. Briefly, the components that make up the novel disinfectant is hydrogen peroxide, surfactants, inorganic salts, hydrogen peroxide booster, and water (Decon Seven Systems, 2020). This product has been shown to be effective against *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa* (Scottsdale, AZ). The novel disinfectant should be used on non-critical surfaces, like in physician offices, operating rooms, and radiology rooms. It can be applied using a foaming apparatus or a sprayer, but it requires that the surface be rinsed with potable water after disinfection to remove any residue. The CDC does not currently have specific guidelines or recommendations for novel disinfectants. The novel disinfectant requires preparation prior to its usage, must be used within 24 hours of its preparation. This product may cause skin burns and is dangerous if aerosolized. Novel disinfectants, such as this, may have potential to be more effective in destroying microbial populations faster than bleach is able to and require more attentive comparisons with other common disinfectants.

Despite recommendations from the CDC, the guidelines for disinfection in the United States remain quite vague, not specifying the frequency, agents, or methods required for disinfection (Sexton, Clarke, O'Neill, Dillane, & Humphreys, 2006). This leads to many discrepancies in the cleanliness of hospitals and a higher incident rate of HAI, as hospital cleaning staff do not have a detailed standard procedure to follow. As such, hospitals have begun relying on internal cleaning audits to ensure cleaning

requirements are met (Sexton et al., 2006). Education and training programs have also been implemented to instruct cleaning staff on optimal disinfection strategies and on how the environment contributes to HAI incidences (Mitchell et al., 2018). It is typical for hospitals to contract infection control professionals to test and maintain hospital standards or to assist housekeeping staff in sustaining expected cleaning and disinfection results (Xu et al., 2015). In order to monitor cleaning, culturing surface samples to determine the number of microbiologic colonies and the use of ATP have been implemented. Taking cultures of the different HTOs in patient rooms allows researchers to determine the amount and type of microbes living on the surfaces, providing more information for which disinfectant should be used and a definitive rate of cleanliness. ATP bioluminescence assays have been used, producing immediate results which allows housekeeping staff to assess their own cleaning competence.

The ATP monitoring system used for this research was the 3M Clean-Trace Surface ATP test. Swabs are used to sample biologic residue left on surfaces. After sampling has been completed, the swab is injected into the enzyme test solution that reacts with any ATP, adenosine triphosphate, to produce light. The swab is then placed into the luminometer, where the light from the enzymatic reaction is read and interpreted as RLUs. The ATP, adenosine triphosphate, that the 3M Clean-Trace Surface ATP test detects includes any living microorganisms and any other source of ATP. Under optimal conditions the RLUs are directly proportional to the amount of ATP detected.

Previously only used in foodservice, hospitals have begun to use ATP, allowing housekeeping staff to monitor their own cleaning efficacy, as the tests are simple to use

and produce results in less than 30 seconds. This allows the housekeeping staff to modify their cleaning habits as they work, increasing the cleanliness of hospital rooms.

A more common measurement of biological contamination is counting CFUs. To find CFUs, one can either use a swab to sample the desired surface area, run the swab over a contact plate, and incubate the plate for 24 hours or use a dipslide, pressing it directly against the surface of interest, and then incubating the dipslide for 24 hours (Obee, Griffith, Cooper, & Bennion, 2007). These samples will grow the bacteria that were living on the surfaces and, after 24 hours, produce the number of the colonies living on the sampled surfaces. This provides environmental services with an objective measure of the cleanliness/contamination of the surface. However, this method of measurement requires time to prepare the contact plates and to allow the bacteria to incubate, preventing environmental services from seeing the immediate results of their work. For this reason, ATP has become more prevalent due to their instant feedback of measuring cleanliness.

ATP is useful due to its quick results, allowing environmental services to augment their cleaning procedures during cleaning. However, since ATP detects all the ATP, adenosine triphosphate, on a surface, no standard has been determined for their usage. The lack of a standard-use luminometer further complicates matters as different brands produce different RLU readings for the same amount of bioburden. Studies have also struggled to find meaningful correlations between CFUs and RLUs, making it difficult to assess the actual cleanliness of the surface in question (Obee et al., 2007). As Obee et al. explains, the ATP bioluminescence assays measure organic and inorganic phosphate, although every type of ATP should be removed by effective cleaning. The research

further states that an ATP assay would prove more useful if a correlation between surface contamination and RLUs could be found. While many studies argue that there is no correlation between CFUs and RLUs, such research has been conducted on surfaces with unknown amounts of bioburden already deposited on them. The purpose of this study is to determine whether there is a correlation between CFUs and RLUs on new and worn surfaces inoculated with a measured amount of MRSA. Using a laboratory experimental research design will allow for an accurate CFU/RLU comparison, informing a recommendation for a standard RLU baseline for cleanliness of hospital surfaces.

CHAPTER THREE

Methods

The research design is experimental, conducted in a laboratory and statistically analyzed. Surface samples were prepared in triplicate for both worn and new surfaces. The surfaces tested were stainless steel, copper, high pressure laminate, homogenous solid surface, and a cupron infused solid surface, all common surfaces in healthcare environments. Stainless steel and laminate continue to be used frequently in hospital settings. The solid surface in this study is homogeneous and is approved for use in the food industry. Copper is known for having antimicrobial properties. The cupron infused solid surface also has antimicrobial properties and since the copper is infused in the surface, it should not oxidize and tarnish like uncoated copper.

Data Collection

Environmental Surface Material Wear Test.

The surfaces were cut into 4" x 4" squares to keep the contamination areas consistent. The samples that were to be considered "worn" were abraded by a Taber Abrader to simulate six years of use, utilizing ASTM G195-18, modified for accelerated wear. This was accomplished at ambient room temperature (69˚F) with a S35 Tungsten Carbide wheel, set to 1000g loading. A vacuum was employed to remove the abraded

residue and was set to a suction level of 60 (100%). The vacuum nozzle was placed 1.5mm above each surface.

The samples were then coded based on material composition and categorized as new (n=120) or worn (n=60). Prior to their use in the experiment, the samples were placed in Chex-all sterilization bags (six samples per bag) and autoclaved for sterilization.

Microbiological Testing.

Specimen preparation. A freeze-dried MRSA pellet was used to produce the bacterial colonies necessary to conduct the study. The pellet was rehydrated with 1ml of tryptic soy agar broth, which was later transferred to 5ml of tryptic soy agar broth and incubated at 37˚C for 24 hours. This solution was used to make a streak plate with a tryptic soy agar plate and was incubated at 35˚C for 24 hours. A colony was then taken from this plate, placed on a plate using the same procedure and media and used to inoculate Fisher Scientific CryoCare specimen preservation beads to be used later.

To prepare an inoculation solution, a Fisher Scientific CryoCare specimen preservation bead was taken and used to create a smear plate by placing the bead on the agar and moving it across the plate. After 24 hours of incubation at 35˚C, a colony was taken from the smear plate and transferred to 10ml of tryptic soy broth, which was incubated for 20 hours at 35˚C. Next, the inoculated broth was transferred to 200ml of tryptic soy broth and allowed to incubate for 30 minutes at 35˚C, containing approximately 1.375 x 10^7 CFU/ml of MRSA in total. This solution was then used to

make a dilution by combining 1 ml of the tryptic soy broth inoculant with 99 ml of ultrapure water, reducing the concentration to 1.375 x 10^5 CFU/ml of MRSA. This final solution was then ready for immediate use.

Sample inoculation. After the solution had been prepared, thirty 100µl (0.1 ml) drops were pipetted onto the surfaces. For the new surfaces, the droplets were placed in a random manner across the surface. For the worn surfaces, all 30 droplets were placed randomly onto the worn ring to produce a more accurate result regarding the impact of viability and ATP production on a worn surface.

Once each surface was inoculated, the samples were placed in the biosafety cabinet (Thermo Scientific, Waltham, MA) at 77˚F and 45% relative humidity for two hours to dry.

Disinfection Intervention.

To disinfect the surfaces, a spray bottle was filled with Clorox Healthcare Bleach Germicidal Cleaner (0.65% bleach solution). The surfaces were sprayed so that they were wet, but not saturated so that the surfaces were dry by the end of the 30-minute disinfection period. No mechanical motions were used in addition to the spray.

ATP Test.

The ATP tests were taken at two points during the experiment. The first test was taken at the end of the two hours after inoculation, before disinfection. This provided time for the MRSA to replicate and produce ATP on the surface samples. The ATP tests were taken when the samples were completely dried to yield accurate results. The ATP swabs were taken and rubbed across the samples in a zig-zag pattern both vertically and

horizontally (*see Appendix Figure 1*). The swabs were then returned to their labeled containers. Once each surface has been sampled, the swabs were plunged into the enzymatic solutions, shaken side to side, and inserted into the 3M Clean-Trace ATP test luminometer one by one. The results were then recorded in a table corresponding to the surface sample.

The second test was taken at the end of the 30 minute drying time after the bleach intervention. The swabs were run across each surface in the same manner as the first test and subsequently placed in their coded tubes. The swabs were plunged into the enzymatic solution, placed in the luminometer one by one, and the results were recorded.

Colony Forming Unit Test.

The samples were inoculated in the same fashion as the ATP test samples and allowed to dry uncovered for two hours as well. After the two hour drying period, tryptic soy agar plates were taken and pressed onto each sample four times to cover the entire surface area and incubated for 24 hours at 35˚C. The next day, the contact plates were taken from the incubator and the colonies on each plate were counted and recorded.

Contact plates were also taken after the 30-minute disinfection period. The plates were pressed onto the surfaces four times (this method allowed data to be collected from the entire surface area of the samples), covered, and incubated for 24 hours at 35˚C. At the end of 24 hours, these plates were also removed from the incubator and the colonies that grew on the plates were counted and recorded. This experiment provided CFU counts that was compared with the ATP RLU counts recorded in the previous experiment.

Sterilization Test.

An additional experiment was conducted on the surface samples to measure biological concentration left on the samples after sterilization and by autoclaving. Initially, the samples were placed in Chex-all sterilization bags and placed in the autoclave for sterilization. The samples were carefully removed from the Chex-all sterilization bags and placed on a clean surface. They were immediately tested using the 3M Clean-Trace ATP test luminometer. Like the previous experiment, the swabs were run over the surfaces in a zig-zag pattern both vertically and horizontally and promptly replaced in their labeled containers. After each surface had been sampled, the swabs were plunged into the enzymatic solution one by one, inserted into the luminometer, and the RLU readings were recorded.

Data Analysis

Environmental Surface Material Wear Test.

Data were analyzed for percent mass loss by type of material and within type of material comparing new and worn. ANOVA was conducted on the data to determine differences in the five environmental surface materials. Since there was a difference in the means of the variables ($p<.0001$), post-hoc multiple comparisons were conducted. Tukey's honestly significant difference (HSD) test for pairwise comparisons was used to compare new and worn within material type and compare for mass loss by type of material.

Microbiological Analysis.

ATP tests. Data were analyzed for differences in the mean ATP and RLU readings before and after the disinfection intervention. Interaction between material and wear within materials and multiple comparisons across the five environmental surface materials were analyzed using the Brown-Forsythe test. The Brown-Forsythe test was used to test for equal population variances, based on the absolute differences within each group from the group median.

CFU tests. After incubation, plates were counted containing 30 to 300 CFUs. Those with less than 30 were listed as too few to count (TFTC) and those over 300 were listed as too many to count (TMTC). The data was treated as ordinal categories (0, TFTC, 30-300, and TMTC).

Sterilization test. Each environmental surface material was tested in triplicate for ATP RLU counts after sterilization by autoclave. With a small sample size (n=15), variances between the means was evaluated using the F-Test between sample types.

CHAPTER FOUR

Results

Environmental Surface Materials

Percent mass loss by type of environmental surface material.

There was an interaction between material type and wear, but only for copper sheet ($p<.0001$). There were statistically significant differences between copper sheet and the acrylic polymer solid surface (p=0.0006), between copper sheet and the solid surface with cupric oxide ($p=0.0151$), and between copper sheet and stainless steel sheet ($p=0.0002$). Significant differences were also found between high pressure laminate and the acrylic polymer solid surface ($p=0.0245$) and stainless steel ($p=0.01$). Multiple comparisons showed no difference in percentage mass loss between stainless steel, acrylic polymer solid surface, or the solid surface with cupric oxide. No difference was shown between the solid surface with cupric oxide and high pressure laminate nor between high pressure laminate and copper sheet. Copper sheet had the most percent mass loss (33%) and the stainless steel had the least percent mass loss (13%) (Figure 1).

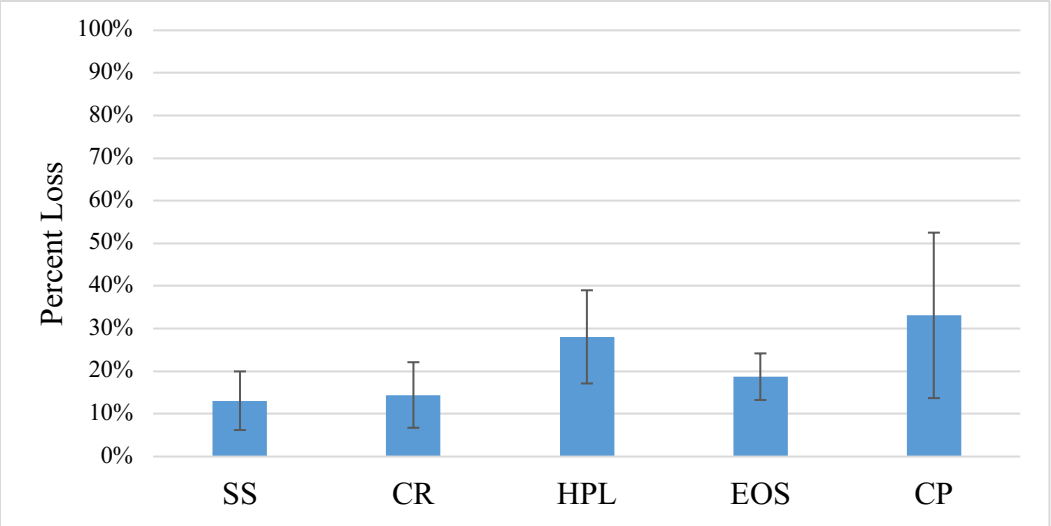

Figure 1. Mean mass percent loss for five environmental surface materials for simulated six years use. Error bars are standard deviation for each set of triplicate surface sample.

Percent mass loss of new and aged samples by environmental surface material type.

When comparing new and worn surfaces of the same material type, all materials except stainless steel indicated a significant difference: 1) copper sheet (p=0.0001); 2) acrylic polymer solid surface (p=0.0353); 3) solid surface with cupric oxide (p=0.0009); and 4) high pressure laminate (p=0.0001).

Microbiological Analysis

ATP tests.

No interaction was found between material type and wear, however the findings indicated that both material (p=0.0007) and wear (p=0.0374) affected mean ATP RLU counts before cleaning. The copper sheet and the solid surface with cupric oxide had the lowest mean ATP RLU counts (Figure 2).

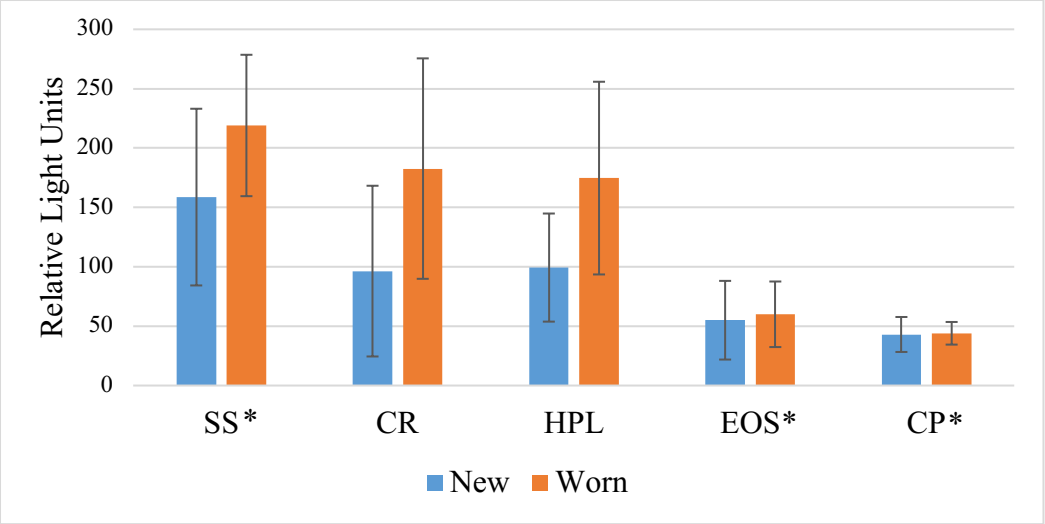

Figure 2. Mean RLU values of environmental surface material type (new and worn) prior to disinfection, two hours after inoculation. Error bars are standard deviation for each set of triplicate surface sample. Note: * designates significance at a confidence of 95%.

When comparing new material type prior to disinfection, there was a significant difference between mean ATP RLU counts of the stainless steel and copper sheet samples (p=.0015). There was also a difference between ATP RLU counts of worn materials, where stainless steel was significantly higher than copper sheet and the solid surface with cupric oxide (p=.0042) (Figures 3 & 4).

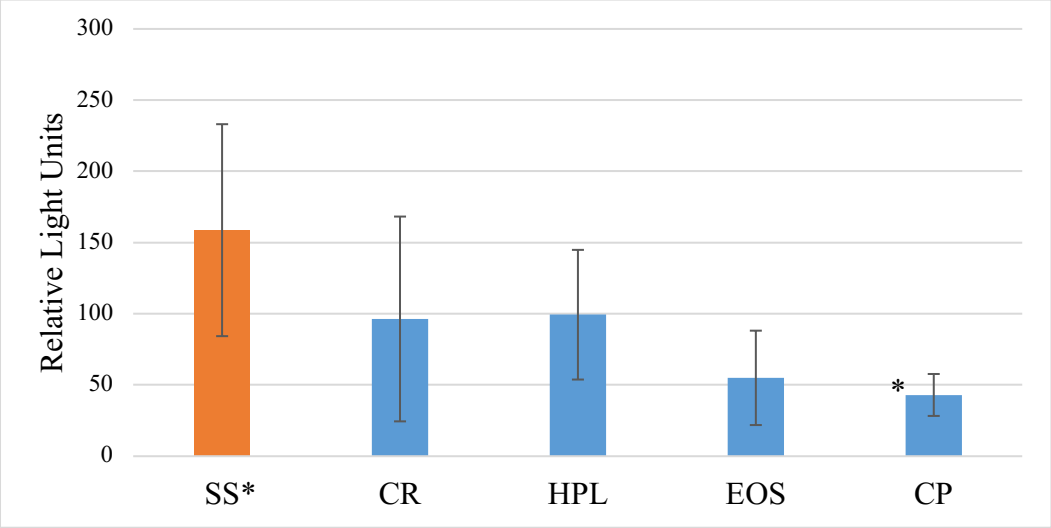

Figure 3. Comparison of new material type prior to disinfection showing a significant higher mean RLU for stainless steel than copper (p=.0015). Error bars are standard deviation for each set of triplicate surface sample. Asterisks denote values with significant differences. Note: * designates significance at a confidence of 95%.

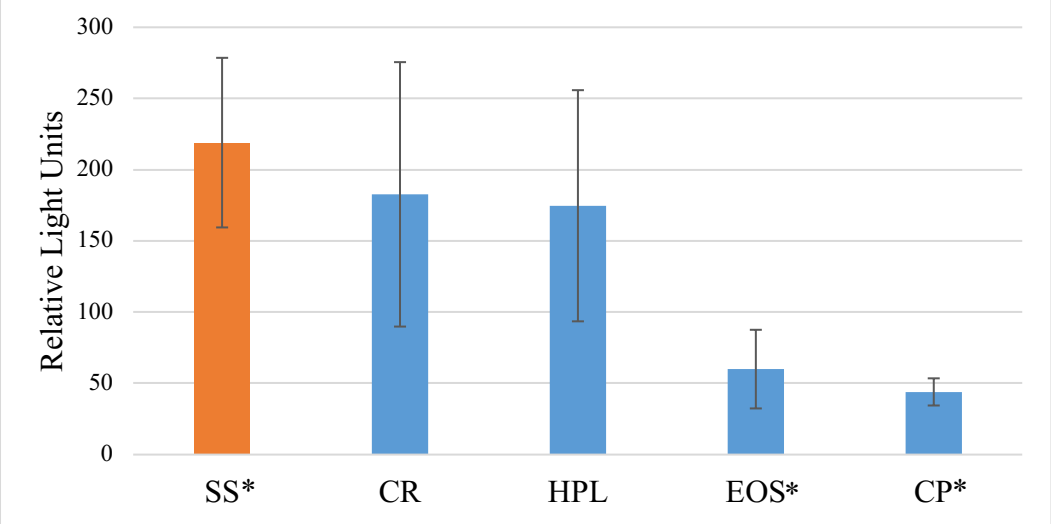

Figure 4. Comparison of worn material type prior to disinfection showing a significant higher mean RLU for stainless steel than copper sheet and the solid surface with cupric oxide and copper (p=.0042). Error bars are standard deviation for each set of triplicate surface sample. Note: * designates significance at a confidence of 95%.

CFU tests.

Prior to the bleach disinfection intervention, all environmental surface material CFUs were too many to count (TMTC). After disinfection, both the new and worn samples of each surface were reduced to zero CFUs. There were no significant differences between material type for CFU counts before or after the disinfection intervention. The disinfectant was successful at killing MRSA.

Comparison of ATP and CFU results.

The CFU data was treated as ordinal categorical data, which compromised the ability to statistically compare the ATP and the CFU results. Before the disinfection intervention, the mean CFU counts were TMTC and after the intervention, the mean CFU counts were all 0. The ATP RLU data showed that before the intervention, all worn samples had higher mean RLU counts; whereas after the intervention the worn samples of three materials (homogeneous solid surface, high pressure laminate, and the solid surface with cupric oxide) showed a mean RLU that was lower than the new materials. Worn homogeneous solid surface and worn high pressure laminate had the highest percent differences at 91% and 88% respectively, whereas new stainless steel had the lowest percent difference at 51% (Table 1). Figure 5 shows the before and after intervention ATP RLU counts for all five environmental surface material types.

Table 1. Percent Mean Differences of Each Material Based on RLU Counts Before and After Disinfection

	New			Worn		
Material	Before	After	% Differential	Before	After	% Differential
Stainless Steel (SS)	158.67	67.00	58%	219.00	77.67	65%
Homogeneous Solid Surface (CR)	96.33	18.00	81%	182.67	17.00	91%
High Pressure Laminate (HPL)	99.33	23.33	77%	174.67	20.67	88%
Solid Surface with Cupric Oxide (EOS)	55.00	17.33	68%	60.00	17.00	72%
Copper Sheet (CP)	43.00	15.33	64%	44.00	17.00	61%

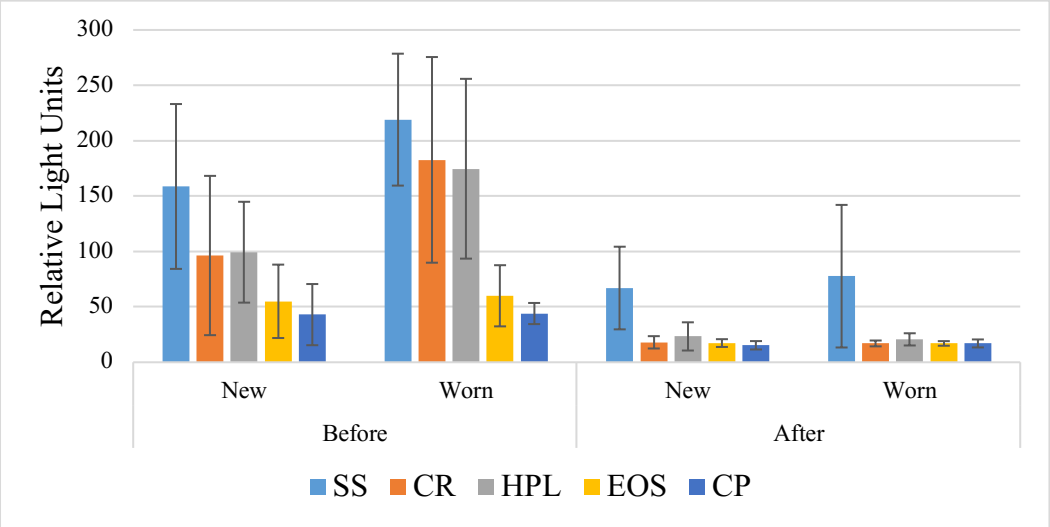

Figure 5. Mean comparison of environmental surface material type before and after disinfection. Error bars are standard deviation for each set of triplicate surface sample.

Sterilization test.

No significant differences were found between sample type for materials sterilized using the autoclave. Generally, there was variability between the RLU counts within each material type. The material with the highest ATP RLU count was high pressure laminate with solid surface with cupric oxide having the second highest; the

lowest ATP RLU count was for stainless steel with homogenous solid surface having the second lowest count. Copper sheet represented the median RLU count (Figure 6).

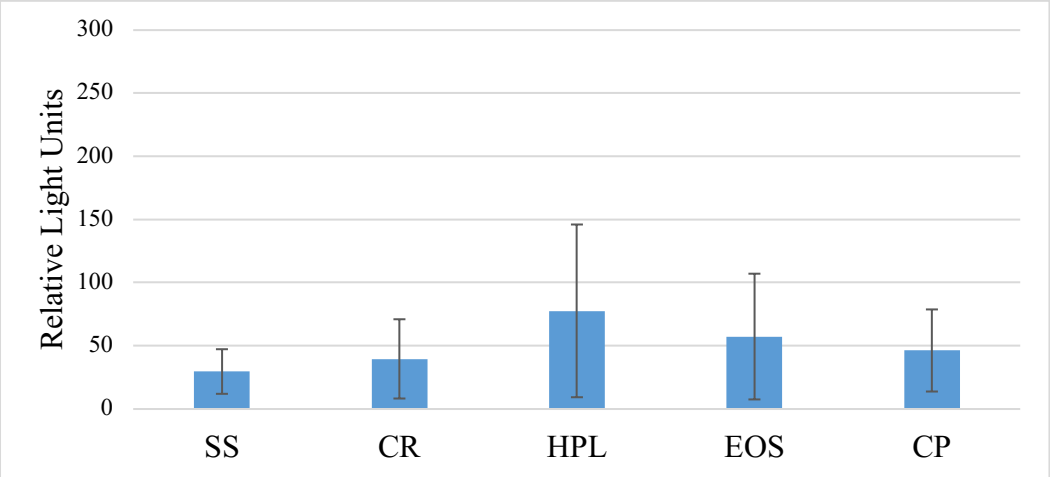

Figure 6. Mean RLU values by material type after sterilization by autoclave. Error bars are standard deviation for each set of triplicate surface sample.

CHAPTER FIVE

Discussion

Environmental Surface Materials

Percent mass loss

When the materials were abraded to produce the effect of wear, copper lost the greatest percent of its mass (33%), with high pressure laminate close behind (28%) and stainless steel lost the least percent of its mass (13%) (Figure 1). These figures would imply that copper and high pressure laminate are the least resistant to wear, making stainless steel appear to be a reasonable choice of material to have in healthcare settings. However, despite this resilience to wear, stainless steel still consistently had the highest RLU values throughout every test except for sterilization test.

Percent mass loss of new and aged samples by environmental surface material type.

Upon microscopic analysis of the surfaces before and after they were abraded, worn copper had a significant loss of material mass (33%) (p=.0014). However, the worn high pressure laminate did not follow this trend, as it was not significantly different from the new high pressure laminate, despite losing 28% of its mass after abrasion. Worn solid surface with cupric oxide, however, also indicated a significant mass loss (p=.0206), though it only lost 19% of its mass during abrasion.

Microbiological Analysis

ATP tests.

As shown in Figure 2, stainless steel, high pressure laminate, and homogeneous solid surface all experienced significant differences (p=0.0374) between the new and worn surface RLU counts. Despite this significant change due to wear, these three materials were still unable to come close to the reduced RLU values of the copper sheet and the solid surface with cupric oxide (Figure 2). Copper is thought to limit microbial viability by releasing toxic radicals that damage the microbes living on the surface (Warnes & Keevil, 2011). This mechanism is likely what caused the decrease in ATP production on the copper sheet and EOScu. This mechanism seems to not be disrupted by wear as both new and worn copper and the solid surface with cupric oxide had very similar RLU values prior to and after disinfection. The disinfection was conducted without mechanical action, so mechanical action, like wiping, may prove to lower the RLU values further by physically removing the ATP from the surface.

CFU tests.

Although every surface, both new and worn, had too many colonies of MRSA to count, studies have shown that replacing commonly touched surfaces with copper reduces the frequency with which MRSA appears on the surfaces (Schmidt et al., 2012). This information lends itself to the efficacy of copper as means to reduce the microbiological colonies in healthcare settings and explains why lower levels of ATP were found on the copper sheet and EOScu.

Comparison of ATP and CFU results.

While no specific correlations could be made between the CFU counts and the RLU values, the percent differences between the new and worn surfaces did show that the worn homogeneous solid surface had the highest percent difference (91%). This shows that homogeneous solid surface may be potentially the easiest surface to disinfect, due to surface conditions. The wear studies indicated a smoothing of the surface over time for the homogeneous solid surface material, which may have influenced the disinfection of the surface. Once again, the new stainless steel presented the lowest values at 51%, demonstrating that it did not have the same disinfection rate as some other materials. Compared to the other samples, stainless steel had the highest post-disinfection RLU values at 67 for new and 77.7 for worn stainless steel. All other surfaces, both new and worn had RLU values below 25 post-disinfection. Not a single surface had an RLU value in the single digits, making the expectation that hospital surfaces should have RLU values of zero unrealistic.

Sterilization test.

This test proved to be one of the most interesting, as the RLU values of all the surfaces, except for the stainless steel, were higher after sterilization than they were after disinfection. These data show that the Clorox Healthcare Bleach Germicidal Cleaner disrupts the ATP left on the surfaces in a way that sterilization cannot. In addition, this was the only instance in which stainless steel had the lowest RLU values of all the surfaces (Figure 6). High pressure laminate, however, continued to have relatively high RLU values (Figure 7). This may imply that stainless steel could be more resistant to picking up ATP from simple human touch.

CHAPTER SIX

Conclusions

Although this study was unable to determine a correlation between CFUs and RLUs, it did determine that, in the absence of mechanical action, the expectation that a clean surface should produce zero RLUs is unrealistic. Most surfaces continued to produce values of 10-20 RLUs after disinfection. The data garnered from the experiment would reject a reading of less than 100 RLUs as a valid "clean" value, as many of the surfaces produced RLU values near or below 100 RLUs prior to disinfection (Anderson et al., 2011). These experiments indicate that the "clean" standard must be lower and closer 25 RLUs, as none of the contaminated surfaces had produced RLUs below 25 and a majority of the surfaces had RLU readings below 25 after disinfection. In addition, this study showed that sterilization does not reduce the ATP bioburden on surfaces like a disinfectant is able to, implying that different "clean" RLU values may be required for each type of cleaning or disinfection. This could be tested by examining non-inoculated surfaces for RLUs before and after sterilization and testing surfaces for RLUs before and after disinfection or simply wiping the surfaces down.

Due to the high number of colonies on each of the surfaces, a correlation between CFUs and RLUs was unable to be determined at this time. However, ATP readings of the copper sheet and solid surface with cupric oxide did confirm the antimicrobial properties of copper as the MRSA's viability appeared to be impeded on these two surfaces. This

would suggest that copper surfaces provide some degree of benefit by being installed in hospital facilities. Further research should be conducted to test the CFU and RLU correlation by changing methods to ensure that MRSA inoculation amounts would produce quantities of CFUs between 30 and 300 to produce a countable number of colonies, making it possible to determine whether there is a true correlation between CFUs and RLUs. The effectiveness of copper could also be tested further by measuring the viability of different common hospital bacteria on it to see if they are similarly reduced like the MRSA.

APPENDIX

APPENDIX

Protocol

CFU Test

Materials

- Stainless steel (SS) sheet –4”x4”, less than 6.5mm (3) new, (3) worn
- Copper sheet (CP) sheet –4”x4”, less than 6.5mm (3) new, (3) worn
- Solid surface with cupric oxide (EO) solid surface-4”x4”, greater than 6.5mm (3) new, (3) worn
- Homogeneous solid surface (CR) solid surface-4”x4”, greater than 6.5mm (3) new, (3) worn
- High pressure laminate (HPL)–4”x4”, less than 6.5mm (3) new, (3) worn
- CryoCare Bead
- Tryptic Soy Agar Plate
- Bottle of 200ml Tryptic Soy Broth
- Test Tube with 10 ml Tryptic Soy Broth
- Ultrapure Sterile Water
- Inoculating Loop
- Tryptic Soy Agar Contact Plates
- Spray Bottle
- Clorox Healthcare Bleach Germicidal Cleaner (0.65% bleach solution)
- 35˚C Incubator
- CFU Counter
- Micropipette
- Micropipette Tips

Specimen Coding

- Specimens were given unique codes for each trial.
- Ex. 3/SS/N/1, Meaning Aim 3 (CFU), Stainless Steel, New, Triplicate Number

Procedure

1. Place one CryoCare bead onto a tryptic soy agar place.
2. Push the bead across the plate.
3. Cover the contact plate.

4. Incubate for 24 hours at 35˚ C.
5. Pour 10ml of tryptic soy agar broth into a test tube.
6. Remove one MRSA colony from the plate and place it into the 10ml of tryptic soy agar broth.
7. Incubate the broth for 20 hours at 35˚C.
8. Prepare a bottle of 200ml tryptic soy agar broth.
9. Flick the 10ml of tryptic soy agar broth to allow the pellet to mix with the broth.
10. Pour the 10ml of tryptic soy agar broth into the 200ml bottle of tryptic soy agar broth.
11. Incubate at 35˚C for 30 minutes.
12. Add 1ml of the 210ml solution to 99ml of ultra-pure sterile water.
13. Place all 30 surface samples into a clean biosafety cabinet.
14. Using a micropipette, inoculate each surface with 30 100µl droplets.
15. Allow surfaces to dry in the biosafety cabinet for 2 hours at 78˚F and 49% relative humidity.
16. Using contact plates, sample each of the surfaces by pressing the plates onto all four quarters of the surfaces.
17. Replace the lids of the contact plates.
18. Incubate the plates at 35˚C for 24 hours.
19. Using a spray bottle, disinfect the surface samples with Clorox Healthcare Bleach Germicidal Cleaner.
20. Surfaces should be wet, but not saturated for at least 10 minutes.
21. Surfaces should be dry after 30 minutes.
22. Use contact plates to obtain cultures of the disinfected surfaces.
23. Incubate the contact plates at 35˚C for 24 hours.
24. Using the CFU counter, count the number of colonies growing on each contact plate.
25. Record each value.

ATP Test

Materials

- Stainless steel (SS) sheet –4”x4”, less than 6.5mm (3) new, (3) worn
- Copper sheet (CP) sheet –4”x4”, less than 6.5mm (3) new, (3) worn
- Solid surface with cupric oxide (EO) solid surface-4”x4”, greater than 6.5mm (3) new, (3) worn
- Homogeneous solid surface (CR) solid surface-4”x4”, greater than 6.5mm (3) new, (3) worn
- High pressure laminate (HPL)–4”x4”, less than 6.5mm (3) new, (3) worn
- CryoCare Bead
- Tryptic Soy Agar Plate
- Bottle of 200ml Tryptic Soy Broth

- Test Tube with 10 ml Tryptic Soy Broth
- Ultrapure Sterile Water
- Inoculating Loop
- Spray Bottle
- Clorox Healthcare Bleach Germicidal Cleaner (0.65% bleach solution)
- 35˚C Incubator
- Micropipette
- Micropipette Tips
- 3M Clean Trace Luminometer
- ATP swabs

Procedure

1. Place one CryoCare bead onto a tryptic soy agar place.
2. Push the bead across the plate.
3. Cover the contact plate.
4. Incubate for 24 hours at 35˚ C.
5. Pour 10ml of tryptic soy agar broth into a test tube.
6. Remove one MRSA colony from the plate and place it into the 10ml of tryptic soy agar broth.
7. Incubate the broth for 20 hours at 35˚C.
8. Prepare a bottle of 200ml tryptic soy agar broth.
9. Flick the 10ml of tryptic soy agar broth to allow the pellet to mix with the broth.
10. Pour the 10ml of tryptic soy agar broth into the 200ml bottle of tryptic soy agar broth.
11. Incubate at 35˚C for 30 minutes.
12. Add 1ml of the 210ml solution to 99ml of ultra-pure sterile water.
13. Place half of the surface samples (15 new and 15 worn) into a clean biosafety cabinet.
14. Using a micropipette, inoculate each surface with 30 100µl droplets.
15. Allow surfaces to dry in the biosafety cabinet for 2 hours at 78˚F and 49% relative humidity.
16. Using the ATP swabs, sample each surface by running the swab over the surface in a zig-zag pattern horizontally and vertically.

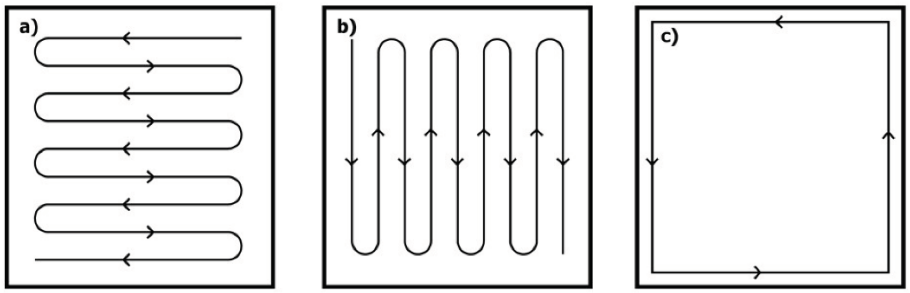

Figure 1. Display of wiping pattern.

17. Replace the ATP swabs back into their respective containers.
18. Repeat with each surface.
19. Using a spray bottle, disinfect the surface samples with Clorox Healthcare Bleach Germicidal Cleaner.
20. Surfaces should be wet, but not saturated for at least 10 minutes.
21. Surfaces should be dry after 30 minutes.
22. Using the ATP swabs, sample each surface by running the swab over the surface in a zig-zag pattern horizontally and vertically.
23. Replace the ATP swabs back into their respective containers.
24. Plunge each ATP swab into the luciferase solution.
25. Shake each container side-to-side to ensure that the solution is properly activated.
26. Promptly place the container with the activated solution into the 3M Clean Trace Luminometer.
27. Record each RLU value.

Sterilization Test

Materials

- Stainless steel (SS) sheet –4”x4”, less than 6.5mm (3) new
- Copper sheet (CP) sheet –4”x4”, less than 6.5mm (3) new
- Solid surface with cupric oxide (EO) solid surface-4”x4”, greater than 6.5mm (3) new
- Homogeneous solid surface (CR) solid surface-4”x4”, greater than 6.5mm (3) new
- High pressure laminate (HPL)–4”x4”, less than 6.5mm (3) new
- Chex-all sterilization bags
- Fisherbrand SterilElite 24 Tabletop Autoclave
- 3M Clean Trace Luminometer
- ATP swabs
- LMS-Z200 Series Stereo Microscope System
- Entris Laboratory Balance

Specimen coding

- Specimens were given unique codes for each trial.
- Ex. S/CP/1, meaning Sterilization, Copper, Triplicate number

Procedure

1. Obtain 15 specimens, three of each type of surface.

2. Inscribe unique identifying codes on each sample.
3. Weigh specimens individually
4. Place samples into Chex-all sterilization bags by surface type.
5. Sterilize samples in the Fisherbrand SterilElite 24 Tabletop Autoclave at 135˚C on the covered setting with drying.
6. Allow samples to dry in the Chex-all sterilization bags.
7. Carefully remove each individual sample.
8. Weigh samples individually on an Entris Laboratory Balance.
9. Record weights.
10. Place each sample beneath an LMS Z200 Series Stereo Microscope System to obtain images of the clean surface.
11. Place samples in a clean biosafety cabinet.
12. Run ATP swabs over each sample in a zig-zag pattern both horizontally and vertically.

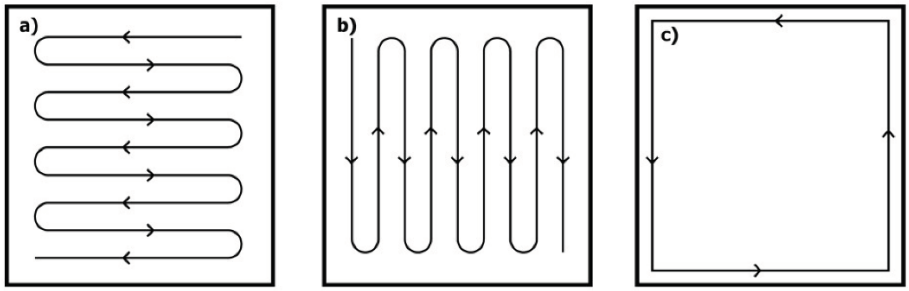

Figure 1. Display of wiping pattern.

13. Return swabs to their individual containers.
14. Plunge swabs individually into luciferase solution after each surface is sampled.
15. Shake container side-to-side to activate the enzyme solution.
16. Promptly place the activated container in the 3M Clean Trace Luminometer.
17. Record RLU readings.

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