Determining the efficacy of hydrogels as a surface sampling method for surfaces

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Introduction

Biological terrorism is the intentional release of toxic biological agents into a population with the goal of causing sickness or even death. This issue has been prevalent for many years, a prime example being the occurrence of the anthrax attacks in 2001. These attacks involved the mailing of letters laced with anthrax spores to individuals across the United States, infecting seventeen and killing five others (Rastogi et al., 2009).

The anthrax attacks brought focus to the importance of analysis of contaminated surfaces after a bioterrorist attack. Analysis relies on surface sampling, which collects microorganisms from exteriors, and it allows conclusions to be drawn about the agent, such as its composition and the quantification of the amount released. Surface sampling results give public leaders the information necessary to make decisions regarding measures that should be taken to minimize adverse impact on public health.

Past studies have been done regarding surface sampling methods and their efficacies. Results from these studies have shown that currently available sampling technologies, such as swabs and wipes, have efficacies of 30–70 percent for smooth, nonporous surfaces, but less than one percent for bumpy, porous surfaces, illustrating the demand for more effective sampling methods for porous surfaces (Frawley et al., 2008).

This study hypothesizes that hydrogels, a crosslinked hydrophilic polymer, would make a more accurate surface sampling tool. The scope of the study involves comparing the efficacies of hydrogels to current technologies on both porous and nonporous surfaces.

Methods and Materials

To determine the difference in recovery efficiencies of hydrogels compared to wipes, twelve separate experiments were done: three wipes and three gels, each on concrete and on plastic. Methods for procedures and data collection were taken from a similar study by Smith et al. (2016).

Gel experiments were conducted over three days. On the first day, coupons were spotted with a *Bacillus globigii* spore suspension and allowed to dry. Hydrogel was applied to the coupons (Figures 1 and 2), and again allowed to dry. The next day, the gel was peeled off using sterile tweezers, placed into conical tubes filled with phosphate buffer containing Tween-80 (PBS-T buffer), and vortexed. Serial dilutions were performed for all coupons, which involved the aliquoting of 100 microliters (µL) of the

Figure 1 (left): A 2-inch by 0.75-inch by 0.75-inch concrete/porous coupon after hydrogel application.

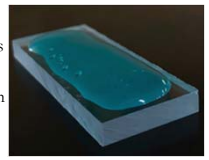


Figure 2 (right): A 2-inch by 1-inch by 0.25-inch plastic/nonporous coupon after hydrogel application.

Methods and Materials (continued)

original suspension into 900 µL of PBS-T buffer and continuing this process until the desired dilution was reached. Select dilutions were plated in duplicate on tryptic soy agar (TSA) plates, and the plates were incubated at ambient room temperature for 48 hours. On the third day, colony-forming units (CFUs) were counted from each plate (Figure 3).

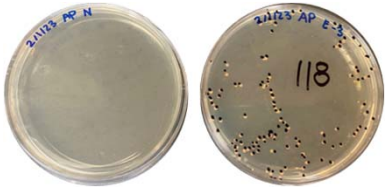


Figure 3 (left): Two TSA plates after counting of the CFUs had been completed. The left plate is the negative control, and therefore, has zero CFUs. The right plate is the extracted control. The CFUs counted were recorded to be 118.

Wipe experiments utilized similar methods. On the first day, coupons were spotted with spore suspension, allowed to dry, and swiped with sterile polyester wipes. The wipes were placed into conical tubes containing PBS-T buffer and vortexed. Serial dilutions were performed, select dilutions were plated in duplicate, and the plates were incubated at ambient room temperature for 48 hours. On the second day, CFUs formed were counted.

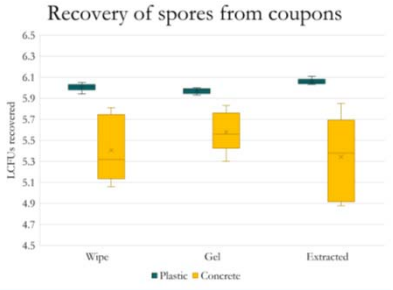
Several controls were done for each experiment. Negative controls used the same methods as wipe/gel experiments, but no spores were applied. Extracted controls used the same methods as wipe/gel experiments but with no extraction method, and they determined the maximum spore extraction. Titer controls were plated aliquots of diluted spore suspension done to determine the total spores deposited on each surface. Log CFUs (LCFU's) were computed using the equation in Figure 4 to give comprehensible values for analysis.

$$\text{LCFUs recovered} = \log\left(\left(\frac{(\text{Plate 1 CFUs})(\text{Plate 2 CFUs})}{2}\right) \div \text{dilution factor}\right)$$

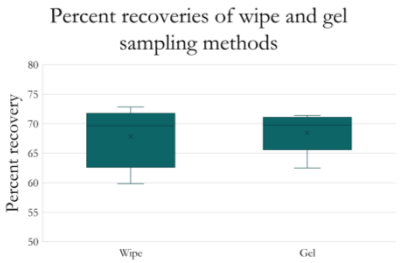
Figure 4 (above): The equation used to calculate LCFU's recovered values. The average CFU's were divided by the dilution factor, and the logarithm of that number was taken.

Results

Graph 1 (right): The recoveries in LCFUs of each of the 18 wipe (test) groups, the 18 gel (test) groups, and the 12 extracted (positive) controls. The 12 negative controls (not depicted in the graph) yielded zero CFUs recovered, proving test sterility. There is no significant difference in LCFUs recovered between the wipe, gel, and extracted groups.



Results (continued)



Graph 2 (left): The percent spore recoveries of the wipe sampling method on both plastic and concrete compared to the gel sampling method on both plastic and concrete. Both groups have very similar percentages of approximately 68%, showing very little difference between the two methods.

A two-sample *t*-test showed there was no significant difference in the LCFUs recovered between the wipe sampling method ($M = 5.71$, $SD = 0.368$) and the gel sampling method ($M = 5.77$, $SD = 0.240$), $t(18) = 0.650$, $p = .518$. Using the alpha level of 0.05, the null hypothesis could not be rejected, indicating there is not a statistically significant difference in the recovery efficacies of wipes and hydrogels.

Conclusions

This study was conducted to serve as a basis to evaluate the applicability of hydrogels as a surface sampling method for various surfaces. The hypothesis projected that hydrogels would serve as a more accurate sampling method compared to wipes on various surfaces. With similar recovery values between the two groups, the data collected in this investigation does not support the hypothesis, but instead that hydrogels are comparable to wipes. Hydrogels do offer a more efficient approach than wipes, however. Their application can easily become an automated process, as it is simpler and requires no prior training. This requires less human intervention, leading to less error. A drawback of hydrogels are their cost and the dry time of over twelve hours for the hydrogel to form a thin film. The suitability of hydrogels as a more accurate sampling method would require future studies to be done using a variety of other surfaces.

References

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