Contamination of polyvinyl chloride acetate-based credit cards using *Bacillus atrophaeus* var. *globigii*

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Introduction

As credit cards become a highly used method of paying for goods in the economy, it increases the possibility of posing a health threat to society. There are limited published studies available addressing the potential of credit cards to transfer microorganisms from one surface to another. By investigating the ability of organisms to be transferred, awareness and understanding of certain health threats posed by credit cards and similar commonly handled objects is publicized. A recent study published by J. Kesavan, Stephens, and M. Kesavan (2016), indicated that a significant number of bacteria were transferred between paper currencies, potentially causing a source for disease transmission and bioterrorism. The current study evaluated how this type of transfer could occur between credit cards.

Bacillus anthracis, the deadly bacteria that causes the infectious disease anthrax, is one of the most likely agents to be used in a biological attack. If surfaces were contaminated with anthrax, large numbers of people can come in contact and become infected.

Spores from the organism *Bacillus atrophaeus* var. *globigii* (Bg spores) were chosen in this study. It is a non-pathogenic, aerobic, spore-forming, Gram positive microorganism. Spores were chosen because of their ability to withstand environmental stresses such as dehydration, since the question of concern is about transfer and not viability. *B. atrophaeus* was also chosen because of its orange color when inoculated on trypticase soy agar (TSA) plates. This characteristic makes it easily distinguishable from other microorganisms.

The purpose of this study was to test for the cross-contamination potential of credit cards and the collection efficiency of *B. atrophaeus* spotted on polyvinyl chloride acetate (PVCA) plastic credit cards. The null hypothesis states that there was no difference in the collection efficiencies of PVCA plastic and glass when sampled with an identical collection swab and there was no difference in the transfer rates between PVCA surfaces when using different transfer methods, sleeve and pressure.

Methods and Materials

The credit cards and one-quarter inch industrial glass were decontaminated using 70% ethanol (EtOH) and the glass was autoclaved (20 minutes, 121 °C). For these surfaces, 2×10^7 colony forming units (CFUs) Bg spores diluted in 1 mL Butterfield's buffer, were individually spotted on the surfaces and then allowed to dry. The surfaces were sampled using pre-moistened Puritan® Sterile Foam Tipped Applicators (Figure 1) and techniques from the Centers for Disease Control and Prevention's procedures for sampling surfaces for *B. anthracis* spores from smooth, non-porous surfaces (CDC, 2012). The swabs were placed in conical tubes with 10 mL of Butterfield's buffer and vortexed for at least one minute. The samples were serially diluted by pipetting 100 µL of the sample into

Materials and Methods (cont.)

900 µL of buffer. The titer control was determined by pipetting 1 mL of 2×10^7 CFU Bg spores into 10 mL of Butterfield's buffer, serially diluting, and plating select dilutions onto a TSA plate. The buffer control was obtained by pipetting 100 µL of buffer directly onto a TSA plate.

Additional experiments were performed to determine the potential of credit cards to transfer microorganisms. First, a decontaminated card was placed in a 3.7 × 2.3 in. transparent soft plastic sleeve. A second decontaminated card was spotted with 2×10^7 Bg spores and set to dry. After drying, the second card was slid into the plastic sleeve with the decontaminated card for trials of either one minute or 24 hours before removal. Both cards were sampled using the same technique previously stated.

Another experiment was conducted to test pressure using a painted cement block (Figure 2). A decontaminated card and spotted card were placed facing each other under the cement block for trials of either one minute or 24 hours. Both cards were then sampled using the same technique stated previously.

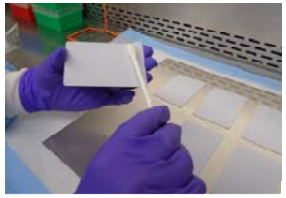


Figure 1 (left): A macrofoam swab was used to sample a PVCA credit card contaminated with Bg spores under a biological safety cabinet.

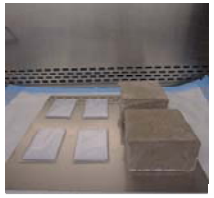
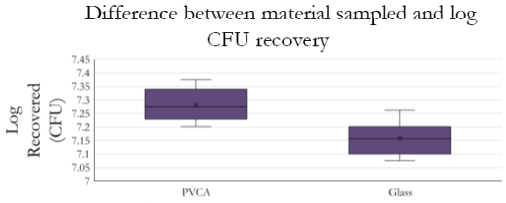
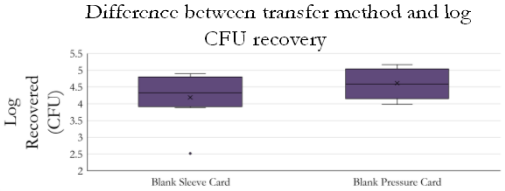


Figure 2 (right): Bg spores spread on a PVCA credit card were placed in a sleeve with a decontaminated card. Another trial was performed with cement block and pressure.

Results

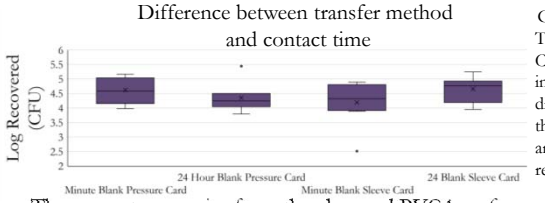


Graph 1 (left): The number of CFU's per sample was displayed logarithmically to show the difference in amount of colonies recovered between materials.



Graph 2 (right): The number of CFU's per sample was displayed logarithmically to show the difference between the transfer method and the colonies recovered.

Results (cont.)



Graph 3 (left): The number of CFU's was shown in logs to show the difference between the transfer time and the colonies recovered.

The percent recoveries from the glass and PVCA surfaces were 69.10% (M = 7.16, SD = 0.06) and 83.97% (M = 7.28, SD = 0.06), respectively. This was determined utilizing the titer controls because they displayed the greatest recovery. The blank sleeve card had a percent recovery of 0.16% and blank pressure card was 0.37%. A 2-sample t-test run on the data in Graph 1 with $\alpha = 0.05$ and $n = 10$ for each material produced a p-value of 0.0002. Using a 2-sample t-test to evaluate the data from Graph 2, where $\alpha = 0.05$ and $n = 9$ for each group, the test yielded a p-value of 0.1982. Using two separate 2-sample t-tests to evaluate the data from Graph 3, where $\alpha = 0.05$ and $n = 7$ for each group, the test yielded a p-value of 0.4455 for the pressure transfer method and 0.1738 for the sleeve transfer method.

Conclusions

This project helped determine if microorganisms can be sampled from PVCA surfaces and the most effective mode of transfer between two PVCA surfaces. There were limited studies that explored the relationship between microorganisms and credit cards. The results of this study displayed that PVCA surfaces have a significantly greater recovery rate of Bg spores when compared to glass, which was used as a control surface. This could be due to the hydrophobic property of PVCA surfaces. When observing the data from the method of transfer experiments, there was no significant difference between the pressure and sleeve data. When observing the data that compared the method of transfer and contact time, there was no significant difference between the one minute and 24 hour trials. This again could be due to the hydrophobic features of PVCA surfaces. Future experiments could test the effect of an embossed credit card on the recovery.

These findings can help the public better understand and bring awareness to the possible threats of cross-contamination of microorganisms through credit and gift cards. Bioterrorism is another possible threat to consider when dealing with commonly handled objects such as credit cards.

References

Kesavan, J., Stephens, A., & Kesavan, M. (2016). Bacterial cross-contamination potential associated with contaminated currency. *Journal of Forensic Sciences, 61*(6), 1639–1642.

Surface sampling procedures for *Bacillus anthracis* spores from smooth, non-porous surfaces. (2012). Retrieved February 8, 2019 from https://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html