PERFORMANCE OF GERMBOT WITH GENERIC *E. COLI*

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Introduction

UV light has been utilized in biosafety cabinets to destroy bacteria, viruses, and parasites. Research has shown that the UV light works to damage DNA and RNA of these organisms (Becker *et al*., 1989). While UV light does not kill these original microorganisms, it renders them unable to replicate and thus unable to cause infection if taken in by a host organism, such as a human. UV light has not only been utilized in biosafety cabinets, but also as a common water disinfection method (NWRI and WRF, 2012). Hospitals across the country also use UV to sterilize hospital surfaces, and been shown to sterilize more effectively than chemical cleaning methods (Andersen *et al*., 2006). Although, areas shadowed or shielded from UV do not result in microbial inactivation. A more recent idea regarding UV light is using it to sterilize surfaces in other venues.

A new design has the potential to make sterilization of large surface areas almost effortless. A trial product referred to as the Germbot® has a design analogous to the iRobot® vacuum cleaner (Figure 1). The technology used to maneuver the robot is similar, however the Germbot® has 6.5 -7 watt UVC light that runs the length of the diameter of the Germbot®. The sustained output of the UVC bulb is 1.5 watts. The manufacturers travel speed is reported to be 300 mm/sec. Given the bulb wattage, and estimates of sustained UVC emissions, the surface should be exposed to 3225 microwatts/cm^2 (Winkler, 2014). However, the Germbot® is programed to typically make three passes randomly over the same surface area. The fluence (or UV dose) are typically reported in microwatt-sec/ cm^2. In one study of biological safety cabinets, the energy output should not be less than 40 µW/ cm^2. The Germbot® meets that criteria. The fluence to inactivate 90% of *E. coli* in BSCs is reported to be on the order of 40,000 µW-sec/ cm^2 (UOttawa, 2013).

In order to determine whether the Germbot® delivers an adequate fluence to disinfect floors, a series of experiments were conducted. A variety of hard surface floor materials (ceramic tile, gym mat, and linoleum) were tested. Since time is a factor that greatly affects fluence, two different travel speeds were tested.

Figure 1. Schematic of Germbot®

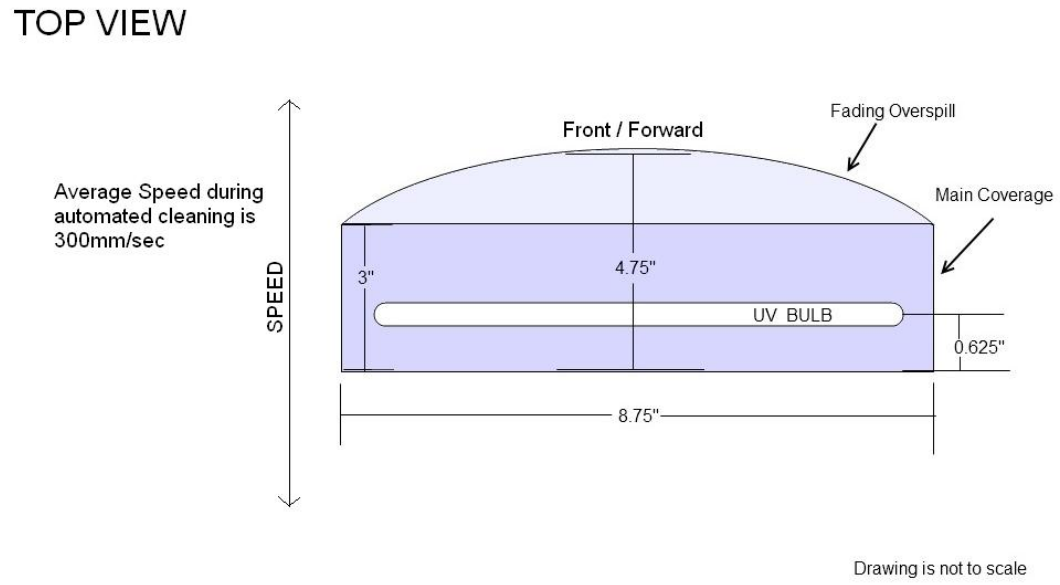


Figure courtesy of Innostrat Group

Methods

A solution of phosphate buffered saline (PBS)/0.01% (v/v) Tween 80 was prepared for suspension and enumeration of bacteria prior to experimental testing. The PBS was prepared from pre-mixed powder packets provided by Sigma-Aldrich Co (St. Louis, MO). One packet of the powder was mixed into each liter Type I laboratory water in an autoclaved 5 liter glass bottle with the aid of a magnetic stir bar and stir plate. The solution was stirred at a moderate speed until no powder was visible. Five hundred mL of water was reserved from the total volume and 100 µL Tween 80 (Sigma-Aldrich Co, St. Louis, MO) per liter total volume was added and stirred until dissolved. This solution was quantitatively added to the balance of the solution in the 5 liter bottle. The solution was well mixed and autoclaved at 121° for 15 minutes. The solution was cooled overnight, and then 100 mL aliquoted to sterile 150 mL bottles and stored in a 4°C until use, typically within one week. The bottles of solution were used to resuspend bacteria from each individual floor material coupon.

The use of PBS-Tween 80 was chosen for multiple reasons. First, PBS has a pH of approximately 7.4. This pH range provides a mild environment for bacteria, which help prevent additional injured *E. coli* cells from dying during resuspension. The mixture contained 0.01% Tween 80, a surfactant, because this detergent facilitates removal of *E. coli* cells from the surface of the material being tested. Therefore, use of this solution minimized the chance of over-estimating the inactivation efficacy of the Germbot® UV light.

The experiments were conducted in a biological safety cabinet to minimize environmental contamination. Prior to the Germbot exposure, all floor materials (ceramic tile, plastic mat coupons, linoleum coupons) and equipment (pipetman, pipet tips, forceps, etc.) were exposed to UV light for at least 20 minutes to ensure all materials were sterile.

The test materials included miniature ceramic tiles, plastic mat coupons and linoleum coupons. Each was approximately one square inch in area. The target inoculum was approximately 1000 *E. coli* (ATCC 11775 type strain) cells in order to assess up to three $\log_{10}$ reduction by UV exposure. Based on the concentration of the cell stock solution, the cell suspension in tryptic soy broth with 25% glycerol was pipetted onto the test material surface and allowed to air dry. For the first experiment (ceramic tile at full speed), 200 µL of cell suspension of approximately 6000 cells/mL was used. It required over an hour for the water to evaporate and the amount of glycerol remaining on the solution was deemed significant. A more concentrated suspension of approximately 133,300 cells/mL was used in all subsequent experiments. Therefore, only 10 µL of cell suspension was needed to inoculate coupons and UV exposure could commence after approximately 15 minutes.

After drying, three replicate tiles/coupons were exposed to one pass of the Germbot, another group was exposed to two passes, and final group experienced three passes of the Germbot UV light. Two travel speeds were tested. The first was the manufacturers speed (full speed) and the other was at one half the manufacturers speed (half speed).

All tiles/coupons that were placed in 100mL of PBS/0.01% (v/v) Tween-80 solution in sterile 150mL bottles. *E. coli* was enumerated using chromogenic substrate in most-probable number format (APHA *et al.,* 2012). The commercial product Colilert® was used in Quanti-Tray® 2000 format (IDEXX Corporation, Westport, ME). The Quanti-Trays® were incubated at 35±1°C incubator for 24 to 28 hours. Production of a yellow color and fluorescence under UV light indicates the presence of *E. coli*.

In all, a total of 18 tiles were used in each of the ceramic tile and plastic mat experiments. Three of the tiles/coupons inoculated with *E. coli* were randomly selected and enumerated prior to operation of the Germbot, allowing for a reference point to determine approximately how many viable *E. coli* were present on the tiles at the start of the experiment. Three tiles/coupons were enumerated after the Germbot exposures were completed, without receiving any exposure to the Germbot UV light. The purpose of this was to account for *E. coli* inactivated during the length of the experiment. The controls for this experiment included a spike of the concentrated *E. coli* suspension. To confirm that the tiles/coupons were sterile before being inoculated with *E. coli,* a tile/coupon was enumerated directly. As another negative control, a bottle of the PBS/Tween-80 solution itself was also enumerated to assure sterility.

Upon examination of the data from the first experiments, the relative positions of the triplicate ceramic tiles to the geometry of the Germbot were noted and tracked through enumeration in the final experiment. This was conducted to assess the heterogeneity in UV exposure resulting from position relative to the UV source.

Results

As discussed above, time of UV light exposure is an important factor in fluence and ultimately inactivation efficiency. The Germbot® is designed to randomly traverse an area up to three times during its operation. Therefore, a comparison between one pass, two passes, and three passes was conducted. In addition, the experimental unit was tested at full-speed and half-speed. Table 1 summarizes all the inactivation data. The goal of this was to determine how much the efficacy of the UV exposure was affected by the number of passes and exposure time. Figure 2 summarizes the means of three replicates for each exposure at full speed, while Figure 3 presents the results at half-speed. Note that the data for linoleum was limited to one replicate. As anticipated the duration of exposure of the Germbot® was an important factor in determining the effectiveness of the UV inactivation. It can be seen clearly in Figure 2 and 3 that *E. coli* inactivation improved with the number of passes. It can also be observed that the total percent inactivation is higher with the Germbot® operated at half-speed over full-speed.

Table 1. Summary of UV Inactivation Data

	Full-Speed (% inactivation)		Half-Speed (% inactivation)		
	2 Passes	3 Passes	1 Pass	2 Passes	3 Passes
Ceramic	73.7	73.7	56.1	97.4	99.4
Gym mat	88.5	96.5	48.4	94.0	99.6
Linoleum*	NT	NT	52.2	82.5	98.5

Data represents the mean of three replicates
*data from only one coupon
NT – not tested

However, it must be noted that there was approximately a ten minute time gap in between the tiles exposed to one pass of the Germbot and the tiles exposed to three passes. Therefore, there was the potential that a percentage died off during this time gap as a result of desiccation. In order to avoid over estimation of the Germbot inactivation, three tiles had the levels of E. coli measured before the experiment and three tiles had the levels of *E. coli* measured after the experiment. The group of three tiles before and the group of three tiles after received no exposure. The *E. coli* levels of the before and after tiles were averaged and used as a reference to determine the approximate number of *E. coli* cells inactivated by the Germbot®.

Figure 2. Comparisons of Number of Passes at Full-Speed

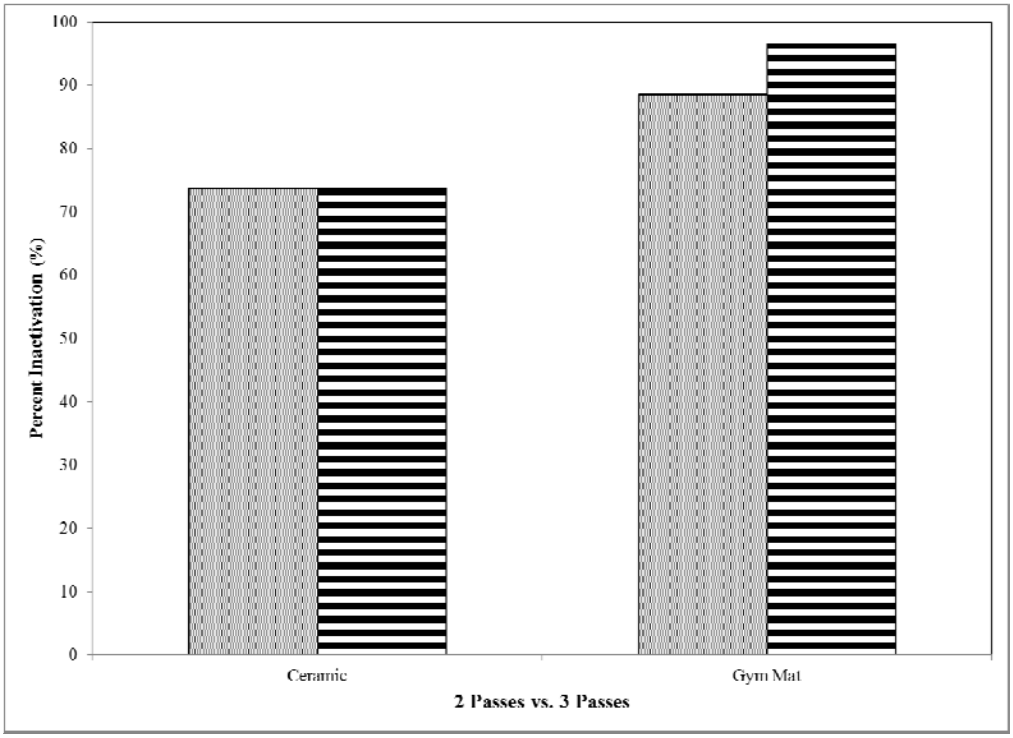
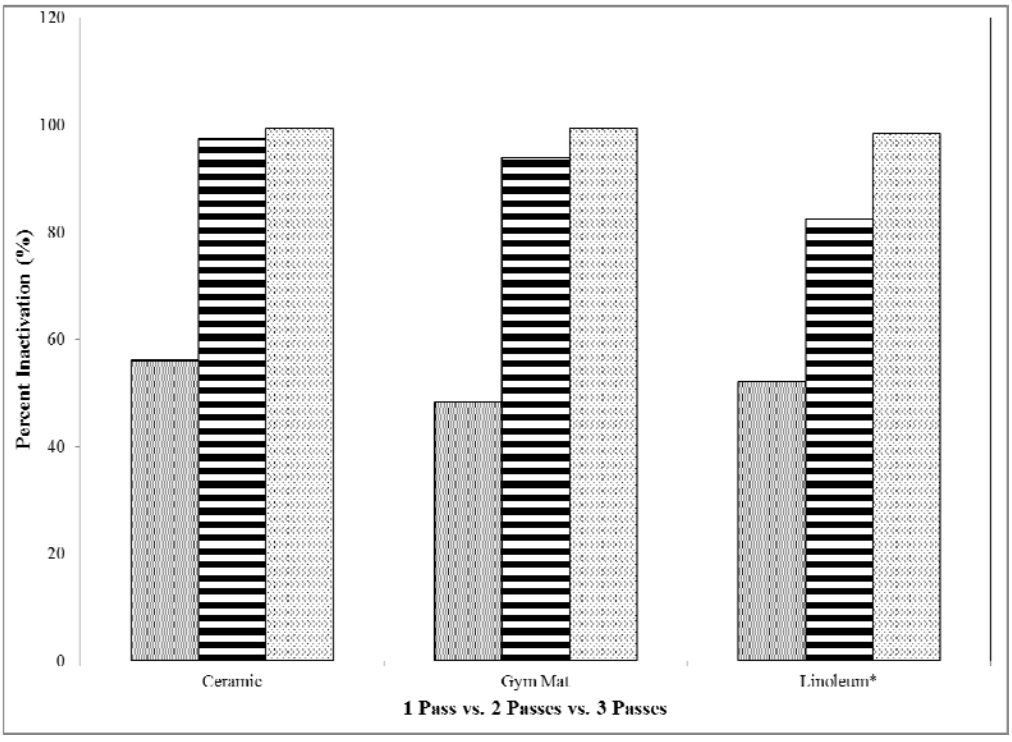


Figure 3. Comparisons of Number of Passes at Half-Speed



Student T-tests were applied to the data. There were no statistical differences after three passes among any of the different factorial tests. However, after only two passes, the levels of inactivation were statistically different between the full-speed and half-speed trials.

Another variable of this experiment was the type of material being tested. The two main materials tested were ceramic tile and gym mat. The results are presented for full-speed in Figure 4 and for half-speed in Figure 5. At first, it would appear inactivation was more effective for the gym mat material than for the ceramic tile when the data at full-speed was examined. However, the numbers were found to be very similar at half-speed. Student T-tests revealed that the differences at both speeds were not statistically significant. Therefore, it seems that the type of material does change the effectiveness of the Germbot® for the types of non-porous surfaces tested.

Figure 4. Comparison of Material at Full-Speed

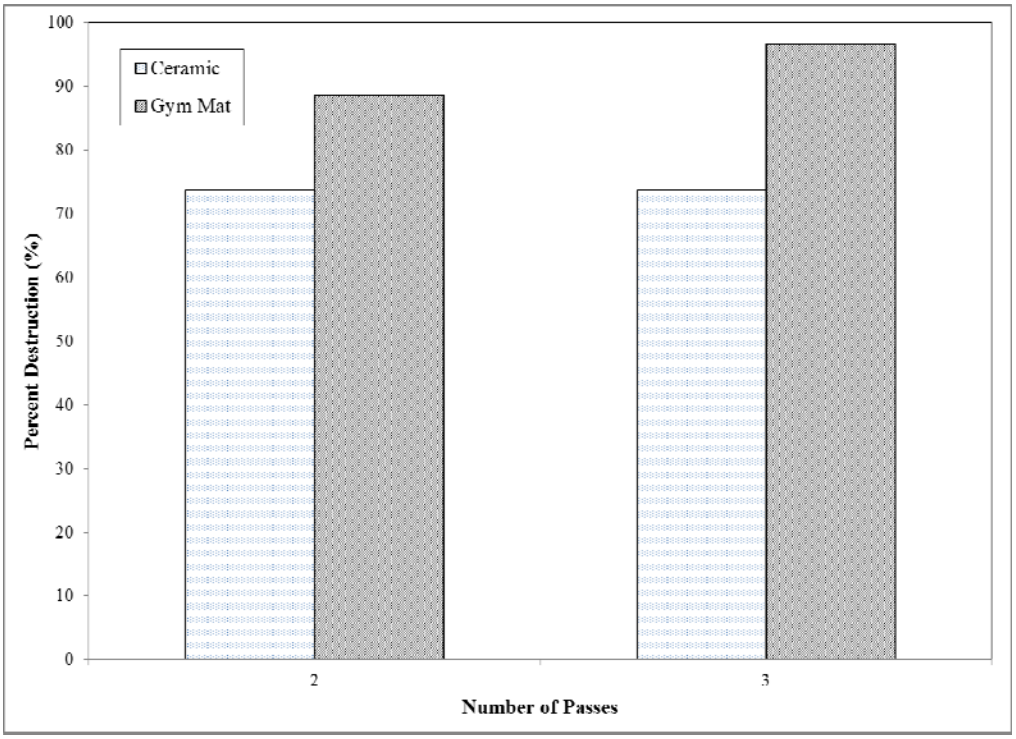
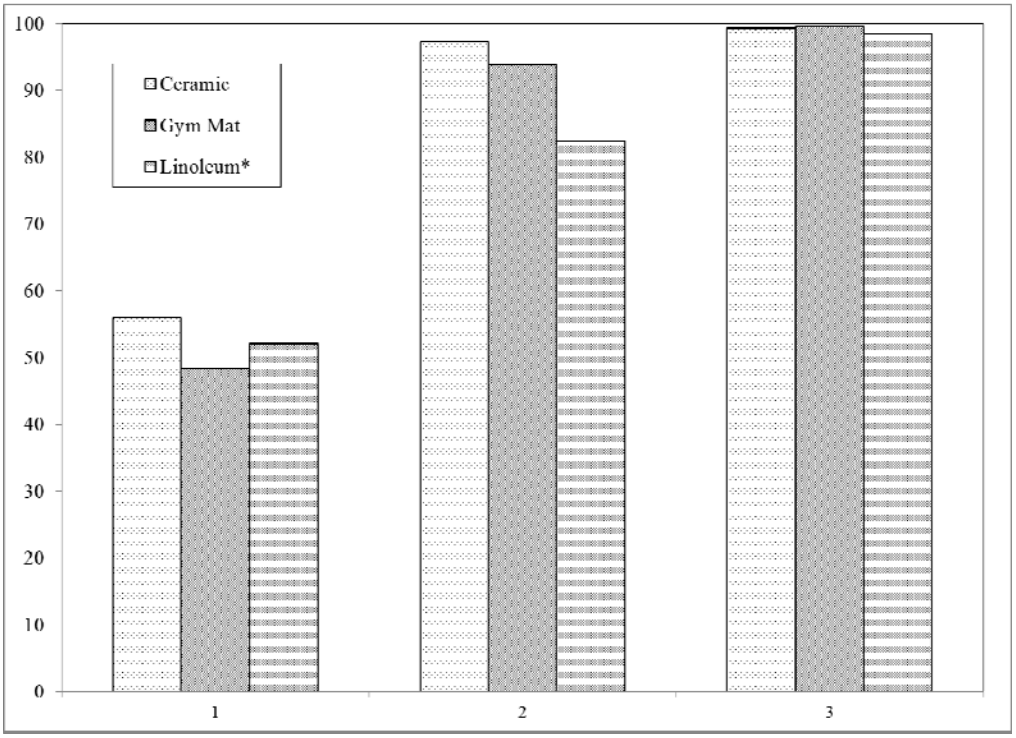
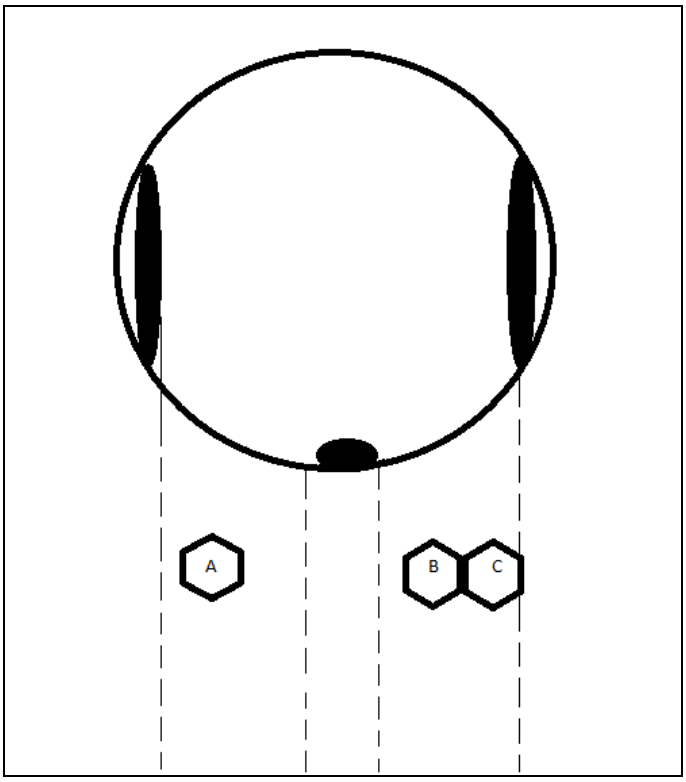


Figure 5. Comparison of Materials at Half-Speed.



When analyzing the data of the first few experiments, a certain pattern type of pattern seemed to develop. Frequently, one tile in each group of three exhibited a much higher destruction level than the other two. Therefore in a final experiment, the relative positioning of the tiles was compared (Figure 6). It was found that positon A had the highest level of destruction in this experiment. However, further experimentation with positioning is necessary to see if this trend is repeated. However based on these results, it seems that the power of Germbot® UV light may be stronger in certain areas, which explains the varying levels of inactivation.

Figure 6. Schematic of Position Experiment



Conclusions and Recommendations

The results of these preliminary experiments indicate that the Germbot® will affect inactivation of *E. coli* bacteria. Decreasing speed and increasing the number of random passes over the same floor area (*i.e.* increasing exposure time) increased bacterial inactivation. Although there appeared to be observational differences among non-porous surface material type, these were likely a result of experimental variability and were not statistically significant.

It is recommended that more rigorous testing with bacterial spores and viruses, organisms demonstrated to be more difficult to inactivate, be conducted. It is also recommended that pathogens of concern, such as multiple antibiotic resistant *S. aureus* be tested as well.

References

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