FACTORS TO CONSIDER IN THE INDOOR ENVIRONMENT WHEN EVALUATING THE RISK OF HUMAN HEALTH AT THE ENVIRONMENTAL DETECTION LIMIT

By

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ABSTRACT

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Quantitative analysis of the limit of detection, sample recovery efficiency (SRE) and characterization of the microbial community are necessary parameters for accurate exposure assessment as part of the quantitative microbial risk assessment framework for indoor environments. To control and remediate an indoor environment from an outbreak, accidental or intentional release of pathogens can be a challenging task. Without understanding the situation in quantitative terms, determination of a site as safe or "clean" especially when a sample result is negative will be unachievable. The negative result may not establish zero risk and can be due to variability in the sampling or detection methods. The release of *Bacillus anthracis* in 2001, the numerous outbreaks from the food industry, hospital settings, and on university campuses have highlighted the lack of quantitative information. A review of the literature for the limits of detection of methods detecting *B. anthracis* provided a distribution to quantify the variability in the instrument limit of detection; however there were only a few articles on the environmental limit of detection. An exponential dose response model estimated the risk at the dose equal to the environmental limit of detection to determine the probability of death as high as 0.52. The SRE of bacteriophage P22 was evaluated at the environmental limit of detection and was most affected by sampling time, fomite surface area, wetting agent and relative humidity. After samples dried on the fomite (20 min), less than 3% was able to be recovered even though the bacteriophage P22 was still active on the fomite. Genetically characterizing the bacterial communities on touched and untouched fomites resulted in two unique bacterial communities.

Touched fomites were more diverse and had a high presence of fecal indicators which demonstrated potential reservoirs for pathogens. This research will improve exposure assessment by indicating the risk and limitations at the environmental limit of detection, enhancement of sampling strategies and the role fomites have in the transmission of infectious diseases.

To my family, I will always be grateful for your unconditional love and support.

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Chapter 1: Introduction

1.1 Exposure assessment

Quantitative microbial risk assessment (QMRA) is a framework and approach that is used to address the spread of microorganisms through the environment (disease outbreak, bioterrorism event, etc.) and to characterize the risk to human health. There are four steps to the QMRA framework which includes hazard identification, dose response, exposure assessment, and risk characterization (5). The focus of the work presented is on obtaining quantitative date for exposure assessments. The exposure assessment quantitatively determines the dose of the organism exposed to the population and the route, concentration and duration of the exposure. The exposure assessment relies on acceptable methods and models for recovery, detection, and quantification. Methods should address sensitivity, specificity, virulence, viability and fate and transport through the environment (5). To analyze a wide range of risk scenarios it is also important to have knowledge on the ecology of microorganisms in the environment (fomites, water, soil, etc.), microbial sources, inactivation rates, survival in the environment, resistance to environmental factors (temperature, relative humidity (RH), UV, etc.) and the movement through the environment (5).

The limit of detection of any method is critical for assessing a negative result during environmental monitoring. While the instrument limit of detection can give quantitative information for ideal conditions, it is the environmental limit of detection that can assess real field scenarios. The environmental limit of detection also points out limitations in the methodologies. Chapter 2 explores the current literature knowledge on the instrument and

environmental detection limits for methods detecting *Bacillus anthracis*. The risk to human health is then calculated by using the limit of detection (instrument and environmental) as the dose of exposure. The results from this work will aid in the evaluation of the preparedness for bioterrorism events.

Sample recovery efficiency (SRE) directly affects the environmental limit of detection. The SRE is especially important to know when analyzing samples at concentrations close to the limit of detection. For instance, to confirm the efficacy of the cleaning protocols during environmental monitoring after an outbreak or bioterrorism event. With the use of pyrosequencing the knowledge of the microbial community is not limited to cultural method capabilities, such as an underestimation of organism (2). In chapter 3, the SRE is evaluated at the environmental limit of detection (large fomite area at low sample concentrations). The results will indicate the parameters that can affect variations in the SRE. This will lead to implementing appropriate sampling and decontamination strategies.

Defining the microbial ecology of any environment is significant for understanding the relationship between microorganisms, humans and the environment. Especially in the indoor environment, the microbial community analysis can demonstrate the effect human behavior and environmental factors on the fate and transport of microbial communities. Chapter 3 defines the bacterial communities in the indoor environment found on touched and untouched fomites. The results will provide information for infectious disease transmission models and improve fomite hygiene interventions.

1.2 The indoor environment

An important environment to study the exposure to a microorganism(s) from a bioterrorism event or disease outbreak is the indoor environment. The data presented in the following chapters evaluate the parameters affecting limit of detection, SRE, and microbial communities in the indoor environment, specifically the role of fomites. However, the limit of detection was also reviewed for the air, soil, and water environments.

The indoor environment is a complex ecosystem with a vast diversity of trillions of microorganisms (4, 6, 7). Humans spend a majority of their time indoors and have designed a closed environmental system to maintain static conditions (temperature and RH) to feel comfortable during seasonal variations (4, 6). In addition, human occupancy and movement, frequency of cleaning fomites and the use of mechanical ventilation systems instead of windows have controlled the microbial community structures of the indoor environment (3, 7). There are a variety of microbial sources (skin, pets, food), environmental factors (ventilation, shoes, body secretions) and dispersal vectors (pH, temperature, fomite material) that affect the microbial diversity (Figure 1.1) (6). Research has suggested that bacteria such as *Staphyloccus epidermis* can help protect against skin infections and bacteria associated with dogs can prevent allergies in children (3, 10). However there are pathogens such as *Salmonella* and *Escherichia coli* that have negative effects on human health. Also, horizontal gene transfer of antibiotic resistant genes of pathogenic bacteria on touched fomites may increase risk of drug resistant infections (12). There is still a great extent of knowledge to obtain on the dynamics of microbial ecology and the effects it has on human health (positive, negative, or neutral) in the indoor environment (2, 4, 6).

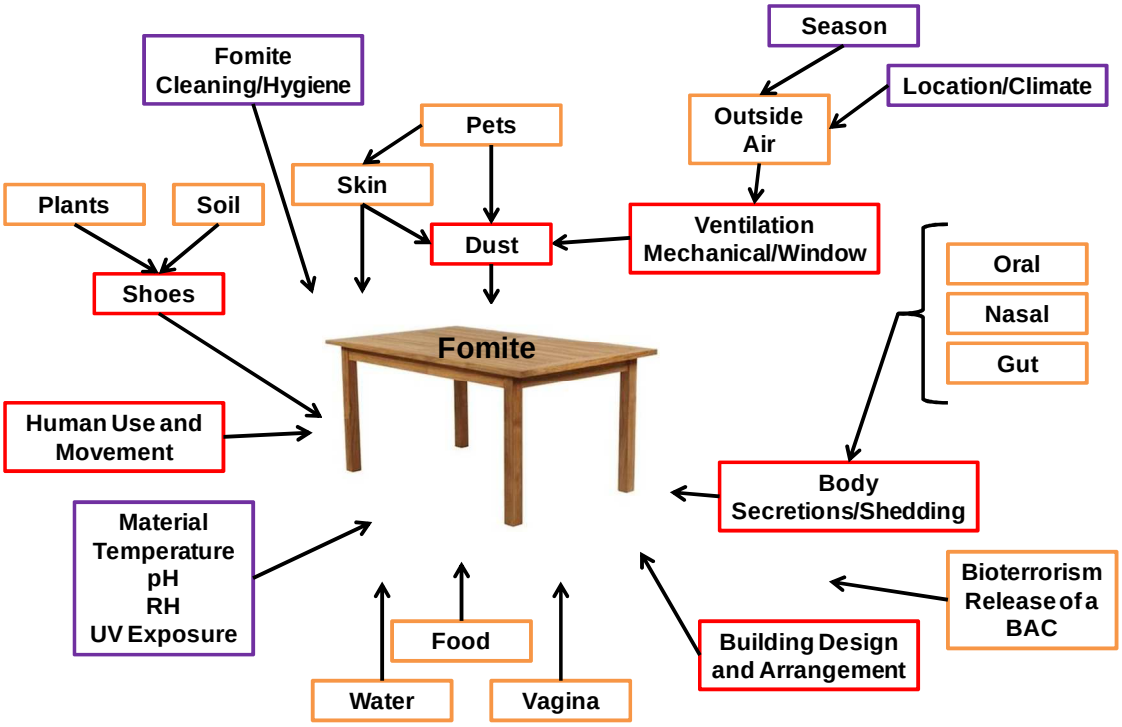


Figure 1.1 Various sources and transmission routes in the indoor environment that can effect microbial diversity. Where orange boxes represent microbiome source, purple boxes are environmental factors and red boxes are dispersal vectors (6).

1.3 Indoor infectious disease transmission

Nonporous fomites can be an important point for transmission of bacterial and viral disease especially for populated indoor environments (1, 11). Pathogens can survive on fomites for hours to months depending on the microorganism, concentration of the microorganism and the indoor environmental factors (1, 11). In general, exposure to live pathogens from the environment may be picked up by susceptible individuals from a fomite by touching (hand to mouth, eye or nose), direct contact from fomite to mouth or through the inhalation of resuspended microorganisms from contaminated fomites (Figure 1.2) (1, 8, 9). Then infected or infectious individuals deposit pathogens into the environment through shedding (saliva, blood, feces, etc.). Individuals (infected or not infected) touching fomites can transfer microorganisms and can inoculate and re-inoculate the fomite through its usage (6). Over time individuals recover, become completely immune or the infection was fatal. In addition, pathogens are eliminated from the environment through natural decay, decontamination processes, or removed through other environmental processes (Figure 1.2) (8).

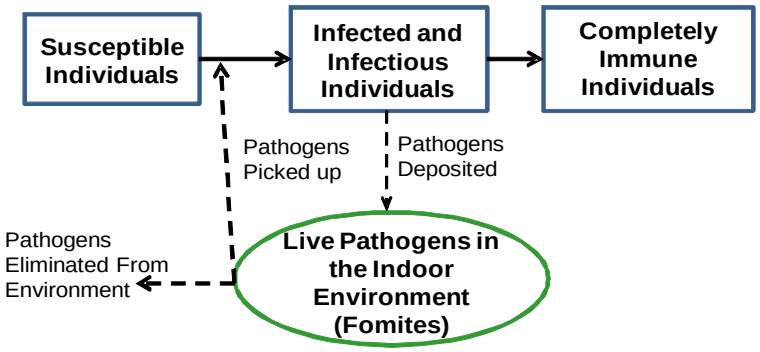


Figure 1.2 Environmental infection transmission model (8).

1.4 Dissertation outline and objectives

The overall objective of these chapters is to gain quantitative data for enhancement of exposure assessments in the QMRA framework for assessing risk to human health.

Chapter 2 – Implications of limit of detection of various methods for *Bacillus anthracis* in computing risk to human health. The instrument limit of detection and environmental limit of detection for all methods detecting *B. anthracis* are reviewed from the literature. Risk estimates are calculated at concentration equal to the instrument and environmental limit of detection. The literature review also identifies current limitations in the knowledge of detection methods for *B. anthracis* in environment matrices (air, soil, water and fomites).

Chapter 3 – Evaluation of sample recovery efficiency of bacteriophage P22 on fomites. Bacteriophage P22 is applied onto fomites at concentrations near the limit of detection to quantify the SRE. The variability in SRE as a function of fomite type, fomite surface area, sampling time, application media, relative humidity and wetting agent is evaluated. Survival of bacteriophage P22 (24 hr) on fomites indicated the enhancement of the sampling method for increased efficiency in sampling at low concentrations dried on the fomite.

Chapter 4 – Genetic characterization of microorganisms on highly touched and untouched fomites. To determine the microbial ecology in the indoor environment samples are collected from university dormitories from touched and untouched fomites and sequenced. Touched and untouched fomites provided unique bacterial communities. Dominate genera indicated interactions with humans and reservoirs of possible pathogen contamination. In addition,

determined the correlation between bacterial community with fomite type, room location and dormitory.

Chapter 5 – Conclusions. Results from the dissertation are summarized. Contributions of the science to the field and the future direction of the work are discussed.

REFERENCES

REFERENCES

1. **Boone, S. A., and C. P. Gerba.** 2007. Significance of fomites in the spread of respiratory and enteric viral disease. Appl. Environ. Microbiol. **73:**1687-1696.

2. **Corsi, R. L., Kinney, K.A., Levin, H.** 2012. Microbiomes of built environments: 2011 symposium highlights and workgroup recommendations. Indoor Air **22:**171-172.

3. **Dunn, R. R., N. Fierer, J. B. Henley, J. W. Leff, and H. L. Menninger.** 2013. Home life: factors structuring the bacterial diversity found within and between homes. PLoS One **8**.

4. **Green, J. L.** 2014. Can bioinformed design promote healthy indoor ecosystems? Indoor Air **24:**113-115.

5. **Haas, C. N., J. B. Rose, and C. P. Gerba.** 1999. Quantitative microbial risk assessment. John Wiley, New York.

6. **Kelley, S. T., and J. A. Gilbert.** 2013. Studying the microbiology of the indoor environment. Genome Biol. **14**.

7. **Kembel, S. W., E. Jones, J. Kline, D. Northcutt, J. Stenson, A. M. Womack, B. J. M. Bohannan, G. Z. Brown, and J. L. Green.** 2012. Architectural design influences the diversity and structure of the built environment microbiome. ISME J **6:**1469-1479.

8. **Li, S., J. N. S. Eisenberg, I. H. Spicknall, and J. S. Koopman.** 2009. Dynamics and control of infections transmitted from person to person through the environment. Am. J. Epidemiol. **170:**257-265.

9. **Lopez, G. U., C. P. Gerba, A. H. Tamimi, M. Kitajima, S. L. Maxwell, and J. B. Rose.** 2013. Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. Appl. Environ. Microbiol. **79:**5728-5734.

10. **Schommer, N. N., and R. L. Gallo.** 2013. Structure and function of the human skin microbiome. Trends Microbiol. **21:**660-668.

11. **Sinclair, R., S. A. Boone, D. Greenberg, P. Keim, and C. P. Gerba.** 2008. Persistence of category A select agents in the environment. Appl. Environ. Microbiol. **74:**555-563.

12. **Warnes, S. L., C. J. Highmore, and C. W. Keevil.** 2012. Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: implications for public health. mBio. **3:**10.

Chapter 2: Implications of limits of detection of various methods for *Bacillus anthracis* in computing risk to human health

This chapter is adapted from our published work in Applied and Environmental Microbiology: Amanda B. Herzog, S. Devin McLennan, Alok K. Pandey, Charles P. Gerba, Charles N. Haas, Joan B. Rose, and Syed A. Hashsham. Implications of Limits of Detection of Various Methods for *Bacillus anthracis* in Computing Risk to Human Health. Applied and Environmental Microbiology. 2009. 75(19):6331-6339. DOI:10.1128/AEM.00288-09.

2.1 Abstract

Used for decades for biological warfare, *Bacillus anthracis* (Category A agent) has proven to be highly stable and lethal. Quantitative risk assessment modeling requires descriptive statistics of the limit of detection to assist in defining exposure. Furthermore, sensitivities of various detection methods in environmental matrices are vital information for first responders. A literature review of peer reviewed journal articles related to methods for detection of *B. anthracis* was undertaken. Articles focused on the development or evaluation of various detection approaches such as polymerase chain reaction (PCR), real-time PCR, immunoassay, etc. Real-time PCR and PCR were the most sensitive methods for the detection of *B. anthracis* with a median instrument limit of detection of 430 and 440 cells/ml, respectively. There were very few peer reviewed articles on the detection methods for *B. anthracis* in the environment. The most sensitive limit of detection for the environmental samples were 0.1 CFU/g for soil using PCR-ELISA, 17 CFU/L for air using ELISA-biochip system, 1 CFU/L for water using cultivation, and 1 CFU/cm^2 for stainless steel fomites using cultivation. An exponential dose response model for

the inhalation of *B. anthracis*, estimates of risk at concentrations equal to the environmental limit of detection determined the probability of death if untreated as high as 0.520. Though more data on the environmental limit of detection would improve the assumptions made for the risk assessment, this study's quantification of the risk posed by current limitations in the knowledge of detection methods should be considered when employing those methods in environmental monitoring and clean up strategies.

2.2 Introduction

According to the Centers for Disease Control (CDC), a category A agent is an organism that poses a risk to national security because it can be easily disseminated or transmitted from person to person, results in high mortality rates, has the potential for major public health impact, might cause public panic and social disruption, and requires special action for public health preparedness (23). Quantitative information on category A agents in environmental matrices (soil, air, fomite, water) are very limited (64). However, from the literature it has been concluded that *B. anthracis* are the most environmentally stable category A agent overall (64).

After the release of *B. anthracis* through mail envelopes in 2001, assessment of the decontamination process revealed an important question: could the detection methods effectively determine if the environment is clean? An evaluation of the effectiveness of sampling methods at a U.S. postal facility in Washington D.C. contaminated with *B. anthracis* spores concluded that neither of the sampling methods used (HEPA vacuum or wipes) were sensitive enough to ensure that spores had been removed completely. In addition, the event exposed the necessity of

quantifying recovery and extraction efficiency during sample collection and processing to improve the method limit of detection (69, 63)

In this literature review, the limit of detection of methods for *B. anthracis* is characterized as either an instrument limit detection or environmental limit of detection. An instrument limit of detection is generally evaluated with pure cultures. An environmental limit of detection is evaluated with cultures/cells spiked into an environmental matrix (soil, air fomites, water), which then undergoes various recovery and concentration procedures (i.e. filtration and extraction, or direct extraction) before detection (Figure A1.1 in the Appendix).

Compared to instrument limit of detection, establishment of environmental limit of detection poses more challenges, including dilute target concentrations, environmental impurities, background inhibitors, organisms in a viable but not cultivable (VNBC) state, and overall processing efficiency. There are many steps in processing environmental samples prior to detection. At each process step, there can be a loss of the initial target organism and thus, each step has a recovery efficiency, which could be interpreted as a set number, distribution or range (Figure A1.1). Since recovery efficiency directly affects the limit of detection, improving recovery efficiency would result in a more sensitive detection method.

In determining if an environmental site is “clean”, another component that should be evaluated is the quantification and characterization of the potential health risk. Quantitative microbial risk assessment (QMRA) is a method to assess the likelihood of infection based on specific exposures to hazardous pathogenic organisms. QMRA risk modeling has been used for water and food and

could be useful for management decisions during a disease outbreak or a bioterrorism attack (37). Environmental monitoring is used to inform the exposure assessment as well as the efficiency of disinfection. The limit of detection is a critical criterion for any method, which dictates the application and usefulness of demonstrating a "zero" during environmental monitoring. The limit of detection of a chosen analytical method is also an input variable for the QMRA model; a statistical distribution quantifying the variability in limit of detection is preferred for realistic modeling.

The objectives of this study were to review, from the literature, the instrument limit of detection and the environmental limit of detection for methods to detect *B. anthracis* and to compare the estimated risk at the instrument limit of detection and the environmental limit of detection. Though the number of articles on *B. anthracis* was extensive there was a paucity of articles that specifically included environmental limit of detection. This information is essential for a QMRA of *B. anthracis* in the establishment of future environmental monitoring strategies and clean up goals.

2.3 Methods and approach

Journal articles were searched on the ISI Web of Science database searching for *B. anthracis* and the following keywords; method, sensitivity, limit of detection, detection limit, limit, water, air, soil, fomite, surface, specificity, PCR sensor, environmental, rapid, assay, diagnostic, immunoassay, antibody, real time, real-time PCR, microfluidic, polymerase, quantitative, bioaerosol, aerosol, microarrays, biosensor, electrochemiluminiscence, Raman spectrometry, and mass spectrometry. Approximately 1700 references (and abstracts, when available) were

retrieved and were saved in an EndNote file. Though the search defaults were set for the years 1900 through 2007, the oldest article used to evaluate the limit of detection was published in 1994. Abstracts were manually screened for information on the detection of *B. anthracis*. Some studies used a surrogate for *B. anthracis* to determine the limit of detection. It is assumed that *B. anthracis* would behave as the surrogate and was included in this review. If the abstract pertained to a detection method then the full article was downloaded, saved in another database, and reviewed for quantitative data describing the limit of detection. The remaining references and abstracts that were not used in this literature review either did not indicate information about detection methods or the articles were not retrievable. At the end, 71 articles were retrieved and analyzed to obtain instrument limit of detection or environmental limit of detection.

2.3.1 Instrument limit of detection

Instrument limit of detection was extracted from the articles describing a method that detected *B. anthracis* in a pure culture without spiking *B. anthracis* into an environmental matrix (soil, air, fomite, water). Raw data extracted were recorded in units of cells, spores, DNA, colony forming units (CFU), protective antigen, and genomic copies in volumes that ranged from liters to microliters. Articles that used units of protective antigens were not used in this literature review due to the unknown conversion factor for antigens to cells. All data were converted into standard units of cells per milliliter of reaction solution and the data by method were graphed and compared.

2.3.2 Environmental limit of detection

In studies reporting environmental limit of detection, *B. anthracis* spores were spiked into the matrix, extracted, and detected using various detection methods. The articles that reported the environmental limit of detection of *B. anthracis* were categorized according to the matrix in which *B. anthracis* was detected (soil, air, fomite, water). Additional parameters extracted from the articles varied with the matrix (Figure A1.2). These included the following parameters (i) For soil, they included the amount of soil, sample concentration, extraction volume, volume of extracted sample added to the reaction, and total volume. In addition, the type of pretreatment or extraction method and soil type or location were noted (Table 2.1). (ii) For air, they included the sample volume, airflow rate, duration, sample concentration, extraction volume, volume of extracted sample added to the reaction and total volume. (iii) For fomites, they included the surface area, sample concentration, surface seeding method, extraction volume, and total volume. In some cases recovery efficiency and extraction efficiency were available and noted. In addition, the type of fomites, sampling method, extraction method and culturing method were noted (Table 2.2). (iv) For water, they included the sample volume, sample concentration, extraction volume, volume of extracted sample added to the reaction and total volume. In addition the condition of the water was noted.

Table 2.1 Parameters for the environmental limit of detection in soil.

Method	Soil (g)	Sample conc.	Pretreatment/ extraction method	Time (hr)	Difficulty level	Extract vol. (µl)	Added to rxn (µl)	Total vol. (µl)	LOD (CFU/g soil)	Soil type/ location	Ref.
PCR-ELISA	100	1-100CFU/100 g	Easy DNA kit (Invitrogen)	2.5	2	100	60	60	0.1	Non-suspicious sites	(10)
	100	1-100CFU/100 g	Easy DNA kit (Invitrogen)	2.5	2	100	60	60	1.0	Contaminated sites w/ organic compounds and tanning agents	(10)
Nested PCR+2x culture	1	0,1,10,10^2,10^3 CFU/g	FastDNA SPIN kit	36	4	--	--	25	1.0	Garden soil w/ 3% peat	(26)
Nested PCR+ culture	1	0,1,10,10^2,10^3 CFU/g	FastDNA SPIN kit	18	3	--	--	25	$1.0x10^2$	Garden soil w/ 3% peat	(26)
Nested PCR	1	0,1,10,10^2,10^3 CFU/g	FastDNA SPIN kit	2	2	--	--	25	$1.0x10^3$	Garden soil w/ 3% peat	(26)
	0.1	10^6 CFU/100 mg	Three freeze thaw cycles/glass beads and glassmilk	3.5	5	30	5	25	$1.0x10^5$	Litter, meadow, cultivated, swamp and lawn	(65)
PCR	1	$2.5x10^3$-$2.5x10^7$ CFU/g	Hot detergent/bead mill homogenization	1	3	100	10	100	$2.5x10^3$	Anthony fine sandy loam from New Mexico agriculture fields	(46)

Table 2.1 (cont'd).

Method	Soil (g)	Sample conc.	Pretreatment/ extraction method	Time (hr)	Difficulty level	Extract vol. (µl)	Added to rxn (µl)	Total vol. (µl)	LOD (CFU/g soil)	Soil type/ location	Ref.
IM	1	10^3-10^7 CFU/g	Aqueous polymer two-phase system	0.75	2	100	20	40	$5.6x10^3$	Sand	(2)
	1	10^3-10^7 CFU/g	Aqueous polymer two-phase system	0.75	2	100	20	40	$1.4x10^4$	Garden	(2)
Real-time PCR	0.1	10^3-10^7 CFU/g	Heat treatment w/ 1.22g/ml sucrose-0.5% TritonX-100	0.75	3	1000	5	25	$1.0x10^4$	National Institute of Health-Korea	(62)
Multiplex PCR	0.1	10^3-10^7 CFU/g	Heat treatment w/ 1.22g/ml sucrose-0.5% TritonX-100	0.75	3	1000	1	25	$1.0x10^5$	National Institute of Health-Korea	(62)
	0.1	10^3-10^7 CFU/g	Heat treated with sterilized water and 10% TritonX-100-PBS	1.5	3	1000	1	25	$1.0x10^8$	National Institute of Health-Korea	(62)

Table 2.1 (cont'd).

Method	Soil (g)	Sample conc.	Pretreatment/ extraction method	Time (hr)	Difficulty level	Extract vol. (µl)	Added to rxn (µl)	Total vol. (µl)	LOD (CFU/g soil)	Soil type/ location	Ref.
IM-ECL	0.001	0-10^6 CFU/assay	IM separation twice and resuspend in PBS	1.5	3	--	--	--	$1.0x10^5$	Moist dark brown to black soil and dry light yellowish sandy soil from diverse military and agriculture fields	(18)
	0.001	0-10^6 CFU/assay	IM separation twice and resuspend in PBS	1.5	3	--	--	--	$1.0x10^6$	Moist dark brown to black soil and dry light yellowish sandy soil from diverse military and agriculture fields	(18)
	0.001	0-10^6 CFU/assay	IM separation twice and resuspend in PBS	1.5	3	--	--	--	$1.0x10^7$	Moist dark brown to black soil and dry light yellowish sandy soil from diverse military and agriculture fields	(18)
Biosensor assay	0.01	$3.2x10^3$-$3.2x10^5$ CFU/ml	Washed with 1 ml PBST	0.25	1	30	5	25	$3.2x10^8$	Talc-based powder, cornstarch, confectioners' sugar, baking soda, and B. *thuringiensis* based pesticide	(71)

Table 2.2 Parameters for the environmental limit of detection on fomites.

	Hodges *et al.* 2006	Rose *et al.* 2004	Brown *et al.* 2007
Surface area	10 cm^2	25 cm^2	25 cm^2
Sample conc.	0.2-3,000 CFU/cm^2	$2x10^4$ CFU/cm^2	100-100,00 CFU/cm^2
Surface seeding	Inoculated 0.5 ml spore solution	Inoculated 0.5 ml spore solution	Dry aerosol deposition
Sampling method	Macrofoam swab	Cotton swab Macrofoam swab	Polyester-rayon blend gauze wipe
Extraction method	Vortex in 5 ml PBST for 2 min at 10 s intervals	Vortex in 5 ml PBST for 2 min at 10 s intervals	Sonication and heat treatment
Extraction vol.	5 ml	5 ml	30 ml
Total vol.	--	100 µl	1 ml
Recovery efficiency (%)	31.7-49.1	41.7: Cotton swab 43.6: Macrofoam swab	35: Stainless steel 29: Painted wall board
Extraction efficiency (%)	93.4	93.9: Cotton Swab 93.4: Macrofoam swab	93
Culture method	Sheep blood agar	Trypticase soy agar w/ 5% sheep blood	Brain heart infusion agar
Limit of detection	12 CFU/cm^2	20 CFU/cm^2 [a]	90 CFU/cm^2: Stainless steel 105 CFU/cm^2:Painted wallboard

[a] Limit of detection not recorded in article and was calculated to be at the least 20 CFU/cm^2

Table 2.2 (cont'd).

Parameter	Brown *et al.* 2007	Brown *et al.* 2007	Buttner *et al.* 2004
Surface area	25 cm^2	100 cm^2	1 m^2
Sample conc.	100-100,00 CFU/cm^2	100-100,00 CFU/cm^2	10^5 CFU/ m^2
Surface seeding	Dry aerosol deposition	Dry aerosol deposition	Inoculated spore solution
Sampling method	Rayon swab	Vacuum filter sock	BiSKit-wet and dry
Extraction method	Sonication and heat treatment	Sonication and heat treatment	Foam compression
Extraction vol.	10 ml	30 ml	3.3 ml: wet sampling 16.1 ml: dry sampling
Total vol.	1 ml	1 ml	1 ml
Recovery efficiency (%)	41: Stainless steel 41: Painted wallboard	29: Stainless steel 25: Painted wallboard 28: Carpet 19: Concrete	11.3: Wet sampling 18.4: Dry sampling
Extraction efficiency (%)	76	--	--
Culture method	Brain heart infusion agar	Petrifilm aerobic plate count media	Trypticase soy agar
Limit of detection	1 CFU/cm^2: Stainless steel and Painted wallboard	105 CFU/m^2: Stainless steel 102 CFU/m^2: Painted wallboard 105 CFU/m^2: carpet 160 CFU/m^2: concrete	42±6 CFU/m^2: wet sampling 100±10 CFU/m^2: dry sampling

2.3.3 Quantifying limits of risk estimates

Risk of mortality by inhalation of *B. anthracis* spores was estimated for concentrations corresponding to the instrument limit of detection and the environmental limit of detection in air. For each limit of detection, a distribution of risks was calculated by the Monte Carlo method using 100,000 replicates in *Crystal Ball*® 7.3.1 (Oracle, 2007). The number of replicates was chosen at the point where the 90% confidence interval was stable over a range from 1/10 to 10 times the number of replicates used.

A recent evaluation of dose response data for *B. anthracis* spores through the inhalation exposure route found that the dose response relationship could be modeled by the exponential equation (4),

$$P(d)=1-e^{-kd} \qquad (2.1)$$

where P(d) is the probability of death (when untreated) at dose d and k is the probability that one organism will survive to initiate the response (4). In this study, a k value generated from a pooled guinea pig and rhesus monkey data set was used. A distribution of 10,000 best-fit k values generated using bootstrap replicates of that data set was provided by Timothy Bartrand of Drexel University and fit to a gamma distribution. Dose was calculated as,

$$d = C_{air} \cdot R \cdot t \qquad (2.2)$$

where C_{air} is the number of spores per cubic meter of air (instrument limit of detection or environmental limit of detection), R is the breathing rate (m^3/hr), and t is the duration of exposure (hr). When C_{air} was evaluated as a range of limits of detection it was modeled as a lognormal distribution; otherwise, it was evaluated as a point estimate. Breathing rate, R, was modeled as a Pareto distribution fit to the short term breathing rates of adults (18 years of age

and up) of both sexes from rest to moderate activity (72). Exposure time, *t*, was modeled as a uniform distribution from 1 min to 8 hr.

Five risk scenarios were evaluated with this model using different values of C_{air}. For each risk scenario, either instrument limit of detection or environmental limit of detection, a sensitivity analysis was generated using *Crystal Ball*® 7.3.1 (Oracle; 2007). The median real-time PCR instrument limit of detection and the range of real-time PCR instrument limit of detection were two scenarios used to explore the effect of instrument limit of detection on risk. For the instrument limit of detection scenarios, it was assume that all *B. anthracis* spores in a cubic meter of air could be collected without any loss and concentrated into 1 ml of solution for analysis. Log transformed real-time PCR and PCR instrument limits of detection were checked for normality with a Lilliefors test and compared using analysis of variance (ANOVA). Then, the range of PCR instrument limits of detection were combined with the range of real-time PCR instrument limits of detection to increase the data in the distribution.

There were three environmental limits of detection scenarios; C_{air} was set to the environmental limits of detection reported for *B. anthracis* detected in air. There were only two articles on the environmental limit of detection in air. Due to the lack of data on the environmental limit of detection, the two limits of detection were referred to as the lower and upper environmental limit of detection. These two risk scenarios were evaluated as point estimates. The last risk scenario assumed that the environmental limit of detection for air fit the same distributions as the lognormal instrument limit of detection, ranging from 17,000 to 50,000 CFU/m^3 (this may not be the true range).

2.4 Results and discussion

2.4.1 Instrument limit of detection

Out of 56 articles on the instrument limit of detection, 17 articles were on real-time PCR (6, 7, 11, 25, 27, 29, 41, 43, 47, 51, 53, 55, 58, 60, 62, 73, 74), 6 were on PCR (13, 33, 50, 59, 78), 10 were on biosensors (1, 3, 21, 22, 35, 38, 42, 54, 74, 75), 5 were on microarray/PCR (5, 19, 52, 66, 77), 6 were on immunoassay (31, 32, 34, 48, 67, 70), 3 were on electrochemiluminescence (17, 36, 79), 2 were on ELISA (12, 28), 3 were on Raman spectroscopy (39, 57, 80), and 4 were on mass spectrometry (8, 9, 30, 45) (Figure 2.1). Limits of detection ranged from 10 cells/ml (for real-time PCR) to 10^8 cells/ml (for mass spectrometry). Considering the median instrument limit of detection, real-time PCR and PCR were the most sensitive methods with the median instrument limits of detection of 430 and 440 cells/ml, respectively. It should be noted that there was one instrument limit of detection ($4.29x10^6$ cells/ml) that was not added to the distribution for real-time PCR because it was a multiplex assay and the other instrument limits of detection in the distribution were of a singleplex assay (44). The least sensitive methods were Raman spectroscopy and mass spectrometry, with median instrument limits of detection of approximately $1.0x10^7$ and $8.0x10^7$ cells/ml, respectively.

The number of journal articles for real-time PCR and biosensor allowed limits of detection to be fit to a statistical distribution. When fewer articles were published, as was true for the other eight methods, assigning distributions were not possible. ECL, ELISA, Raman spectroscopy, and mass spectrometry (having less than 4 articles) were the methods with the least sensitive instrument limits of detection. With limited information on these methods, the median instrument limit of detection may not properly represent these detection methods' capabilities for

detecting *B. anthracis*. For example, the instrument limit of detection for ECL had only three published articles with limits of detection ranging from 10^2 cells/ml to 10^6 cells/ml. For some emerging techniques, such as immuonmagnetic-ECL (IM-ECL) and aptamer-magnetic bead-ECL (AM-ECL), limits of detection differed by 4 orders of magnitude. While the instrument limit of detection gives insight to the instruments capabilities, when evaluating cleanup goals and assessing risk, the environmental limits of detection are needed to understand the challenges and capabilities for addressing the contamination.

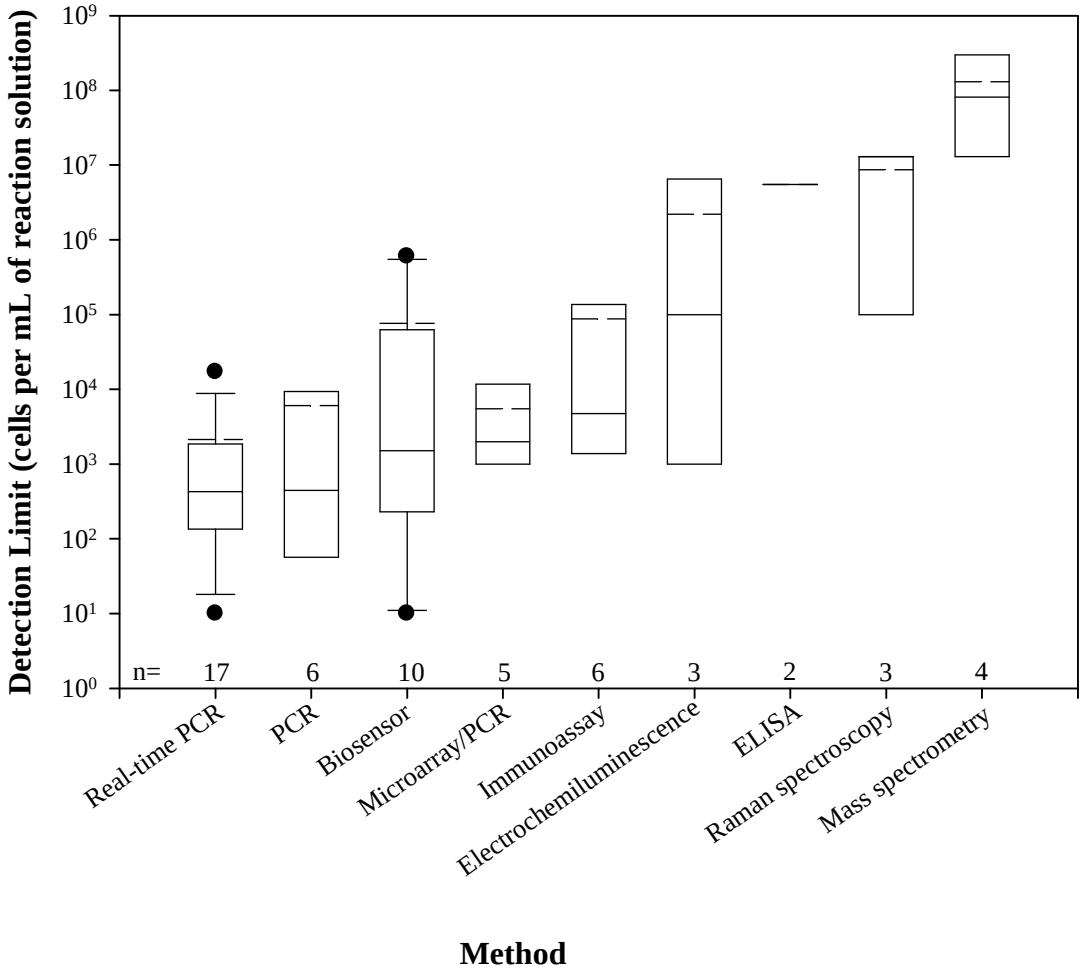


Figure 2.1 Distribution of the instrument limit of detection for various methods. On the box plot, the solid line represents the median result, and the dashed line represents the mean result. The box plot whiskers above and below the box indicate the 90^{th} and 10^{th} percentiles, respectively. The solid circles represent the outlying limits of detection, and n represents the number of journal articles available on each detection method for *Bacillus anthracis*.

2.4.2 Environmental limit of detection

Out of 15 articles on the environmental limit of detection, 8 articles were on detection in soil (2, 10, 18, 26, 46, 62, 65, 71), 2 were on detection in air (49, 68), 4 were on detection on fomites (15, 20, 40, 61), and 1 was on detection in water (56). The results for the environmental limit of detection could not be reported as distributions due to the limited number of articles for each matrix. The two most predominant methods used for the environmental limit of detection were cultivation and PCR based methods.

2.4.2.1 Soil

The environmental limit of detection of *B. anthracis* spiked into soil ranged from 0.1 (reported as 10 CFU/100g of soil) to 3.2×10^8 CFU/g of soil, with a median limit of detection of 1.2×10^4 CFU/g soil (Table 2.1). The median environmental limit of detection for soil should be used with caution, since there is a 9 orders of magnitude range due to the many approaches used to evaluate the environmental limit of detection. The approximate time for the extraction method (Table 2.1) was the time for one sample to be processed based on the information reported. If it was not an automated extraction procedure, then with the increase in samples there would be an increase in extraction process time. The difficulty level for the extraction process (1 easy to 5 difficult) was based on the number of steps in the procedure, the preparation time and the approximated time for the extraction (Table 2.1). The biosensor assay, the easiest extraction method, resulted in the poorest limit of detection (3.2×10^8 CFU/g of soil). The detection methods with the most sensitive limits of detection (PCR-ELISA, Nested PCR, and PCR) had extraction methods with difficulty levels ranging from 2 to 5 (Table 2.1).

The environmental limit of detection depended highly on the pretreatment/extraction process; for instance Ryu *et al.* 2003 (62) used multiplex PCR, and reported a difference of 3 orders of magnitude between heat treatment with 1.22 g/ml sucrose-0.5% TritonX-100 and heat treatment with sterilized water and 10% TritonX-100-phosphate buffered saline (PBS) (Table 2.1). Similar results were found for Bruno and Yu 1996 (18) when using IM-ECL as the detection method.

Differences in the environmental limit of detection were also based on the location or the type of soil. Beyer *et al.* 1999 (10) reported that the PCR-ELISA method was more sensitive when using soils from non-suspicious locations compared to former tannery sites. While Agarwal *et al.* 2002 (2) reported that the immunofluorescence assay was more sensitive when spores were spiked into sand (10^3) rather than into garden soil (10^4). For the IM-ECL method, Bruno and Yu 1996 (18), reported differences due to different strains, Sterne (10^5) being more sensitive in the assay than Ames (10^6) and Vollum B1 (10^7).

2.4.2.2 Air

There were only two studies on the evaluation of aerosolized *B. anthracis* spores collected by an air sampler and extracted for detection. The ELISA-biochip system coupled with a portable bioaerosol collection system collected aerosolized spores at an air sampling rate of 150 L/min for 2 min into 5 ml of phosphate buffered saline (PBS). The ELISA-biochip system consisted of an ELISA for antibody-based identification in combination with the biochip detection instrument. The environmental limit of detection of the ELISA-biochip system was 17 CFU/L. For the ELISA-biochip system, the efficiency of the air sampler was reported as approximately 50% but the distribution was not fully described (68). The anthrax smoke detector collected aerosolized

spores using a bioaerosol collection system at a rate of 15 L/min for 1 min onto a glass fiber filter tape. The detection of the spores using the lifetime-gated fluorimeter occurred after a thermal lysis and addition of $TbCl_3$. The environmental limit of detection of the anthrax smoke detector was 50 CFU/L (49).

2.4.2.3 Fomites

Spores were seeded on fomites (stainless steel, plastic, laminar, wood, glass, etc.), recovered, extracted, and detected by cultivation. The environmental limit of detection was evaluated from stainless steel fomites ranging in surface area from 10 cm^2 to 1 m^2 (Table 2.2). Brown *et al.* 2007 (14-16) also evaluated the environmental limit of detection on painted wallboard. In addition, the vacuum filter sock study tested porous fomites, carpet and concrete (14).

The sampling methods evaluated in the articles were macrofoam swab, cotton swab, polyster-rayon blend gauze wipe, rayon swab, vacuum filter sock and a biological sampling kit (BiSKit) (Table 2.2). Sampling methods such as cotton, macrofoam, polyester, and rayon swabs were all tested by Rose *et al.* 2004 (61). It was concluded that the cotton and macrofoam swabs produced the highest recovery when the swabs were premoistened as opposed to dry. Similarly, Buttner *et al.* 2004 (20) tested the BiSKit, cotton swab, and foam swab. The BiSKit was designed to do wet and dry sampling of large surfaces for bacteria, viruses, and toxins. BiSKit resulted in the highest recovery of the three methods. Using a wetting agent to recover spores from the surfaces enhanced the recovery and environmental limit of detection. Brown *et al.* 2007 (15, 16) used sterilized deionized water (except when using the vacuum filter sock), Buttner et al. 2004 (20) used potassium phosphate buffer with 0.05% Tween-20 (PBT), and the other two articles used

phosphate buffered saline with 0.04% Tween-80 (PBST). According to the CDC the recommended wetting agents were sterile water, a sterile saline solution, or a sterile phosphate-buffered solution (24).

The detection method for all fomite studies was cultivation; however, a different agar was used in each study. The focus of Rose *et al*. 2004 (61) article was on achieving the best recovery, and did not determine an environmental limit of detection. From the information given in the article, the environmental limit of detection was calculated by using the initial suspension concentration, the surface area and the lowest recovery reported. The calculated environmental limit of detection was approximately 20 CFU/cm^2.

The recovery efficiencies for all the fomite studies ranged from 10 to 50%, and the extraction efficiency ranged from 75 to 99%. Recovery of *B. anthracis* spores from fomites depends on many parameters such as fomite type, sampling procedure and sampling processing for detection. The recovery efficiency from the sampling method was primarily the controlling factor in determining the limit of detection and secondarily the efficiency from the extraction method.

Interestingly, in survival studies using cultivation as the detection method on fomites, surface characteristics, relative humidity, and temperature were the most important contributors to viability (64). It was not clear whether recovery and limit of detection changed with time in the environment, as this was difficult to differentiate from survival/degradation of the target. However, this distinction could be made by adding a marker along with the biological agent that does not degrade. For environmental monitoring, the separate time dependence of survival and

recovery will be critical to define in future studies. Only the articles from Brown *et al.* 2007 (14-16), reported and maintained the relative humidity and temperature in the fomite studies at 30 ± 10% and 25 ± 2 ^{o}C, respectively. Determining and maintaining the relative humidity and temperature that are most optimal for viability may increase recovery efficiency. In addition, this information could be used at a contaminated site to inform first responders of the possible viability of remaining levels of the biological agent of concern.

2.4.2.4 Water

The spores were spiked into a volume of water, filtered through a 0.2 to 0.45 µm pore size filter, extracted from the filter and then detected by various methods. The main challenge for detection of *B. anthracis* in water was the ability to concentrate the sample. If the sample was too dilute, then the number of *B. anthracis* cells per liter of water could fall below the environmental limit of detection. When the sample is concentrated, some loss of the initial cells is likely.

There was only one article that evaluated the limit of detection of *B. anthracis* in water; the lack of articles could be due to this matrix being less likely a vehicle for transmission (64). Perez *et al*. 2005 (56), spiked *B. anthracis* spores into tap and source water in volumes ranging from 0.1 to 10 L. Sample concentrations were detected using sheep red blood cell agar plate, *B. anthracis* chromogenic agar plate (R&F Laboratories), PCR or nested PCR.

Cultivation was used to determine the viability of the organisms in the sample, and PCR was used to confirm the identity of any suspect colonies. When using the cultivation approach for the source water samples (Chesapeake Bay and Patuxent River), overgrowth of non-targeted flora

occurred in all studies. PCR was only successful for testing source water when the sample concentrations were at least 26 CFU/ml. The environmental limit detection for tap water was reported as 10 CFU/10L using the cultivations methods, while for PCR based methods, the environmental limit of detection decreased to 534 CFU/L. Though the PCR based methods have a rapid detection time compared to the cultivation methods (more than 24 hr), in this case, PCR was less sensitive. Challenges, such as loss of initial cells, could occur when concentrating large sample volumes (i.e., 10 L) into 5 to 10 μl for the PCR reactions.

2.4.3 Quantifying limits of risk estimates

Five risk scenarios using instrument limits of detection and environmental limits of detection for C_{air} were evaluated. Log transformed PCR and real-time PCR instrument limits of detection were normally distributed (Lilliefors test, p = 0.65 PCR, p = 0.78 real-time PCR) and were not significantly different (ANOVA, p = 0.94). Therefore, the PCR and real-time PCR instrument limit of detection distributions were combined to increase the data set for the real-time PCR instrument limit of detection. With the assumption of 100% recovery, the median risk when C_{air} equaled the median real-time PCR instrument limit of detection was 0.006. When C_{air} was modeled with a lognormal distribution of real-time PCR instrument limits of detection, the estimated risk was 0.0062. The median risk of death from the inhalation of the entire dose of *B. anthracis* at the environmental limit of detection in air was 0.22 at the lower reported environmental limit of detection and 0.52 at the upper environmental limit of detection. Assuming that the environmental limit of detection would have a similar distribution as the instrument limit of detection (lognormal) and ranged from 17,000 to 50,000 spores/m^3, the median risk of death was 0.32 (Table 2.3). This assumption should be further evaluated with

environmental studies to confirm that the environmental limit of detection would have the same distribution as the instrumental limit of detection. These risk estimates assumed that 100% of the spore sample was inhalable. Risk estimates were also reported for the percentages of 66.5%, 10%, and 1% of spores in the sample that were inhalable or respirable (Table 2.3). Approximately 70% of inhaled air volume actually contacts alveoli in the lungs, allowing spores to enter the body (68). In addition, the 5^{th} and 95^{th} percentiles of each risk distribution were used to define a 90% confidence interval for each risk estimate (Table 2.3).

Table 2.3 Risk estimates using instrument limit of detection and environmental limit of detection scenarios.

Risk scenario	Analyzed limit of detection	Percentile	Estimates of risk for percent of sample inhaled			
			100 %	66.5 %	10 %	1 %
Real-time PCR, median instrument limit of detection	429 cells/ml	5th	0.007	0.0047	0.0007	0.00007
		Median	0.006	0.0042	0.00063	0.000063
		95th	0.037	0.025	0.0038	0.00038
Real-time PCR, instrument limits of detection	10-34,300 cells/ml	5th	0.0001	0.000067	0.00001	0.000001
		Median	0.0062	0.0041	0.00062	0.000062
		95th	0.28	0.19	0.032	0.0032
Lower environmental limit of detection in air	17,000 CFU/m^3	5th	0.026	0.017	0.0026	0.00026
		Median	0.22	0.15	0.025	0.0025
		95th	0.78	0.63	0.14	0.015
Upper environmental limit of detection in air	50,000 CFU/m^3	5th	0.075	0.051	0.0078	0.00078
		Median	0.52	0.39	0.071	0.0073
		95th	0.998	0.98	0.46	0.06
Assumed environmental limits of detection in air	17,000-50,000 CFU/m^3	5th	0.03	0.02	0.003	0.00031
		Median	0.32	0.23	0.038	0.0038
		95th	0.94	0.85	0.25	0.028

A sensitivity analysis of the risk model was generated by *Crystal Ball*® 7.3.1 (Oracle; 2007) for each of the five risk scenarios. For the real-time PCR instrument limit of detection lognormal distribution, the limit of detection (79.4%) was the most sensitive factor in determining risk, followed by the exposure time (11.7%) and breathing rates (8.4%). The dose response function parameter *k* (0.5%) had the least impact on the risk estimates. Similarly, for the assumed environmental limit of detection lognormal distribution, the analysis resulted in the exposure time (45%) being the most significant factor, followed by the limit of detection (27.5%), breathing rates (26.1%) and the *k* parameter (0.9%). The median real-time PCR instrument limit of detection and the two environmental limits of detection (lower and upper) scenarios resulted with the exposure time as the dominant factor in determining risk, followed by breathing rates, and the *k* parameter. C_{air} values in these scenarios are point estimates rather than a distribution; therefore, the limit of detection was not a measured parameter in the sensitivity analysis.

Even assuming perfect sample collection and processing (no loss in initial concentration), the estimated risk at the instrument limit of detection was far above the commonly used 1:10,000 level. Environmental limits of detection increase due to the imperfect efficiency of sample collection and processing, increasing the risk at these higher detectable concentrations. These risk estimates show that, using current techniques reported in the literature, even allowing for all possible improvements in collection technology, any detectable *B. anthracis* constitutes an unacceptable risk. Moreover, these estimates define the lowest risk that could be determined from measurement, quantifying the risk that can exist even when no *B. anthracis* was detected.

Finding significant risk at *B. anthracis* limits of detection suggests that direct measurement will rarely be adequate for declaring a contaminated site "clean" and alternative approaches (e.g., extrapolating from demonstrated log reductions) are needed. For fomites, soil, and water, further work is needed regarding the probability of infection by ingestion and contact before one can adequately address limits of detection and risk estimates. Direct measurement could, at best, reveal a catastrophic failure of decontamination. With respect to preventative monitoring, these estimates showed that significant risk was posed by undetectable concentrations of *B. anthracis* spores. This means that a low concentration *B. anthracis* release would be more likely to be detected by the symptoms in exposed humans rather than by current sampling technology. Where there was danger or suspicion of a *B. anthracis* release, close monitoring of human health would be needed in addition to environmental sampling in order to ensure timely medical treatment. Health monitoring alone may be preferred where resources are limited.

The risk assessment approach presented here could be further improved if an experimental probability distribution of the estimated dose was available. However, such a probability distribution was not available even for the most common matrix (soil). To obtain such a distribution, a large number (e.g., 30) of different true doses must be spiked in the environmental matrix of interest, and the sample processed through an entire protocol. This time-consuming process has not yet been reported.

2.5 Conclusion

Instrument and environmental limits of detection are necessary for QMRA when evaluating exposure to human pathogens in a contaminated environment. Due to the lack of pertinent data

on the detection of *B. anthracis*, the environmental limit of detection could not be represented as a distribution. These distributions were necessary in estimating the risk at the environmental limit of detection. Even so, it was clear that environmental samples may be expected to have broad distributions due to the many challenges in sample processing that affect the limit of detection. More environmental limit of detection studies need to be conducted in order to produce distributions similar to those of the instrument limit of detection. This would improve the risk assessment and improve the applicability of the information in regard to survival and clean up goals, providing valuable information for first responders.

APPENDIX

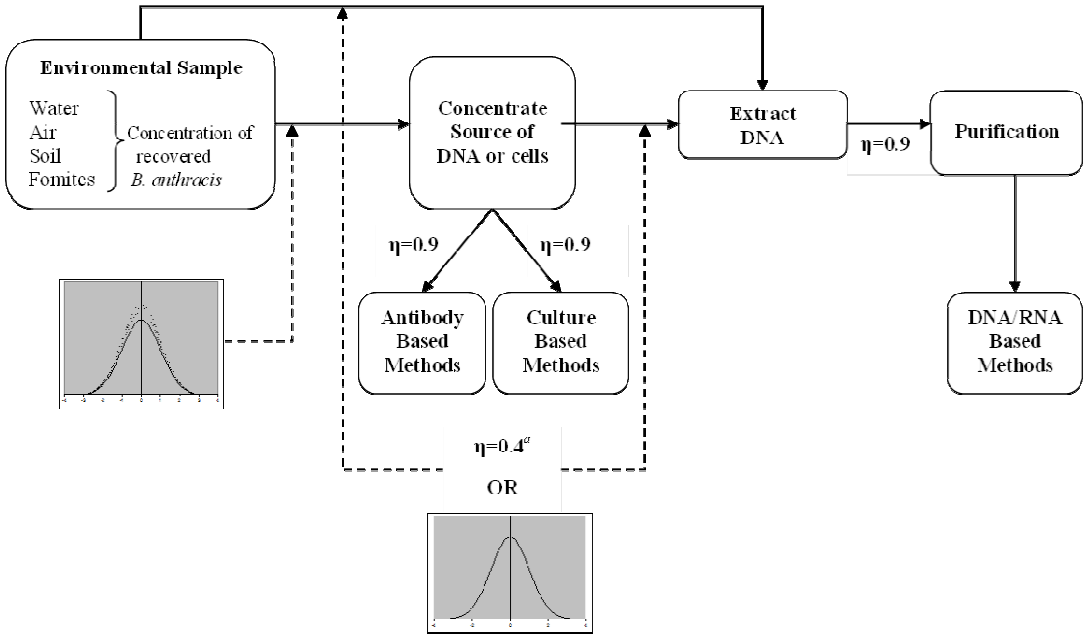


Figure A1.1 General schematic of the efficiency of sample processing and the effect on the environmental limit of detection. $^{\alpha}\eta$ is an example of the average recovery efficiency along with the potential normal distribution.

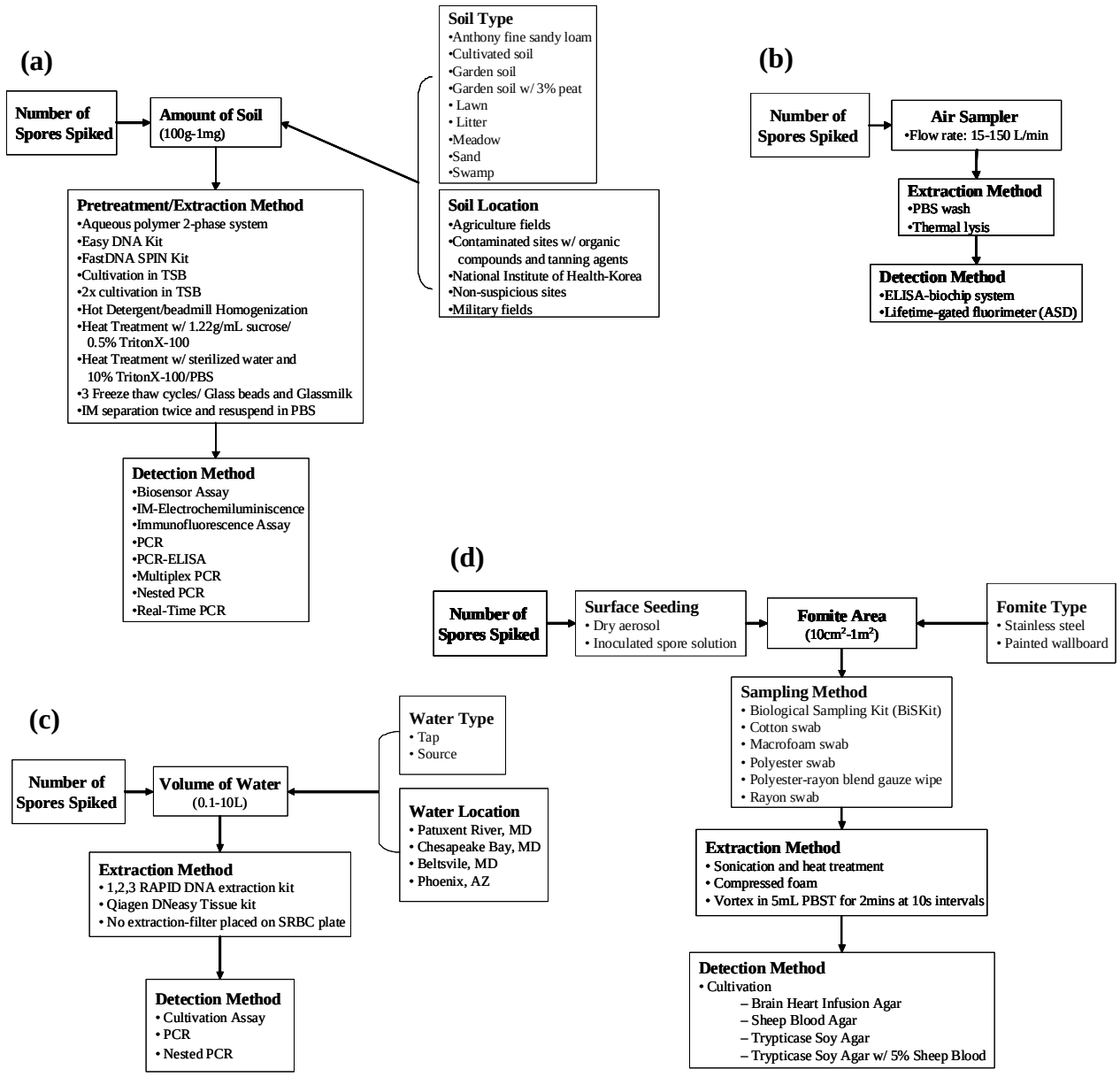


Figure A1.2 Flow diagrams to illustrate the sample processing. Flow diagrams similar to these were used as tools to assist in the calculation of the environmental limit of detection (a) soil, (b) air, (c) water, (d) fomite.

REFERENCES

REFERENCES

1. **Acharya, G., D. D. Doorneweerd, C. L. Chang, W. A. Henne, P. S. Low, and C. A. Savran.** 2007. Label-free optical detection of anthrax-causing spores. J. Am. Chem. Soc. **129:**732-733.

2. **Agarwal, G. S., D. V. Kamboj, S. I. Alam, M. Dixit, and L. Singh.** 2002. Environmental detection of *Bacillus anthracis* spores. Curr. Sci. **83:**697-699.

3. **Baeumner, A. J., J. Pretz, and S. Fang.** 2004. A universal nucleic acid sequence biosensor with nanomolar detection limits. Anal. Chem. **76:**888-894.

4. **Bartrand, T. A., M. H. Weir, and C. N. Haas.** 2008. Dose-response models for inhalation of *Bacillus anthracis* spores: Interspecies comparisons. Risk Anal. **28:**1115-1124.

5. **Belgrader, P., W. Benett, D. Hadley, G. Long, R. Mariella, F. Milanovich, S. Nasarabadi, W. Nelson, J. Richards, and P. Stratton.** 1998. Rapid pathogen detection using a microchip PCR array Instrument. Clin. Chem. **44:**2191-2194.

6. **Belgrader, P., C. J. Elkin, S. B. Brown, S. N. Nasarabadi, R. G. Langlois, F. P. Milanovich, B. W. Colston, and G. D. Marshall.** 2003. A reusable flow-through polymerase chain reaction instrument for the continuous monitoring of infectious biological agents. Anal. Chem. **75:**3446-3450.

7. **Bell, C. A., J. R. Uhl, T. L. Hadfield, J. C. David, R. F. Meyer, T. F. Smith, and F. R. Cockerill.** 2002. Detection of *Bacillus anthracis* DNA by LightCycler PCR. J. Clin. Microbiol. **40:**2897-2902.

8. **Beverly, M. B., F. Basile, K. J. Voorhees, and T. L. Hadfield.** 1996. A rapid approach for the detection of dipicolinic acid in bacterial spores using pyrolysis mass spectrometry. Rapid Commun. Mass Spectrom. **10:**455-458.

9. **Beverly, M. B., K. J. Voorhees, and T. L. Hadfield.** 1999. Direct mass spectrometric analysis of *Bacillus* spores. Rapid Comm. Mass Spectrom. **13:**2320-2326.

10. **Beyer, W., S. Pocivalsek, and R. Bohm.** 1999. Polymerase chain reaction-ELISA to detect *Bacillus anthracis* from soil samples - limitations of present published primers. J. Appl. Microbiol. **87:**229-236.

11. **Bode, E., W. Hurtle, and D. Norwood.** 2004. Real-time PCR assay for a unique chromosomal sequence of *Bacillus anthracis*. J. Clin. Microbiol. **42:**5825-5831.

12. **Borthwick, K. A. J., T. E. Love, M. B. McDonnell, and W. T. Coakley.** 2005. Improvement of immunodetection of bacterial spore antigen by ultrasonic cavitation. Anal. Chem. **77:**7242-7245.

13. **Brightwell, C., M. Pearce, and D. Leslie.** 1998. Development of internal controls for PCR detection of *Bacillus anthracis*. Mol. Cell. Probes **12:**367-377.

14. **Brown, G. S., R. G. Betty, J. E. Brockmann, D. A. Lucero, C. A. Souza, K. S. Walsh, R. M. Boucher, M. Tezak, and M. C. Wilson.** 2007. Evaluation of vacuum filter sock surface sample collection method for *Bacillus* spores from porous and non-porous surfaces. J. Environ. Monit. **9:**666-671.

15. **Brown, G. S., R. G. Betty, J. E. Brockmann, D. A. Lucero, C. A. Souza, K. S. Walsh, R. M. Boucher, M. Tezak, M. C. Wilson, and T. Rudolph.** 2007. Evaluation of a wipe surface sample method for collection of *Bacillus* spores from nonporous surfaces. Appl. Environ. Microbiol. **73:**706-710.

16. **Brown, G. S., R. G. Betty, J. E. Brockmann, D. A. Lucero, C. A. Souza, K. S. Walsh, R. M. Boucher, M. Tezak, M. C. Wilson, T. Rudolph, H. D. A. Lindquist, and K. F. Martinez.** 2007. Evaluation of rayon swab surface sample collection method for *Bacillus* spores from nonporous surfaces J. Appl. Microbiol. **103:**1074-1080.

17. **Bruno, J. G., and J. L. Kiel.** 1999. In vitro selection of DNA aptamers to anthrax spores with electrochemiluminescence detection. Biosens. Bioelectron. **14:**457-464.

18. **Bruno, J. G., and H. Yu.** 1996. Immunomagnetic-electrochemiluminescent detection of *Bacillus anthracis* spores in soil matrices. Appl. Environ. Microbiol. **62:**3474-3476.

19. **Burton, J. E., J. Oshota, E. North, M. J. Hudson, N. Polyanskaya, J. Brehm, G. Lloyd, and N. J. Silman.** 2005. Development of a multipathogen oligonucleotide microarray for detection of *Bacillus anthracis*. Mol. Cell. Probes **19:**349-357.

20. **Buttner, M. P., P. Cruz, L. D. Stetzenbach, A. K. Klima-Comba, V. L. Stevens, and P. A. Emanuel.** 2004. Evaluation of the biological sampling kit (BiSKit) for large-area surface sampling. Appl. Environ. Microbiol. **70:**7040-7045.

21. **Campbell, G. A., and R. Mutharasan.** 2007. Method of Measuring *Bacillus anthracis* spores in the presence of copious amounts of *Bacillus thuringiensis* and *Bacillus cereus*. Anal. Chem. **79:**1145-1152.

22. **Campbell, G. A., and R. Mutharasan.** 2006. Piezoelectric-excited millimeter-sized cantilever (PEMC) sensors detect *Bacillus anthracis* at 300 spores/mL. Biosens. Bioelectron. **21:**1684-1692.

23. **Centers for Disease Control and Prevention (CDC).** 2008. Emergency Preparedness & Response: Bioterroism Agents/Diseases http://emergency.cdc.gov/agent/agentlist-category.asp.

24. **Centers for Disease Control and Prevention (CDC).** 2002. Emergency Preparedness & Response: Comprehensive Procedures for Collecting Environmental Samples for Culturing *Bacillus anthracis* http://www.bt.cdc.gov/Agent/Anthrax/environmental-sampling-apr2002.asp.

25. **Charrel, R. N., B. La Scola, and D. Raoult.** 2004. Multi-pathogens sequence containing plasmids as positive controls for universal detection of potential agents of bioterrorism. BMC Microbiol. **4**.

26. **Cheun, H. I., S. I. Makino, M. Watarai, J. Erdenebaatar, K. Kawamoto, and I. Uchida.** 2003. Rapid and effective detection of anthrax spores in soil by PCR. J. Appl. Microbiol. **95:**728-733.

27. **Christensen, D. R., L. J. Hartman, B. M. Loveless, M. S. Frye, M. A. Shipley, D. L. Bridge, M. J. Richards, R. S. Kaplan, J. Garrison, C. D. Baldwin, D. A. Kulesh, and D. A. Norwood.** 2006. Detection of biological threat agents by real-time PCR: Comparison of assay performance on the RAPID, the LightCycler, and the smart cycler platforms. Clin. Chem. **52:**141-145.

28. **Dang, J. L., K. Heroux, J. Kearney, A. Arasteh, M. Gostomski, and P. A. Emanuel.** 2001. *Bacillus* spore inactivation methods affect detection assays. Appl. Environ. Microbiol. **67:**3665-3670.

29. **Ellerbrok, H., H. Nattermann, M. Ozel, L. Beutin, B. Appel, and G. Pauli.** 2002. Rapid and sensitive identification of pathogenic and apathogenic *Bacillus anthracis* by real-time PCR. FEMS Microbiol. Lett. **214:**51-59.

30. **English, R. D., B. Warscheid, C. Fenselau, and R. J. Cotter.** 2003. *Bacillus* spore identification via proteolytic peptide mapping with a miniaturized MALDI TOF mass spectrometer. Anal. Chem. **75:**6886-6893.

31. **Farrell, S., H. B. Halsall, and W. R. Heineman.** 2005. *Bacillus globigii* bugbeads: A model simulant of a bacterial spore. Anal. Chem. **77:**549-555.

32. **Farrell, S., H. B. Halsall, and W. R. Heineman.** 2005. Immunoassay for *B.globigii* spores as a model for detecting *B. anthracis* spores in finished water. Analyst **130:**489-497.

33. **Fasanella, A., S. Losito, R. Adone, F. Ciuchini, T. Trotta, S. A. Altamura, D. Chiocco, and G. Ippolito.** 2003. PCR assay to detect *Bacillus anthracis* spores in heat-treated specimens. J. Clin. Microbiol. **41:**896-899.

34. **Floriano, P. N., N. Christodoulides, D. Romanovicz, B. Bernard, G. W. Simmons, M. Cavell, and J. T. McDevitt.** 2005. Membrane-based on-line optical analysis system for rapid detection of bacteria and spores. Biosens. Bioelectron. **20:**2079-2088.

35. **Fujinami, Y., Y. Hirai, I. Sakai, M. Yoshino, and J. Yasuda.** 2007. Sensitive detection of *Bacillus anthracis* using a binding protein originating from gamma-phage. Microbiol. Immunol. **51:**163-169.

36. **Gattomenking, D. L., H. Yu, J. G. Bruno, M. T. Goode, M. Miller, and A. W. Zulich.** 1995. Sensitive Detection of Biotoxoids and Bacterial-Spores Using an Immunomagnetic Electrochemiluminescence Sensor. Biosens. Bioelectron. **10:**501-507.

37. **Haas, C. N., J. B. Rose, C. P. Gerba.** 1999. Quantitative microbial Risk Assessment. John Wiley & Sons Inc., New York

38. **Hartley, H. A., and A. J. Baeumner.** 2003. Biosensor for the specific detection of a single viable *B-anthracis* spore. Anal. Bioanal. Chem.**376:**319-327.

39. **Haynes, C. L., C. R. Yonzon, X. Y. Zhang, and R. P. Van Duyne.** 2005. Surface-enhanced Raman sensors: early history and the development of sensors for quantitative biowarfare agent and glucose detection. J. Raman Spectrosc. **36:**471-484.

40. **Hodges, L. R., L. J. Rose, A. Peterson, J. Noble-Wang, and M. J. Arduino.** 2006. Evaluation of a macrofoam swab protocol for the recovery of *Bacillus anthracis* spores from a steel surface. Appl. Environ. Microbiol. **72:**4429-4430

41. **Hoffmaster, A. R., R. F. Meyer, M. P. Bowen, C. K. Marston, R. S. Weyant, K. Thurman, S. L. Messenger, E. E. Minor, J. M. Winchell, M. V. Rassmussen, B. R. Newton, J. T. Parker, W. E. Morrill, N. McKinney, G. A. Barnett, J. J. Sejvar, J. A. Jernigan, B. A. Perkins, and T. Popovic.** 2003. Evaluation and validation of a real-time polymerase chain reaction assay for rapid identification of *Bacillus anthracis*. Emerg. Infect. Dis. **9:**511-511.

42. **Hoile, R., M. Yuen, G. James, and G. L. Gilbert.** 2007. Evaluation of the rapid analyte measurement platform (RAMP) for the detection of *Bacillus anthracis* at a crime scene. Forensic Sci. Int. **171:**1-4.

43. **Hurtle, W., E. Bode, D. A. Kulesh, R. S. Kaplan, J. Garrison, D. Bridge, M. House, M. S. Frye, B. Loveless, and D. Norwood.** 2004. Detection of the *Bacillus anthracis* gyrA gene by using a minor groove binder probe. J. Clin. Microbiol. **42:**179-185.

44. **Kim, K., J. Seo, K. Wheeler, C. Park, D. Kim, S. Park, W. Kim, S. I. Chung, and T. Leighton.** 2005. Rapid genotypic detection of *Bacillus anthracis* and the *Bacillus cereus* group by multiplex real-time PCR melting curve analysis. FEMS Immunol. Med. Microbiol. **43:**301-310.

45. **Krebs, M. D., A. M. Zapata, E. G. Nazarov, R. A. Miller, I. S. Costa, A. L. Sonenshein, and C. E. Davis.** 2005. Detection of biological and chemical agents using differential mobility spectrometry (DMS) technology. IEEE Sens. J. **5:**696-703.

46. **Kuske, C. R., K. L. Banton, D. L. Adorada, P. C. Stark, K. K. Hill, and P. J. Jackson.** 1998. Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. Appl. Environ. Microbiol. **64:**2463-2472.

47. **Lee, M. A., G. Brightwell, D. Leslie, H. Bird, and A. Hamilton.** 1999. Fluorescent detection techniques for real-time multiplex strand specific detection of *Bacillus anthracis* using rapid PCR. J. Appl. Microbiol. **87:**218-223.

48. **Lee, S. H., D. D. Stubbs, J. Cairney, and W. D. Hunt.** 2005. Rapid detection of bacterial spores using a quartz crystal microbalance (QCM) immunoassay. IEEE Sens. J. **5:**737-743.

49. **Lester, E. D., G. Bearman, and A. Ponce.** 2004. A second-generation anthrax "smoke detector". IEEE Eng. Med. Biol. Mag. **23:**130-135.

50. **Merrill, L., J. Richardson, C. R. Kuske, and J. Dunbar.** 2003. Fluorescent heteroduplex assay for monitoring *Bacillus anthracis* and close relatives in environmental samples. Appl. Environ. Microbiol. **69:**3317-3326.

51. **Moser, M. J., D. R. Christensen, D. Norwood, and J. R. Prudent.** 2006. Multiplexed detection of anthrax-related toxin genes. J. Mol. Diagn. **8:**89-96.

52. **Nubel, U., P. M. Schmidt, E. Reiss, F. Bier, W. Beyer, and D. Naumann.** 2004. Oligonucleotide microarray for identification of *Bacillus anthracis* based on intergenic transcribed spacers in ribosomal DNA. FEMS Microbiol. Lett. **240:**215-223.

53. **Pai, S., A. D. Ellington, and M. Levy.** 2005. Proximity ligation assays with peptide conjugate 'burrs' for the sensitive detection of spores. Nucleic Acids Res. **33**.

54. **Pal, S., E. C. Alocilja, and F. P. Downes.** 2007. Nanowire labeled direct-charge transfer biosensor for detecting *Bacillus* species. Biosens. Bioelectron. **22:**2329-2336.

55. **Patra, G., L. E. Williams, Y. Qi, S. Rose, R. Redkar, and V. G. DelVecchio.** 2002. Rapid genotyping of *Bacillus anthracis* strains by real-time polymerase chain reaction. Ann. N.Y. Acad. Sci. **969:**106-111

56. **Perez, A., C. Hohn, and J. Higgins.** 2005. Filtration methods for recovery of *Bacillus anthracis* spores spiked into source and finished water. Water Res. **39:**5199-5211.

57. **Premasiri, W. R., D. T. Moir, M. S. Klempner, N. Krieger, G. Jones, and L. D. Ziegler.** 2005. Characterization of the Surface Enhanced Raman Scattering (SERS) of bacteria. J. Phys. Chem. B **109:**312-320.

58. **Qi, Y. A., G. Patra, X. D. Liang, L. E. Williams, S. Rose, R. J. Redkar, and V. G. DelVecchio.** 2001. Utilization of the rpoB gene as a specific chromosomal marker for real-time PCR detection of *Bacillus anthracis*. Appl. Environ. Microbiol. **67:**3720-3727.

59. **Reif, T. C., M. Johns, S. D. Pillai, and M. Carl.** 1994. Identification of Capsule-Forming *Bacillus-Anthracis* Spores with the PCR and a Novel Dual-Probe Hybridization Format. Appl. Environ. Microbiol. **60:**1622-1625.

60. **Reiman, R. W., D. H. Atchley, and K. J. Voorhees.** 2007. Indirect detection of *Bacillus anthracis* using real-time PCR to detect amplified gamma phage DNA. J. Microbiol. Methods **68:**651-653.

61. **Rose, L., B. Jensen, A. Peterson, S. N. Banerjee, and M. J. Arduino.** 2004. Swab materials and *Bacilius anthracis* spore recovery from nonporous surfaces. Emerg. Infect. Dis. **10:**1023-1029.

62. **Ryu, C., K. Lee, C. Yoo, W. K. Seong, and H. B. Oh.** 2003. Sensitive and rapid quantitative detection of anthrax spores isolated from soil samples by real-time PCR. Microbiol. Immunol. **47:**693-699.

63. **Sanderson, W. T., M. J. Hein, L. Taylor, B. D. Curwin, G. M. Kinnes, T. A. Seitz, T. Popovic, H. T. Holmes, M. E. Kellum, S. K. McAllister, D. N. Whaley, E. A. Tupin, T. Walker, J. A. Freed, D. S. Small, B. Klusaritz, and J. H. Bridges.** 2002. Surface sampling methods for *Bacillus anthracis* spore contamination. Emerg. Infect. Dis. **8:**1145-1151.

64. **Sinclair, R., S. A. Boone, D. Greenberg, P. Keim, and C. P. Gerba.** 2008. Persistence of category A select agents in the environment. Appl. Environ. Microbiol. **74:**555-563.

65. **Sjostedt, A., U. Eriksson, V. Ramisse, and H. Garrigue.** 1997. Detection of *Bacillus anthracis* spores in soil by PCR. FEMS Microbiol. Ecol. **23:**159-168.

66. **Song, L. N., S. Ahn, and D. R. Walt.** 2005. Detecting biological warfare agents. Emerg. Infect. Dis. **11:**1629-1632.

67. **Stopa, P. J.** 2000. The flow cytometry of *Bacillus anthracis* spores revisited. Cytometry **41:**237-244.

68. **Stratis-Cullum, D. N., G. D. Griffin, J. Mobley, A. A. Vass, and T. Vo-Dinh.** 2003. A miniature biochip system for detection of aerosolized *Bacillus globigii* spores. Anal. Chem. **75:**275-280.

69. **Subcommittee on Homeland Security, Committee on Appropriations, House of Representatives.** 2007. Anthrax detection: DHS cannot ensure that sampling activities will be validated. Statement of Keith Rhodes, Chief Technologist, Center for Technology

and Engineering Applied Research and Methods, United States Government Accountability Office (GAO-O-070687T). U.S. Government Accountability Office, Washington, D.C.

70. **Taitt, C. R., G. P. Anderson, B. M. Lingerfelt, M. J. Feldstein, and F. S. Ligler.** 2002. Nine-analyte detection using an array-based biosensor. Anal. Chem. **74:**6114-6120.

71. **Tims, T. B., and D. V. Lim.** 2004. Rapid detection of *Bacillus anthracis* spores directly from powders with an evanescent wave fiber-optic biosensor. J. Microbiol. Methods **59:**127-130.

72. **U.S. Environmental Protection Agency (EPA).** 1997. Exposure Factor Handbook. National Center for Environmental Assessment Washington DC.

73. **Ulrich, M. P., D. R. Christensen, S. R. Coyne, P. D. Craw, E. A. Henchal, S. H. Sakai, D. Swenson, J. Tholath, J. Tsai, A. F. Weir, and D. A. Norwood.** 2006. Evaluation of the Cepheid GeneXpert (R) system for detecting *Bacillus anthracis*. J. Appl. Microbiol. **100:**1011-1016.

74. **Van Ert, M. N., W. R. Easterday, T. S. Simonson, J. M. U'Ren, T. Pearson, L. J. Kenefic, J. D. Busch, L. Y. Huynh, M. Dukerich, C. B. Trim, J. Beaudry, A. Welty-Bernard, T. Read, C. M. Fraser, J. Ravel, and P. Keim.** 2007. Strain-specific single-nucleotide polymorphism assays for the *Bacillus anthracis* Ames strain. J. Clin. Microbiol. **45:**47-53.

75. **Wan, J. H., B. Fiebor, B. A. Chin, I. H. Chen, J. Brigati, And V. A. Petrenko.** 2005. Landscape phage-based magnetostrictive biosensors for detecting *Bacillus anthracis* spores. pg 1308-1311. In Sensors, IEEE, Los Alamitos, CA.

76. **Wan, J. H., H. H. Shu, S. C. Huang, B. Fiebor, I. H. Chen, V. A. Petrenko, and B. A. Chin.** 2007. Phage-based magnetoelastic wireless biosensors for detecting *Bacillus anthracis* spores. IEEE Sens. J. **7:**470-477.

77. **Wang, S. H., J. K. Wen, Y. F. Zhou, Z. P. Zhang, R. F. Yang, J. B. Zhang, J. Chen, and X. E. Zhang.** 2004. Identification and characterization of *Bacillus anthracis* by multiplex PCR on DNA chip. Biosens. Bioelectron. **20:**807-813.

78. **Wilson, W. J., A. M. Erler, S. L. Nasarabadi, E. W. Skowronski, and P. M. Imbro.** 2005. A multiplexed PCR-coupled liquid bead array for the simultaneous detection of four biothreat agents. Mol. Cell. Probes **19:**137-144.

79. **Yu, H., J. W. Raymonda, T. M. McMahon, and A. A. Campagnari.** 2000. Detection of biological threat agents by immunomagnetic microsphere-based solid phase fluorogenic- and electro-chemiluminescence. Biosens. Bioelectron. **14:**829-840.

80. **Zhang, X. Y., M. A. Young, O. Lyandres, and R. P. Van Duyne.** 2005. Rapid detection of an anthrax biomarker by surface-enhanced Raman spectroscopy. J. Am. Chem. Soc. **127:**4484-4489.

Chapter 3: Evaluation of sample recovery efficiency of bacteriophage P22 on fomites

This chapter is adapted from our published work in Applied and Environmental Microbiology: Amanda B. Herzog, Alok K. Pandey, David Reyes-Gastelum, Charles P. Gerba, Joan B. Rose, and Syed A. Hashsham. Evaluation of Sample Recovery Efficiency of Bacteriophage P22 on Fomites. Applied and Environmental Microbiology. 2012. 78(22):7915-7922. DOI:10.1128/AEM.01370-12.

3.1 Abstract

Fomites are known to play a role in the transmission of pathogens. Quantitative analysis of the parameters that affect sample recovery efficiency (SRE) at the limit of detection of viruses on fomites will aid in improving quantitative microbial risk assessment (QMRA) and infection control. The variability in SRE as a function of fomite type, fomite surface area, sampling time, application media, relative humidity (RH), and wetting agent was evaluated. To quantify the SRE, bacteriophage P22 was applied onto the fomites at average surface densities of 0.4 ± 0.2 and 4 ± 2 PFU/cm^2. Surface areas 100 and 1000 cm^2 of nonporous fomites found in indoor environments (acrylic, galvanized steel, and laminate) were evaluated with pre-moistened antistatic wipes. The parameters with the most effect on the SRE were sampling time, fomite surface area, wetting agent, and RH. At time zero (initial application of bacteriophage P22), the SRE for 1000 cm^2 fomite surface area was, on average, 40% lower than that for the 100 cm^2 fomite surface area. For both fomite surface areas, application media trypticase soy broth (TSB) and/or the laminate fomite predominantly resulted in a higher SRE. After the applied samples dried on the fomites (20 min), the average SRE was less than 3%. A TSB wetting agent applied

on the fomite improved the SRE for all samples at 20 min. In addition, RH greater than 28% generally resulted in a higher SRE than RH less than 28%. Parameters impacting SRE at the limit of detection have the potential to enhance sampling strategies and data collection for QMRA models.

3.2 Introduction

Nonporous fomites (inanimate or nonliving objects) can be an important vehicle in the transmission of viral disease, especially for populated indoor environments, such as schools, daycare centers, nursing homes, hospitals, food preparation settings, or any civil infrastructure (4-6, 25, 32). Human exposure can be through touching and transfer of pathogens present on the fomite to the hands and then to the mouth, nasopharynx, and eyes (5, 24). Exposure can also be from the inhalation of re-aerosolized organisms from contaminated fomites (5, 25). Controlling and remediating an indoor environment from an outbreak resulting from an accidental or intentional release of viruses can be challenging tasks (1, 25).

To declare an indoor environment "clean" after decontaminating it, quantification of the loss due to sample recovery that is specific to the method(s) used is essential for verifying the efficacy of the decontamination (16). Quantitative analyses of the parameters that affect SRE from fomites are vital for implementing efficient sampling and detection methods (16). Infection transmission models that include the environmental dynamics (environmental conditions, human behavior, survival characteristics of the agent in the environment, etc.) can be used to make decisions on interventions for preventing viral outbreaks (19). Without a quantitative assessment of the abundance of such agents in the environment, generic intervention recommendations could be

ineffective (4, 35).

Survival and SRE studies with viruses have generally been conducted on fomites at surface densities of 10^2 PFU/cm^2 or higher by applying virus stocks in volumes ranging from 5 to 500 µl on fomite areas ranging from 0.38 to 32 cm^2 (Table 3.1). The use of higher initial titers is known to extend the viral survival rate on fomites (5). Under these optimal conditions, results may represent the upper limits of SRE. The surface densities may also be lower than what has been studied so far and pose significant risk (Table 3.1). However, parameters affecting survival at very low surface densities are less well studied. To our knowledge, only two survival studies (3, 7) and two SRE studies (17, 36) have been conducted at surface densities ranging from 0.02 to 50 PFU or at the 50% tissue culture infective dose ($TCID_{50}$)/cm^2 (Table 3.1 and Table A2.1 in the Appendix). These factors may have a significant effect on quantifying the risk to human health after decontamination.

The objective of this study was to evaluate the parameters that affect SRE of bacteriophage P22, a surrogate for DNA viruses (20, 30), at concentrations close to the limit of detection. Bacteriophage P22 was chosen because it is a surrogate for DNA viruses such as adenovirus (13, 30), it meets many of the desired characteristics of a surrogate (20, 30, 33), and it has been used successfully by our group in environmental release and recovery studies (20, 30). We evaluated the variability of SRE from the parameters, such as fomite type, fomite surface area, sampling time, application media, wetting agent, and RH. The results presented here have implications for sampling strategies and subsequent microbial risk assessment at low concentrations.

Table 3.1 Parameters from survival and SRE studies evaluating viruses applied to fomites.

Organism	Sample conc.	Fomite area (cm^2)	App. vol. (µl)	Surface density	Application medium	RH (%)	Temp. (^oC)	Survival SRE	Ref.
Survival studies									
Alphaviruses Ebola virus Lassa virus	$1.5x10^7$-$4.5x10^{10}$ PFU/ml	0.25	NR[a]	$6.4x10^6$ PFU/cm^2, $7.6x10^7$ PFU/cm^2, $5.6x10^7$ PFU/cm^2	NR	30-40	20, 25	6-14 d	(25)
Astrovirus	$1x10^5$-$5x10^5$ PFU	1,3	20, 50	$3.3x10^4$-$1.6x10^5$ PFU/cm^2	PBS or 20% FS	90±5	4, 20	10-90 d	(2)
Avian metapneumovirus Avian influenza	$3.1x10^6$-$6.3x10^6$ $TCID_{50}$/ml[b]	1	10	$3.4x10^4$-$6.3x10^4$ $TCID_{50}$/cm^2	NR	NR	NR	1-6 d	(38)
Bacteriophage P22	$1x10^9$ PFU/ml	10	10	10^7 PFU/cm^2	NR	50	25	859 hr	(20)
Calicivirus	10^7 $TCID_{50}$/ml	1	20	$2.0x10^5$ $TCID_{50}$/cm^2	NR	NR	NR	4-72 hr	(10)
Coronavirus	10^4-10^5 MPN[c]	1	10	10^4-10^5 MPN/cm^2	Cell culture medium	20±3, 50 ±3, 80 ±3	4, 20, 40	0.25-28 d	(8)
Coronavirus	10^7 PFU/ml	0.79	10	$1.3x10^5$ PFU/cm^2	PBS	55, 70	21	3-6 hr	(34)
Feline calicivirus Norwalk virus	10^9 PFU/ml, 10^6 RT-PCRU/ml	25	NR	$4x10^7$ PFU/cm^2, $4x10^2$ RT-PCRU/cm^2	20% FS in PBS	75–88	22±2	7 d	(11)
Hepatitis A virus Polio virus	10-fold dilution	0.79	10	N/A	10% FS in saline	25±5, 55±5, 80±5, 95±5	5, 20, 35	4-96 hr 4-12 hr	(21)

a. NR-not reported
b. TCID50-median tissue culture infective dose
c. MPN-most probable number
d. N/A-not applicable, not able to calculate surface density from information reported

Table 3.1 (cont'd).

Organism	Sample conc.	Fomite area (cm^2)	App. vol. (µl)	Surface density	Application medium	RH (%)	Temp. (^oC)	Survival SRE	Ref.
Hepatitis A virus Human rotavirus Enteric adenovirus Poliovirus	NR	1	20, 50, 100	N/A	PBS or FS	50±5, 85±5, 90±5	4, 20	30-60 d, 30-60 d, 5-60 d, 5-60 d	(1)
Rotavirus Poliovirus Bacteriophage f2	10^3–10^5 PFU/ml	250	Misted	N/A	Distilled water, distilled water with 10% FS	NR	NR	0.75-1.5 hr	(18)
Influenza A virus	$2x10^8$ PFU/ml	NR	20	N/A	NR	50-60	22±2	6-24 hr	(22)
Influenza A virus	10^6 $TCID_{50}$/ml	1	10	10^4 $TCID_{50}/cm^2$	Eagle minimal essential medium with 25mM HEPES and Earle's salts	30-50	21-28	2 hr-17 d	(37)
Influenza A virus	$1.5x10^8$ $TCID_{50}$/ml	2	10	$7.5x10^5$ $TCID_{50}/cm^2$	1% BSA	23-24	17-21	4-9 hr	(14)
Influenza A and B virus	10^3–10^4 $TCID_{50}$/0.1 ml	7.07–19.63	100	50-$1.4x10^3$ $TCID_{50}/cm^2$	NR	35-40, 55-56	27.8-28.3, 26.7-28.9	24-48 hr	(3)

Table 3.1 (cont'd).

Organism	Sample conc.	Fomite area (cm^2)	App. vol. (µl)	Surface density	Application medium	RH (%)	Temp. (^oC)	Survival SRE	Ref.
Parainfluenza	$1.5x10^0$, $1.5x10^3$, $1.5x10^4$ $TCID_{50}$/ml	32	500	$0.02–2.0x10^2$ $TCID_{50}/cm^2$	Minimal essential medium with Earle's salts	NR	22	6-10 hr	(7)
Rhinovirus	10^7 PFU/ml	0.79	10	$1.3x10^5$ PFU/cm^2	Tryptose phosphate broth, bovine mucin, human nasal discharge	20±5, 50±5, 80±5	20±1	2-25 hr	(27)
Zaire Ebola virus Lake Victoria Marburg virus	$1x10^6$ $TCID_{50}$/ml	0.38	20	$5.2x10^4$ $TCID_{50}/cm^2$	Guinea pig sera, tissue culture media	55±5	4, 22	14-50 d	(23)

Table 3.1 (cont'd).

Organism	Sample conc.	Fomite area (cm^2)	App. vol. (µl)	Surface density	Application medium	RH (%)	Temp. (^oC)	Survival SRE	Ref.
SRE studies									
Bacteriophage MS2	$1x10^6$ PFU/ml	25	5	3.7 PFU/cm^2	50% solution of TSB and dilution buffer (5 mM NaH2PO4 and 10 mM NaCl)	45-60	20-22	7-40%	(17)
Feline calicivirus	$7.0x10^5$-$1.3x10^6$ $TCID_{50}$/100 µl	25.8, 929, 5,290	20	26-10^4 $TCID_{50}$/cm^2	10% FS in PBS	NR	NR	3-71%	(36)
Rotavirus Poliovirus Bacteriophage f2	10^3-10^5 PFU/ml	250	Misted	N/A	Distilled water, distilled water with 10% FS	NR	NR	16.8±6%, 42.3±1.9%, 10.6±5.7%	(18)
Norovirus Rotavirus	$2.0x10^7$ RT-PCRU/ml $2.0x10^5$ RT-PCRU/ml	10	100	$2.0x10^3$, $2.0x10^4$ RT-PCRU/cm^2 $2.0x10^1$, $2.0x10^2$ RT-PCRU/cm^2	10% PBS	NR	NR	10.3±13.0-51.9±38.5% 5.4±1.5-57.7±25.9%	(28)
Rhinovirus	10^7 PFU/ml	0.79	10	$1.3x10^5$ PFU/cm^2	Tryptose phosphate broth, bovine mucin, human nasal discharge	50±5	22	40.3-98.4%	(27)

3.3 Materials and methods

3.3.1 Bacteriophage P22: preparation, application and sample recovery

Bacteriophage P22, which infects the bacterial host *Salmonella enterica* serovar Typhimurium LT2 (ATCC 19585), was provided by Charles P. Gerba (University of Arizona). Bacteriophage P22 is a double stranded DNA (dsDNA) icosahedral-shaped virus with a short tail (52 to 60 nm in size) and belongs to the family *Podoviridae* (30). To prepare bacteriophage P22, 1 ml of bacteriophage P22 stock was added to 25 ml of the bacterial host, *S.* Typhimurium, at log phase in TSB (Difco, Becton Dickinson and Company, Sparks, MD). After a 24 hr incubation at 37 ^{o}C, 0.1 ml of lysozyme and 0.75 ml of EDTA were added to the solution and centrifuged at 2390 x g for 10 min. The supernatant was filtered through a 0.45 µm filter (Millipore) to remove the bacterial cells and debris (30). Bacteriophage P22 was then diluted in suspensions of phosphate buffered saline Tween-80 (Fisher Scientific, NJ) (PBST), TSB or sterile distilled water.

The fomites, simulating an indoor environment, included acrylic (Optix; Plaskolite Inc., Columbus, OH), galvanized steel (type 28 GA galvanized; MD Building Products, Oklahoma City, OK) and laminate (type 350, no. 60 mate finish; Wilsonart International Inc., Temple, Texas) with surface areas of 100 and 1000 cm^2. The fomites and testing area were disinfected with 70% ethanol, rinsed with sterile distilled water, and dried. Bacteriophage P22 was applied in PBST, TSB or water on the fomite in a grid formation comprising of fifty 1 µl droplets. The average amount of bacteriophage P22 applied to the fomite was 433.1 ± 194.5 PFU, approximately 8.66 PFU/droplet, with average surface densities of 4.3 ± 1.9 PFU/cm^2 for the 100 cm^2 fomite and 0.4 ± 0.2 PFU/cm^2 for 1000 cm^2 fomite. The recovery materials, pre-moistened Fellowes screen cleaning wipes (no. 99703; Fellowes, Itasca, IL), are generally used to remove

dirt, dust and finger prints from office equipment and are antistatic, non-toxic and alcohol free. The pre-moistened wipes are made of crepe fabric (crepe material is treated as a trade secret by Fellowes) and wetted by the manufacture with water and detergent (propylene glycol ethers). The pre-moistened wipes were cut into 48 cm^2 pieces using sterilized scissors and stored in sterile Whirl-Pak bags at room temperature during the experiment, lasting no more than 12 hr. Fresh pieces were cut and used each day. The sampling was done by moving the pre-moistened wipes over the entire fomite twice (in perpendicular directions to each other). Two samples were taken, one immediately after the initial application (referred to as 0 min) and another after the samples were visibly dry (which was 20 min). The control experiments conducted with bacteriophage P22 suspensions to determine if the moistening agent had an effect on the viability of the virus indicated that, on average, 95% (range, 80 to 125%) of bacteriophage P22 could be recovered with inoculation directly onto the wipe and dissolution with PBST. Very high recovery rates were also seen at time zero on fomites with no drying.

After sampling, the recovery material was placed into a 50 ml tube containing 5 ml of PBST and vortexed for 30 s. Bacteriophage P22 was assayed using a double agar layer method (39). The sample containing bacteriophage P22 (1 ml) was added to 2.5 ml of melted 1% agar overlay (1 g bacto agar/100 ml TSB) (Bacto agar; Difco, Becton, Dickinson and Company, Sparks, MD) with 0.3 ml of *S.* Typhimurium in the log phase. The solution was rolled by hand for mixing and immediately dispensed evenly onto 1.5% Trypticase soy agar (TSA) (Difco, Becton, Dickinson and Company, Sparks, MD) plates. After the overlay agar solidified, the plates were incubated at 37 ^oC for 24 hr; the number of PFU was then counted. A total of 324 plates were used in the conduction of the SRE experiments. These experiments included 3 fomite types, 2 sampling

times, 3 application media, and 2 fomite surface areas. Each SRE measurement was made in triplicate and repeated on three different days. Because the PFUs recovered were already very low, dilution of samples was not necessary. For each sample recovery experiment, positive control experiments were conducted in triplicate. Fifty 1 µl droplets of bacteriophage P22 inoculated in 950 µl of PBST (same as extraction solution) were dispensed into a 1.5 ml microcentrifuge tube. The 1 ml bacteriophage P22 control was dispensed as described above.

3.3.2 Single agar layer method to separate bacteriophage P22 survival from recovery

When evaluating the survival of bacteriophage P22 on fomites, fifty 5 µl droplets containing an estimated 3.96 PFU/droplet suspended either in TSB or water were applied on polystyrene Petri dish surface (100 by 15 mm) in a grid formation. An average of 198 ± 65 PFU was applied to each plate, with an average surface density of 2.5 ± 0.9 PFU/cm^2. For this experiment, the time of first sampling (other than the initial at time zero) was changed to 1 hr instead of 20 min, because the 5 µl droplets took longer to visibly dry on the petri dish. The samples were evaluated at 0, 1, 2, 4, 8, 12, and 24 hr by implementing a single agar layer method. This method allowed us to evaluate the PFU remaining but eliminated the need to recover them from a surface, because bacteriophage P22 was directly applied on the petri dish surface. The assay consisted of dispensing 3 ml of melted 1% agar overlay (1 g Bacto agar/100 ml TSB) with 0.5 ml of *S.* Typhimurium in the log phase and 2 ml of TSB onto the petri dish surface where bacteriophage P22 was applied. After the overlay agar solidified, the plates were incubated at 37 ^{o}C for 24 hr, at which point the number of PFU was then counted. The experiment was conducted twice using six replicates per time point spanning 7 sampling time points and 2 application media (thus using a total of 168 plates). For each survival experiment, a positive

control experiment, which consisted of fifty 5 µl droplets of bacteriophage P22 inoculated in 750 µl of PBST in 1.5 ml microcentrifuge tubes, was also included in triplicate. One millilter of this positive control was plated as described above.

3.3.3 Relative humidity and TSB wetting agent

The RH and temperature in the laboratory were measured before each experiment with a digital RH and temperature meter (VWR Scientific Products). The average temperature of the laboratory during these experiments was 20.8 ± 0.23 ^{o}C (mean ± standard deviation). The RH ranges of 9 to 23% and 28 to 32% were the natural RHs of the laboratory during the winter and summer months, respectively (Figure 3). For RH range of 55 to 58%, a small laboratory space (14 ft by 7 ft by 9 ft) was equipped with a humidifier (Bionaire, Milford, MA).

Previous studies support that use of a wetting agent applied to the recovery material (wipe or swab) to enhance the SRE (9, 17, 18, 28). In a preliminary experiment, PBST and TSB were compared as wetting agents applied on the fomite surface to evaluate their effects on SRE enhancement at 20 min. There was no statistical differences between the SREs when PBST or TSB as wetting agent was applied on the fomite ($p=0.232$, $n=27$, Student's t-test, data not shown). Hence, in further SRE experiments, a TSB wetting agent was used (this step is referred to as TSB wetting). Using a disposable spreader, 200 µl of TSB was applied and uniformly distributed over 100 cm^2 fomite surface area. The recovery material sampled both the disposable spreader and the fomite. The recovery materials were processed as described above. This experiment used a total of 162 plates consisting of 2 wetting conditions, 1 fomite surface area, 1 sampling time, 3 fomites, 3 application mediums, and 3 RHs. Each measurement was

made in triplicate. A positive control was also conducted in triplicate as described previously for the SRE experiments.

3.3.4 Percent sample recovery efficiency computations and statistical analysis

Percent sample recovery efficiency was calculated as,

$$\%SRE = \frac{N_{assay}(D)}{N_{control}} \times 100 \quad (3.1)$$

where $\%SRE$ s the sample recovery efficiency from the fomite, N_{assay} is the number of PFU counted on the agar plate from sampling the fomite, D is the dilution factor (the total extraction volume divided by the volume of sample assayed), and $N_{control}$ is the number of units on the agar plate from the control experiment.

The data (%SRE) had considerable differences in variance, especially between 0 min and 20 min. Due to this, the data were transformed by adding 1 (to account for the zero values) and converted to a log scale. After analyzing the residuals, it was determined that the normality assumption of the residual did not fit the equation; therefore, the residuals were fitted under the assumption of a gamma distribution. Two equations for the transformed outcome were used to study the relationships between fomite type, application media, RH, and wetting condition.

$$\text{Log}(\%\text{SRE} + 1) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + e \quad (3.2)$$

For equation 3.2, log (%SRE +1) is the log transformed SRE, X_1 is an independent variable that denotes the fomite type so X_1 is a nominal variable with no numerical value (acrylic, laminate, and galvanized steel as categories) for which laminate was taken as the reference category in the analyses, X_2 is an independent variable that denotes the application media so X_2 is a nominal variable (PBST, TSB, and water as categories) for which water was taken as the reference category in the analyses, X_1X_2 is the interaction between fomite type and application media, and e is the error term. The intercept β_0 represents the average value of the reference group, in this case, is the average value of the log of the reference categories laminate fomite and water medium. The terms β_1, β_2, and β_3 are the regression coefficients known as the effect for the corresponding independent variable X_1, X_2, and X_1X_2, respectively.

$$\text{Log (\%SRE +1)} = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_1X_2 + \beta_6X_2X_3 + \beta_7X_1X_3 + e \qquad (3.3)$$

In equation 3.3, log(%SRE +1) is the log transformed SRE, X_1 and X_2 are defined as in equation (3.2), X_3 is an independent variable that denotes RH range so X_3 is a nominal variable (9 to 23%, 28 to 32%, and 55 to 58% as categories) for which 55 to 58% was taken as the reference category in the analyses, X_4 is an independent variable that denotes the use of TSB wetting for the sample collection so X_4 is a nominal variable (no wetting and TSB wetting as categories) for which TSB wetting was taken as the reference category in the analyses, and e is the error term. As previously explained, the intercept β_0 represents the average value of the log of the reference categories laminate fomite, water medium, 55 to 58% RH, and TSB wetting. The interaction terms are X_1X_2 (fomite type and application medium), X_2X_3 (application medium and RH), and

X_1X_3 (fomite type and RH). The regression coefficients (β_{1-7}) are known as the effect for the corresponding independent variable X_{1-4}, X_1X_2, X_2X_3, and X_1X_3, respectively.

Because the independent variables used were nominal, dummy variables were used to compare the different categories to the corresponding reference categories. The dummy variable described the set of experimental conditions consisting of fomite type, application media, surface area, sampling time, RH, and wetting condition as single entity and evaluate the SRE for each set to the next by treating two such sets as reference (laminate and water). A regression was run using SAS 9.2 with the GLIMMIX procedure to evaluate the equations. The data was analyzed to evaluate the type III test of fixed effects (emanating from the factors being investigated) to determine the significance of each of the parameters specified in the model statement (26). Analyses of the model were performed on wetting condition, fomite surface area and sampling time groups. The patterns in the experimental data indicated differences to explore certain effects. This limited the error rates and avoided cancellation of significant effects.

3.4 Results

3.4.1 SRE of bacteriophage P22 from various fomites

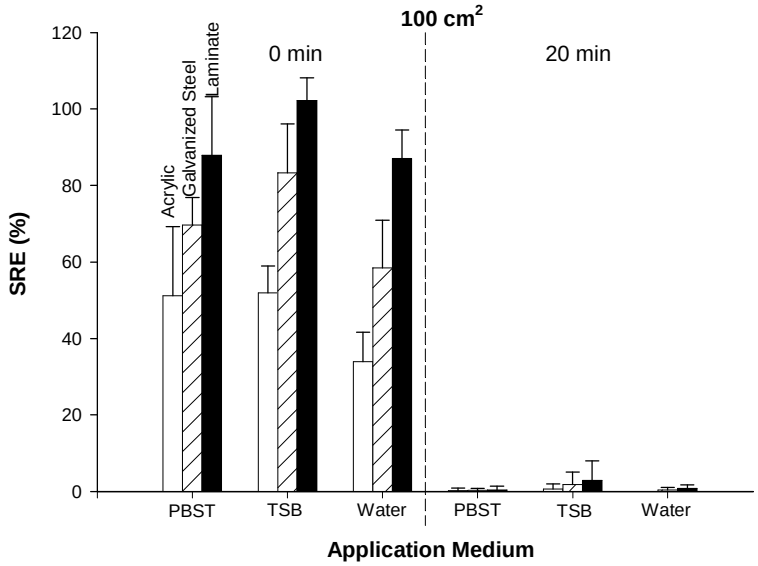
For 100 cm^2 fomite surface area and the three application media (PBST, TSB, or water), the average SRE for the experimental data at 0 min were 46 ± 6.9% (SRE ± standard deviation) for acrylic, 70 ± 7.7% for galvanized steel, and 92 ± 6.4% for laminate (Figure 3.1a). The type III test of fixed effects (equation 3.2) for 100 cm^2 fomite surface area at 0 min was significant for fomite type ($p<0.0001$), application medium ($p<0.0001$) and the interaction between fomite type and application medium ($p=0.0128$) (Table A2.2). Based on equation 3.2, laminate yielded the

highest SRE, while acrylic gave the lowest SRE regardless of which application media was used. However, use of TSB did result in a higher SRE than did the other media. PBST and TSB performed similarly on acrylic, while PBST and water performed similarly on laminate (Table A2.3). At 20 min, the average SRE for acrylic, galvanized steel and laminate were all less than 1 ± 0.9% for all application media (Figure 3.1a). The type III test of fixed effects for 100 cm^2 fomite surface area at 20 min was significant for fomite type ($p=0.0047$) and application media ($p<0.0001$) but not significant ($p=0.3589$) for the interaction between fomite type and application media (Table A2.4). For these conditions, the application media significantly affected the SRE, and a higher SRE was observed from application media TSB. Similar to 0 min, laminate resulted with a higher SRE, while acrylic resulted in a lower SRE. Similar results were observed on acrylic and galvanized steel when applied in PBST medium (Table A2.3).

Considering all application media for 1000 cm^2 fomite surface area, the average SRE for the experimental data at 0 min were 21 ± 6.9% for acrylic, 26 ± 3.1% for galvanized steel, and 42 ± 19.2% for laminate (Figure 3.1b). The type III test of fixed effects for 1000 cm^2 fomite surface area at 0 min was significant for fomite type ($p<0.0001$), application medium ($p=0.0037$) and the interaction between fomite type and application medium ($p=0.0998$) (Table A2.5). The laminate fomite yielded the highest SRE, while acrylic fomite gave the lowest SRE irrespective of the application medium. The use of TSB resulted in higher SRE, while PBST and water had statistically equivalent SREs (Table A2.6). At 20 min, the average SRE for the 1000 cm^2 fomite surface area were 2 ± 1.4% or less for all surfaces and application media (Figure 3.1b). The type III test of fixed effects for 1000 cm^2 fomite surface area at 20 min was significant for fomite type ($p<0.0001$) and application medium ($p=0.0053$) but not significant for the interaction between

fomite type and application medium (p=0.3720) (Table A2.7). The laminate fomite had the highest SRE, while acrylic and galvanized steel had lower and comparable SREs. The use of TSB and water as application media resulted in a higher SRE than the use of PBST (Table A2.6).

(a)



(b)

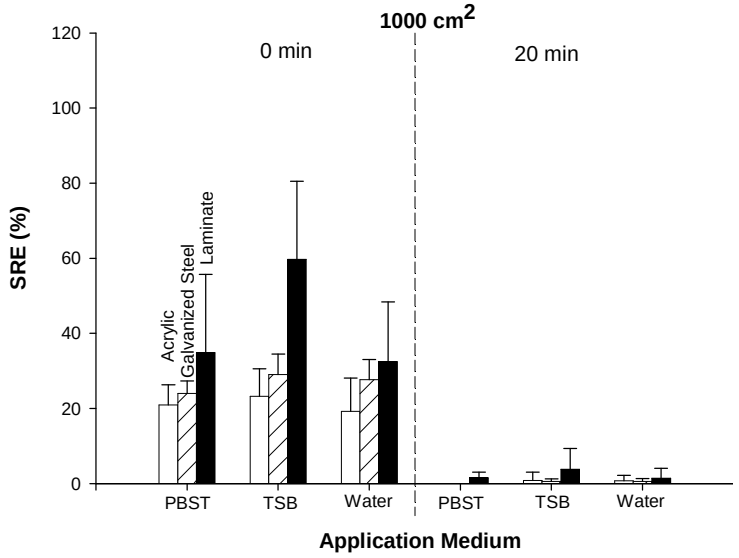


Figure 3.1 Experimental SRE of bacteriophage P22 from fomites acrylic, galvanized steel, and laminate. Bacteriophage P22 was applied in media PBST, TSB and water to fomites surface areas of (a) 100 cm^2 and (b) 1000 cm^2. A pre-moistened wipe recovered bacteriophage P22 at the initial application time (0 min) and after drying (20 min). Each bar represents the average of nine plates, and the error bars represent their standard deviation.

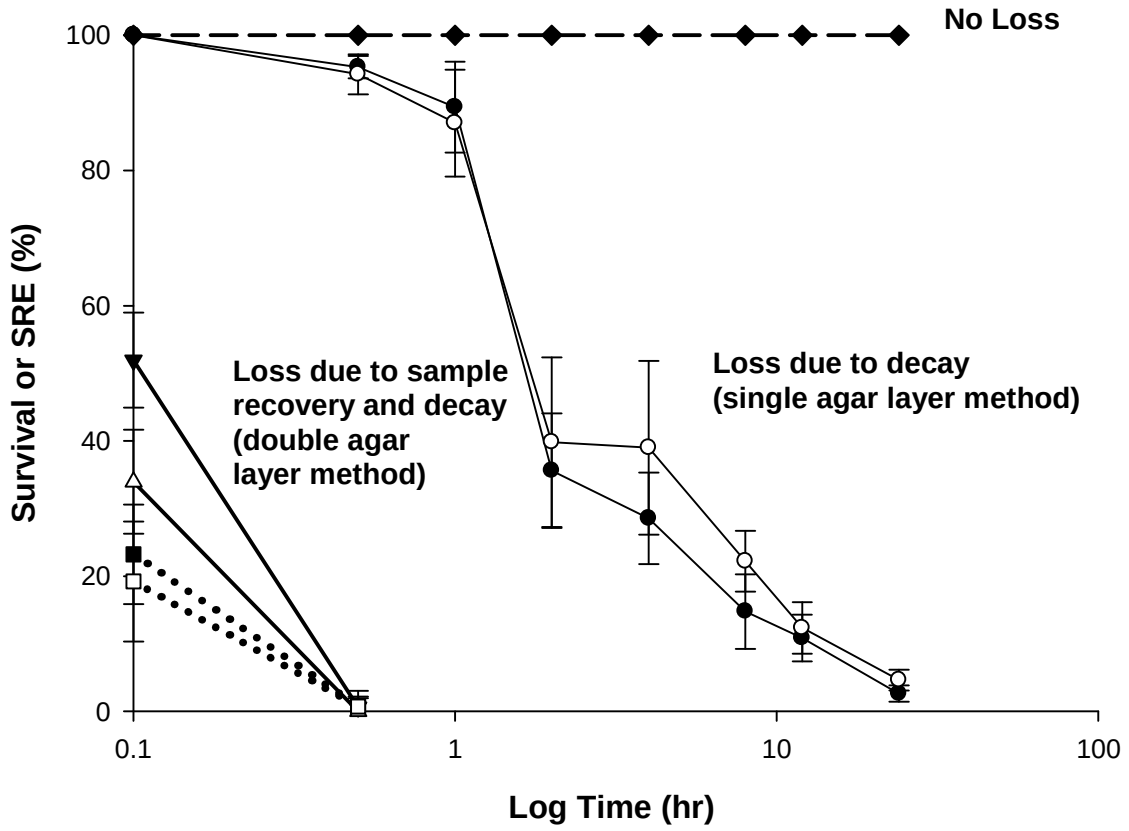


Figure 3.2 Loss of bacteriophage P22 due to decay versus the loss due to sample recovery and decay. The survival of bacteriophage P22 are signified by circles (●, applied in TSB; ○, applied in water), and each point represents the mean and standard deviation of 12 plates. SREs from 100 cm^2 fomite surface area are signified with triangles (▼, applied in TSB; Δ applied in water), and the SREs from 1000 cm^2 fomite surface area are signified by squares (■, applied in TSB; □, applied in water). Each point of the SRE data represents the mean and standard deviation of 9 plates.

3.4.2 SRE versus decay for bacteriophage P22

The method employed to determine SREs included the loss due to decay. To separate this loss from the SRE, bacteriophage P22 was directly applied onto a petri dish (using TSB and water), and decay was quantified as described in the material and methods section using the single agar layer method. The decay rates for bacteriophage P22 were 7.97×10^{-2} hr^{-1} when applied in TSB and 6.81×10^{-2} hr^{-1} when applied in water. After 1 hr, when the 5 μl droplets were visibly dry on the petri dish, the majority of the applied bacteriophage P22 was still infective (89.4 ± 6.7% in TSB and 87 ± 7.9% in water). These SREs were substantially higher than the SREs detectable at 20 min by employing the double agar layer method, which was 0% in water and 0.62 ± 1.3% in TSB for the 100 cm^2 acrylic fomite and 0.76 ± 1.6% in water and 0.69 ± 1.5% in TSB for the 1000 cm^2 acrylic fomite. Even at 24 hr, 2 to 5% of bacteriophage P22 was detectable using the single agar method (Figure 3.2). These results indicate that low or zero SRE may not always indicate an absence of the target, because SREs also include loss due to sample recovery.

3.4.3 Impact of wetting agent at varying relative humidity

From the described experiment, it was clear that significant portion of the bacteriophage P22 was still active on the fomite at 20 min and the recovery material was unable to recover the dried sample. To enhance recovery, TSB was applied to the fomite as a wetting agent for SREs at 20 min (Figure 3.3). Each point on the distribution represents the experimental data for each fomite type, application medium, RH and TSB wetting combination. The type III test of fixed effects using equation 3.3 was significant for application medium ($p<0.0001$), RH ($p<0.0001$), the interaction between fomite type and application medium ($p=0.0001$), the interaction between fomite type and RH ($p=0.0048$) and the interaction between application medium and RH

($p<0.0001$). It was not significant for fomite type ($p=0.7634$). The results of using the TSB wetting step were significantly different from those when it was not used ($p<0.0001$) (Table A2.8). The TSB wetting step improved the mean SRE for all cases. For both TSB wetting and no TSB wetting, bacteriophage P22 applied in TSB medium resulted in a higher SRE than when applied in the PBST and water. The exception to this was the acrylic and galvanized steel, where water gave higher SRE at 55 to 58% RH range (Table A2.9).

Overall, regardless of wetting agent, higher average SREs were primarily observed at RH of 28 to 32% and 55 to 58%. The SRE values for both these humidity ranges were not statistically different from each other. The mean predicted SRE was predominantly lower for RH range of 9 to 23% than for those at the other two ranges. When water was used as the application medium, the highest average SRE was always obtained for 55 to 58% RH range and the lowest in the 9 to 23% RH range (Table A2.9). The effects of RH on SREs with the other application media (TSB and PBST) were less obvious than those with water.

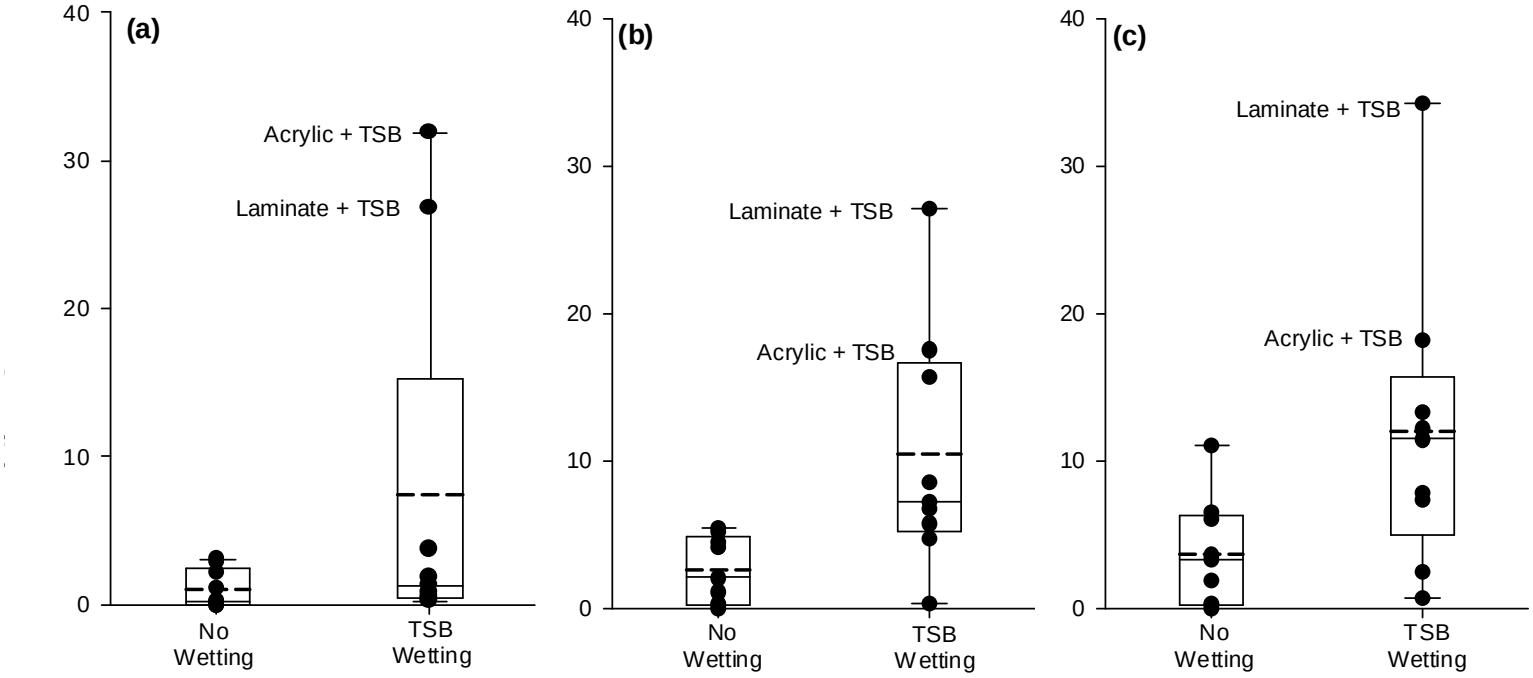


Figure 3.3 The experimental impact of RH and TSB wetting agent on the SREs of bacteriophage P22 after drying (20min) on 100 cm^2 fomite surface area. Bacteriophage P22 was sampled with pre-moistened wipes at RH ranges of (a) 9 to 23%, (b) 28 to 32%, and (c) 55 to 58%. Each dot on the distribution represents the SRE from a single fomite (acrylic, galvanized steel, and laminate) and application medium (PBST, TSB, and water) combination. Those with the highest SREs are labeled. The solid line in the box plot represents the median SREs, and the dashed lines represent the mean SREs. The box plot whiskers above and below the box indicate the 90th and 10th percentiles, respectively.

3.5 Discussion

Once decontamination has been conducted on an indoor site due to a viral outbreak or bioterrorism event, environmental monitoring and quantitative microbial risk assessment modeling will help determine the risk to human health and if the indoor site can be declared "clean" (15, 16). When monitoring fomites for viruses near the limit of detection, the results from the linear regression equation suggest that the sampling priority should be for 100 cm^2 laminate fomite. At both sampling times (0 min and 20 min) and both fomite surface areas evaluated (100 cm^2 and 1000 cm^2), laminate resulted in a higher SRE than those resulting from the other fomites under the same conditions. An increase in the fomite surface area from 100 to 1000 cm^2 decreased the average SRE at 0 min by approximately 25% for acrylic, 40% for galvanized steel, and 50% for laminate (Figure 3.1). A lower SRE for the larger surface area was expected, because the surface density was also lower. Previous studies suggest that one method may not fit all scenarios, and in sampling for larger fomite surface areas the use of alternative recovery material may be more appropriate (12). Wipe methods are generally used for fomite surface areas of 10 to 25 cm^2, but it is unknown what influence fomite surface area may have on the SRE (12). Low surface densities will require sampling of larger surface areas. Given that the SRE at 1000 cm^2 area was lower than the SRE at 100 cm^2 area and SRE includes decay, sampling at low surface densities must be carried out with caution.

In general, the application medium TSB produced higher SREs than PBST and water. TSB is an organic medium used for the growth of bacteria and may have properties that were more stabilizing for the bacteriophage P22 on the fomite than on other media. It has been suggested that suspension in more complex media may affect resistance to desiccation (29). Most of the

SRE studies reported earlier used organic media to suspend viral particles before applying to the fomites (Table 3.1). The higher SREs in the TSB application medium suggest that the application medium may also influence the SRE, especially at low surface densities.

The most dramatic reduction in the average SRE of bacteriophage P22 from the fomite was with time (0 min vs. 20 min). Initially, inactivation of bacteriophage P22 could be the main reason for this loss in SRE when the sample was dry on the fomite (20 min). Most of the rapid inactivation occurs during the period of desiccation when bacteriophage P22 becomes less stable on the fomites than in a liquid medium (20). In addition, the concentration that was applied to the fomite was rather low and close to the limit of detection of the plaque assay. Viral survival rates increase with increases in concentration, which can stabilize the virus against environmental stressors (5). On average, less than 3% of bacteriophage P22 was recoverable after 20 min on the fomite. The SREs reported at 20 min varied widely from 3 to 98.4% (Table 3.1). Each of the studies had a different experimental approach for determining the SREs from fomites which may account for the broad range of SREs reported. Keswick *et al.* 1983 (19), using cotton swabs, recovered rotavirus, poliovirus and bacteriophage f2 immediately after applying the samples to the fomite. Similarly, cotton swabs were used to recover norovirus and rotavirus dried for 15 min on the fomite by Scherer *et al.* 2009 (28). Taku *et al.* 2002 (36) evaluated three methods, moistened cotton swabs or nylon filter, fomite contact with elution buffer and aspiration, and scraping with aspiration, to recover feline calicivirus dried for 15 min on the fomite. The recovery materials antistatic cloth, cotton swab and polyester swab were evaluated by sampling bacteriophage MS2 dried for 45 min on the fomite (17). Sattar *et al.* 1987 (27), analyzed the SREs of human rhinovirus 14 dried on 1 cm diameter disks for 1 hr and then eluted the virus by

submerging the disk in 1 ml of tryptose phosphate broth and sonicating. It is evident that the number of parameters influencing the SREs is rather large, posing a challenge for simple comparison.

A positive sample result indicates surface contamination and potential risk of exposure. However, a negative result does not entirely ensure the absence of infectious agents and the absence of the potential risk of exposure (28). Following the same protocol, Masago *et al.* 2008 (20) found bacteriophage P22 to survive for 36 hr on 10 cm^2 fomites (aluminum, ceramic, glass, plastic, stainless steel, and laminate) when applied at a surface density of approximately 10^7 PFU/cm^2 (Table 3.1). The decay rate of bacteriophage P22, reported in Masago *et al.* 2008 (20), for the plastic fomite was $5.2x10^{-3}$ hr^{-1}. When eliminating the recovery method by applying the bacteriophage P22 (surface density 2.5 ± 0.9 PFU/cm^2) directly onto the petri dish (plastic fomite), 2 to 5% of bacteriophage P22 could be detected at 24 hr. The decay rates for bacteriophage P22 on petri dish were estimated to be $7.97x10^{-2}$ hr^{-1} when applied in TSB and $6.81x10^{-2}$ hr^{-1} when applied in water. The differences between the decay rates in Masago *et al* 2008 (20) and this study were most likely due to the sample concentrations, since higher initial titers have shown to extend survival on fomites (5). As seen in Figure 3.2, at 1 hr (5 µl droplets were visibly dry) an average of 88.2 ± 7.3% of the applied bacteriophage P22 was still active. The majority of the loss (40 to 60%) occurred between hours 1 and 2. Compared to this, the average SRE from the 100 cm^2 and 1000 cm^2 acrylic surfaces at 20 min (1 µl droplets visibly dry) was less than 1% (Figure 3.2). The survival of organisms on fomites is known to be agent specific and ranges from 0.75 hr for rotavirus to 90 days for astrovirus (Table 3.1). Temperature, RH, fomite surface area, and sample concentration are all known to affect survival (5, 32, 40).

Knowledge of the organisms' response to environmental stress on the fomite is important in determining the appropriate detection methods and employing clean up strategies.

The results of the experiment designed to separate the decay from sample recovery (Figure 3.2) revealed that for surface densities of 0.4 to 4 PFU/cm^2, SREs were low due to poor efficiency of the recovery method rather than decay. The TSB wetting step improved the SREs for all cases at 20 min (Table A2.9). The SRE results doubled in the majority of the cases, especially when the application medium was TSB. However, this TSB wetting step, the combination of the scraping from the disposable spreader and the application of the TSB wetting solution applied onto the fomite, may have physically dislodged the viral particles, resulting with a higher SRE than without the TSB wetting step (36). It can also be speculated that the bacteriophage P22 may adhere strongly to the fomite surface after drying or may attach to an imperfection on the fomite so that the sampling material cannot desorb the virus off the fomite. Surface roughness has been shown to influence adhesion and cell retention to fomites, which can affect recovery (31, 38). In this study, surface roughness was not measured. The addition of the TSB wetting step demonstrates the potential to further desorb viruses from the fomite and improve SRE.

The RH and temperature are crucial parameters for viral survival on fomites (Table 3.1) (4, 5, 32). Higher SREs were observed for bacteriophage P22 at RH ranges of 28 to 32% and 55 to 58% regardless of the use of a wetting agent (Figure 3.3). At RH range of 9 to 23%, the lowest SREs were obtained (Table A2.9). The combination of application medium and RH may also play a significant role in SRE. Bacteriophage P22 applied in water consistently had the highest SREs at 55 to 58% and the lowest SREs at 9 to 23%. However, for the RH ranges evaluated, its

effect was not as obvious for the other application media. The interaction between RH and application media may be a useful parameter in implementing sampling strategies.

3.6 Conclusions

In summary, efficient sample recovery and detection methods are essential in determining the exposure of humans to viruses and the resulting risk in a contaminated indoor environment. The SREs of bacteriophage P22 from fomites at concentrations near the limit of detection were influenced most by time of sampling, fomite surface area, the use of a wetting agent and RH. The observations made here using bacteriophage P22 as a surrogate highlight some of the factors that must be considered when sampling for very low surface densities of threat agents. Understanding the contributions of decay and recovery in the overall measured SREs under various conditions and the parameters affecting them will assist in implementing appropriate sampling methods and decontamination strategies.

APPENDIX

Table A2.1 Comparison of Julian *et al.* 2011 to this manuscript.

Parameter	Julian *et al.* 2011	This manuscript
Organism	Bacteriophage MS2	Bacteriophage P22
Surface density	3.7 ± 0.13 PFU/cm^2	4.3 ± 1.9 PFU/cm^2 for 100 cm^2, 0.4 ± 0.2 PFU/cm^2 for 1000 cm^2
Fomites	PVC, Type 304 Stainless Steel	Acrylic, galvanized steel, laminate
Fomite area	25 cm^2	100 cm^2, 1000 cm^2
Application vol.	5 µl	Fifty 1 µl droplets
Application medium	50% solution of TSB and dilution buffer (5 mM NaH2PO4 and 10 mM NaCl)	PBST, TSB and sterile distilled water
Fomite seeding	In the center of the fomite	Fifty 1 µl droplets places in grid formation on the fomite
Sampling time	45 min (dry)	0 min (initial) and 20 min (dry)
Relative humidity	45 to 60%	9 to 23%, 28 to 32% and 55 to 58%
Sampling material/implements	Cotton swab, polyester swab, pre-moistened antistatic wipe	Pre-moistened antistatic wipe
Eluents (applied to sampling material)	Ringer's solution, saline solution, viral transport media, and 1mM sodium hydroxide solution	N/A
Fomite wetting agent (applied to fomite)	N/A	TSB (PBST evaluated in preliminary experiments)
Extraction medium	Ringer's solution, saline solution, viral transport media, and 1 mM sodium hydroxide solution	PBST
Detection method	Culture and qPCR	Culture

Table A2.2 Fit statistic, type III test of fixed effects and parameter estimates for 100 cm^2 at 0 min.

100 cm^2 at 0 min

Fit statistics

-2 Log likelihood	627.83
AIC	647.83
BIC	671.77

Type III test of fixed effects

Effect	Num DF[a]	Den DF[b]	F-value	p-value
Fomite	2	72	106.56	<0.0001
Media	2	72	19.67	<0.0001
Fomite*Media	4	72	3.42	0.0128

Parameter estimates

Effect	Estimate	SE[c]	DF	t-value	p-value
Intercept	4.4785	0.05883	72	76.12	<0.0001
Fomite (Laminate)[d]					
Acrylic	-0.9253	0.0832	72	-11.12	<0.0001
G. Steel	-0.3935	0.0832	72	-4.73	<0.0001
Media (Water)					
PBST	-0.0041	0.0832	72	-0.05	0.9608
TSB	0.1271	0.0832	72	1.53	0.1309
Acrylic*PBST	0.4053	0.1177	72	3.44	0.0010
Acrylic*TSB	0.2884	0.1177	72	2.45	0.0167
G. Steel*PBST	0.1775	0.1177	72	1.51	0.1359
G. Steel*TSB	0.2227	0.1177	72	1.89	0.0624

a. Num DF-numerator degrees of freedom
b. Den DF-denominator degrees of freedom
c. SE-standard error
d. (Laminate or Water)-reference category

Table A2.3 %SRE for fomites 100 cm^2 at 0 and 20 min. Estimates reported from log transformed data. Mean and 95% confidence interval (CI) reported in %SRE.

100 cm^2 at 0 min				
Media	**Fomite**	**Mean**	**95% CI**	
PBST	Acrylic	51.16	45.39	57.66
PBST	G. Steel	69.69	61.87	78.49
PBST	Laminate	86.74	77.03	97.66
TSB	Acrylic	51.92	46.06	58.50
TSB	G. Steel	83.33	74.00	93.83
TSB	Laminate	99.04	87.97	100.00
Water	Acrylic	33.93	30.06	38.27
Water	G. Steel	58.44	51.86	65.83
Water	Laminate	87.10	77.35	98.06
100 cm^2 at 20 min				
PBST	Acrylic	0.22	0.00	0.77
PBST	G. Steel	0.26	0.00	0.83
PBST	Laminate	0.35	0.00	0.96
TSB	Acrylic	0.62	0.11	1.35
TSB	G. Steel	1.79	0.92	3.06
TSB	Laminate	2.86	1.66	4.62
Water	Acrylic	0.00	0.00	0.45
Water	G. Steel	0.40	0.00	1.04
Water	Laminate	0.80	0.23	1.61

Table A2.4 Fit statistic, type III test of fixed effects and parameter estimates for 100 cm^2 at 20 min.

100 cm^2 at 20 min

Fit statistics

-2 Log likelihood	199.63
AIC	219.63
BIC	243.58

Type III test of fixed effects

Effect	Num DF[a]	Den DF[b]	F-value	p-value
Fomite	2	72	5.77	0.0047
Media	2	72	13.08	<0.0001
Fomite*Media	4	72	1.11	0.3589

Parameter estimates

Effect	Estimate	SE[c]	DF	t-value	p-value
Intercept	0.5853	0.1880	72	3.11	0.0027
Fomite (Laminate)[d]					
Acrylic	-0.5853	0.2658	72	-2.20	0.0309
G. Steel	-0.2488	0.2658	72	-0.94	0.3524
Media (Water)					
PBST	-0.2852	0.2658	72	-1.07	0.2869
TSB	0.7662	0.2658	72	2.88	0.0052
Acrylic*PBST	0.4841	0.3760	72	1.29	0.2020
Acrylic*TSB	-0.2852	0.3760	72	-0.76	0.4506
G. Steel*PBST	0.1781	0.3760	72	0.47	0.6371
G. Steel*TSB	-0.07744	0.3760	72	-0.21	0.8374

a. Num DF-numerator degrees of freedom
b. Den DF-denominator degrees of freedom
c. SE-standard error
d. (Laminate or Water)-reference category

Table A2.5 Fit statistic, type III test of fixed effects and parameter estimates for 1000 cm^2 at 0 min.

1000 cm^2 at 0 min

Fit statistics

-2 Log likelihood	593.08
AIC	613.08
BIC	637.03

Type III test of fixed effects

Effect	Num DF[a]	Den DF[b]	F-value	p-value
Fomite	2	72	25.57	<0.0001
Media	2	72	6.07	0.0037
Fomite*Media	4	72	2.03	0.0998

Parameter estimates

Effect	Estimate	SE[c]	DF	t-value	p-value
Intercept	3.5090	0.1106	72	31.72	<0.0001
Fomite (Laminate)[d]					
Acrylic	-0.5045	0.1564	72	-3.22	0.0019
G. Steel	-0.1526	0.1564	72	-0.98	0.3327
Media (Water)					
PBST	0.06866	0.1564	72	0.44	0.6621
TSB	0.5963	0.1564	72	3.81	0.0003
Acrylic*PBST	0.0129	0.2212	72	0.06	0.9537
Acrylic*TSB	-0.4139	0.2212	72	-1.87	0.0654
G. Steel*PBST	-0.2064	0.2212	72	-0.93	0.3541
G. Steel*TSB	-0.5511	0.2212	72	-2.49	0.0150

a. Num DF-numerator degrees of freedom
b. Den DF-denominator degrees of freedom
c. SE-standard error
d. (Laminate or Water)-reference category

Table A2.6 %SRE for fomites 100 cm^2 at 0 and 20 min. Estimates reported from log transformed data. Mean and 95% confidence interval (CI) reported in %SRE.

1000 cm^2 at 0 min				
Media	**Fomite**	**Mean**	**95% CI**	
PBST	Acrylic	20.89	16.56	26.29
PBST	G. Steel	24.00	19.05	30.16
PBST	Laminate	34.79	27.71	43.62
TSB	Acrylic	23.21	18.42	29.19
TSB	G. Steel	29.01	23.07	36.42
TSB	Laminate	59.66	47.66	74.62
Water	Acrylic	19.18	15.18	24.15
Water	G. Steel	27.69	22.01	34.76
Water	Laminate	32.41	25.80	40.66
1000 cm^2 at 20 min				
PBST	Acrylic	0.00	0.00	0.49
PBST	G. Steel	0.00	0.00	0.49
PBST	Laminate	1.52	0.70	2.75
TSB	Acrylic	0.76	0.18	1.61
TSB	G. Steel	0.53	0.03	1.27
TSB	Laminate	3.79	2.22	6.11
Water	Acrylic	0.69	0.14	1.51
Water	G. Steel	0.55	0.05	1.31
Water	Laminate	1.42	0.63	2.60

Table A2.7 Fit statistic, type III test of fixed effects and parameter estimates for 1000 cm^2 at 20 min.

1000 cm^2 at 20 min

Fit statistics

-2 Log likelihood	220.86
AIC	240.86
BIC	264.81

Type III test of fixed effects

Effect	Num DF[a]	Den DF[b]	F-value	p-value
Fomite	2	72	16.36	<0.0001
Media	2	72	5.64	0.0053
Fomite*Media	4	72	1.08	0.3720

Parameter estimates

Effect	Estimate	SE[c]	DF	t-value	p-value
Intercept	0.8847	0.1984	72	4.46	<0.0001
Fomite (Laminate)[d]					
Acrylic	-0.3606	0.2806	72	-1.29	0.2028
G. Steel	-0.4436	0.2806	72	-1.58	0.1183
Media (Water)					
PBST	0.04088	0.2806	72	0.15	0.8846
TSB	0.6814	0.2806	72	2.43	0.0177
Acrylic*PBST	-0.5649	0.3968	72	-1.42	0.1589
Acrylic*TSB	-0.6426	0.3968	72	-1.62	0.1097
G. Steel*PBST	-0.4820	0.3968	72	-1.21	0.2285
G. Steel*TSB	-0.6980	0.3968	72	-1.76	0.0829

a. Num DF-numerator degrees of freedom
b. Den DF-denominator degrees of freedom
c. SE-standard error
d. (Laminate or Water)-reference category

Table A2.8 Fit statistic, type III test of fixed effects and parameter estimates for the impact of TSB wetting at varying relative humidity.

100 cm^2 at 20 min – Impact of TSB wetting at varying relative humidity

Fit statistics

-2 Log likelihood	750.05
AIC	792.05
BIC	856.89

Type III test of fixed effects

Effect	Num DF[a]	Den DF[b]	F-value	p-value
Fomite	2	142	0.27	0.7634
Media	2	142	64.58	<0.0001
RH	2	142	11.19	<0.0001
Wetting	1	142	69.43	<0.0001
Fomite*Media	4	142	6.14	0.0001
Fomite*RH	4	142	3.91	0.0048
Media*RH	4	142	7.33	<0.0001

Parameter estimates

Effect	Estimate	SE[c]	DF	t-value	p-value
Intercept	2.3953	0.2441	142	9.81	<0.0001
Fomite (Laminate)[d]					
Acrylic	0.6651	0.2960	142	2.25	0.0262
G. Steel	0.3896	0.3071	142	1.27	0.2067
Media (Water)					
PBST	-1.2233	0.2972	142	-4.12	<0.0001
TSB	0.3315	0.3062	142	1.08	0.2807
RH (55-58%)					
9-23%	-1.7154	0.3009	142	-5.70	<0.0001
28-32%	-0.3712	0.3006	142	-1.23	0.2190
Wetting (TSB wetting)					
No TSB wetting	-0.9655	0.1159	142	-8.33	<0.0001
Acrylic*PBST	-1.2437	0.3303	142	-3.77	0.0002
Acrylic*TSB	-0.9918	0.3302	142	-3.00	0.0032
G. Steel*PBST	-0.03479	0.3300	142	-0.11	0.9162
G. Steel*TSB	-0.7465	0.3318	142	-2.25	0.0260
Acrylic*9 – 23%	0.6897	0.3319	142	2.08	0.0395
Acrylic*28 – 32%	-0.3686	0.3308	142	-1.11	0.2670
G. Steel*9 – 23%	-0.2752	0.3320	142	-0.83	0.4085
G. Steel*28 – 32%	-0.3207	0.3311	142	-0.97	0.3344
PBST*9 – 23%	1.3659	0.3305	142	4.13	<0.0001
PBST*28 – 32%	0.8506	0.3298	142	2.58	0.0109
TSB*9 – 23%	1.6817	0.3333	142	5.05	<0.0001
TSB*28 – 32%	0.9008	0.3316	142	2.72	0.0074

a. Num DF-numerator degrees of freedom
b. Den DF-denominator degrees of freedom
c. SE-standard error
d. (Laminate, Water, 55 to 58%, TSB wetting)-reference category

Table A2.9 %SRE for no wetting vs. wetting at varying RH. Estimates reported from log transformed data. Mean and 95% confidence interval (CI) reported in %SRE. Bacteriophage P22 was sampled at RH ranges of 9 to 23%, 28 to 32%, and 55 to 58%.

100 cm^2 at 20 min			***No Wetting***			***Wetting***		
Fomite	**Media**	**RH**	**Mean**	**95% CI**		**Mean**	**95% CI**	
Acrylic	PBST	9 – 23%	0.00	0.00	0.54	1.54	0.57	3.13
Acrylic	PBST	28 – 32%	0.00	0.00	0.27	1.02	0.22	2.35
Acrylic	PBST	55 – 58%	0.00	0.00	0.11	0.81	0.09	2.00
G. Steel	PBST	9 – 23%	0.00	0.00	0.52	1.46	0.51	3.02
G. Steel	PBST	28 – 32%	1.05	0.26	2.35	4.40	2.39	7.58
G. Steel	PBST	55 – 58%	0.75	0.05	1.94	3.60	1.81	6.55
Laminate	PBST	9 – 23%	0.00	0.00	0.43	1.28	0.37	2.78
Laminate	PBST	28 – 32%	0.99	0.21	2.25	4.21	2.23	7.41
Laminate	PBST	55 – 58%	0.23	0.00	0.99	2.23	1.03	4.14
Acrylic	TSB	9 – 23%	7.09	3.93	12.28	20.25	12.25	33.07
Acrylic	TSB	28 – 32%	3.93	2.07	6.92	11.95	7.12	19.66
Acrylic	TSB	55 – 58%	3.20	1.53	5.95	10.03	5.54	17.57
G. Steel	TSB	9 – 23%	1.99	0.79	4.00	6.85	3.71	12.11
G. Steel	TSB	28 – 32%	4.02	2.01	7.38	12.18	6.96	20.85
G. Steel	TSB	55 – 58%	3.07	1.60	5.39	9.70	5.85	15.71
Laminate	TSB	9 – 23%	4.63	2.46	8.15	13.78	8.35	22.37
Laminate	TSB	28 – 32%	8.88	5.03	15.20	24.96	15.12	40.81
Laminate	TSB	55 – 58%	4.82	2.46	8.79	14.28	8.24	24.27
Acrylic	Water	9 – 23%	1.91	0.76	3.82	6.65	3.57	11.79
Acrylic	Water	28 – 32%	2.88	1.37	5.33	9.18	5.25	15.58
Acrylic	Water	55 – 58%	7.13	4.16	11.80	20.34	12.15	33.62
G. Steel	Water	9 – 23%	0.00	0.00	0.33	1.21	0.36	2.61
G. Steel	Water	28 – 32%	2.09	0.91	3.99	7.11	4.02	12.09
G. Steel	Water	55 – 58%	5.16	2.68	9.33	15.20	8.59	26.35
Laminate	Water	9 – 23%	0.00	0.00	0.22	0.97	0.20	2.25
Laminate	Water	28 – 32%	1.88	0.76	3.71	6.57	3.64	11.36
Laminate	Water	55 – 58%	3.18	1.59	5.74	9.97	5.77	16.77

REFERENCES

REFERENCES

1. **Abad, F. X., R. M. Pinto, and A. Bosch.** 1994. Survival of enteric viruses on environmental fomites Appl. Environ. Microbiol. **60:**3704-3710.

2. **Abad, F. X., C. Villena, S. Guix, S. Caballero, R. M. Pinto, and A. Bosch.** 2001. Potential role of fomites in the vehicular transmission of human astroviruses. Appl. Environ. Microbiol. **67:**3904-3907.

3. **Bean, B., B. M. Moore, B. Sterner, L. R. Peterson, D. N. Gerding, and H. H. Balfour.** 1982. Survival of influenza viruses on environmental surfaces J. Infect. Dis. **146:**47-51.

4. **Boone, S. A., and C. P. Gerba.** 2010. The prevalence of human parainfluenza virus 1 on indoor office fomites. Food Environ. Virol. **2:**41-46.

5. **Boone, S. A., and C. P. Gerba.** 2007. Significance of fomites in the spread of respiratory and enteric viral disease. Appl. Environ. Microbiol. **73:**1687-1696.

6. **Boxman, I. L. A., R. Dijkman, N. Loeke, G. Hagele, J. Tilburg, H. Vennema, and M. Koopmans.** 2009. Environmental swabs as a tool in norovirus outbreak investigation, including outbreaks on cruise ships. J. Food Prot. **72:**111-119.

7. **Brady, M. T., J. Evans, and J. Cuartas.** 1990. Survival and disinfection of parainfluenza viruses on environmental surfaces. Am. J. Infect.Control **18:**18-23.

8. **Casanova, L. M., S. Jeon, W. A. Rutala, D. J. Weber, and M. D. Sobsey.** 2010. Effects of Air Temperature and Relative Humidity on Coronavirus Survival on Surfaces. Appl. Environ. Microbiol. **76:**2712-2717.

9. **Centers for Disease Control and Prevention (CDC).** 2002. Emergency preparedness and response: comprehensive procedures for collecting environmental samples for culturing *Bacillus anthracis* http://www.bt.cdc.gov/Agent/Anthrax/environmental-sampling-apr2002.asp.

10. **Clay, S., S. Maherchandani, Y. S. Malik, and S. M. Goyal.** 2006. Survival on uncommon fomites of feline calicivirus, a surrogate of noroviruses. Am. J. Infect. Control **34:**41-43.

11. **D'Souza, D. H., A. Sair, K. Williams, E. Papafragkou, J. Jean, C. Moore, and L. Jaykus.** 2006. Persistence of caliciviruses on enviromnental surfaces and their transfer to food. Int. J. Food Microbiol. **108:**84-91.

12. **Edmonds, J. M.** 2009. Efficient methods for large-area surface sampling of sites contaminated with pathogenic microorganisms and other hazardous agents: current state, needs, and perspectives. Appl. Microbiol. Biotechnol. **84:**811-816.

13. **Enriquez, C., A. Alum, E. M. Suarez-Rey, C. Y. Choi, G. Oron, and C. P. Gerba.** 2003. Bacteriophages MS2 and PRD1 in turfgrass by subsurface drip irrigation. J. Environ. Engin. ASCE **129:**852-857.

14. **Greatorex, J. S., P. Digard, M. D. Curran, R. Moynihan, H. Wensley, T. Wreghitt, H. Varsani, F. Garcia, J. Enstone, and J. S. Nguyen-Van-Tam.** 2011. Survival of influenza A(H1N1) on materials found in households: implications for infection control. PLoS One **6:**1-6.

15. **Haas, C. N., J. B. Rose, and C. P. Gerba.** 1999. Quantitative microbial risk assessment. John Wiley, New York.

16. **Herzog, A. B., S. D. McLennan, A. K. Pandey, C. P. Gerba, C. N. Haas, J. B. Rose, and S. A. Hashsham.** 2009. Implications of limits of detection of various methods for *Bacillus anthracis* in computing risks to human health. Appl. Environ. Microbiol. **75:**6331-6339.

17. **Julian, T. R., F. J. Tamayo, J. O. Leckie, and A. B. Boehm.** 2011. Comparison of surface sampling methods for virus recovery from fomites. Appl. Environ. Microbiol. **77:**6918-6925.

18. **Keswick, B. H., L. K. Pickering, H. L. Dupont, and W. E. Woodward.** 1983. Survival and detection of rotaviruses on environmental surfaces in day care centers. Appl. Environ. Microbiol. **46:**813-816.

19. **Li, S., J. N. S. Eisenberg, I. H. Spicknall, and J. S. Koopman.** 2009. Dynamics and control of infections transmitted from person to person through the environment. Am. J. Epidemiol. **170:**257-265.

20. **Masago, Y., T. Shibata, and J. B. Rose.** 2008. Bacteriophage P22 and *Staphylococcus aureus* attenuation on nonporous fomites as determined by plate assay and quantitative PCR. Appl. Environ. Microbiol. **74:**5838-5840.

21. **Mbithi, J. N., V. S. Springthorpe, and S. A. Sattar.** 1991. Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. Appl. Environ. Microbiol. **57:**1394-1399.

22. **Noyce, J. O., H. Michels, and C. W. Keevil.** 2007. Inactivation of influenza A virus on copper versus stainless steel surfaces. Appl. Environ. Microbiol. **73:**2748-2750.

23. **Piercy, T. J., S. J. Smither, J. A. Steward, L. Eastaugh, and M. S. Lever.** 2010. The survival of filoviruses in liquids, on solid substrates and in a dynamic aerosol. J. Appl. Microbiol. **109:**1531-1539.

24. **Rusin, P., S. Maxwell, and C. Gerba.** 2002. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. J. Appl. Microbiol. **93:**585-592.

25. **Sagripanti, J. L., A. M. Rom, and L. E. Holland.** 2010. Persistence in darkness of virulent alphaviruses, Ebola virus, and Lassa virus deposited on solid surfaces. Arch. Virol. **155:**2035-2039.

26. **SAS Institute Inc.** 2012. SAS/STAT(R) 9.22 user's guide. Type III test of fixed effects. http://support.sas.com/documentation/cdl/en/statug/63347/HTML/default/viewer.htm#statug_glimmix_a0000001479.htm.

27. **Sattar, S. A., Y. G. Karim, V. S. Springthorpe, and C. M. Johnsonlussenburg.** 1987. Survival of human rhinovirus type 14 dried onto nonporous inanimate surfaces: effect of relative humidity and suspending medium. Can. J. Microbiol. **33:**802-806.

28. **Scherer, K., D. Made, L. Ellerbroek, J. Schulenburg, R. Johne, and G. Klein.** 2009. Application of a swab sampling method for the detection of norovirus and rotavirus on artificially contaminated food and environmental surfaces. Food Environ. Virol. **1:**42-49.

29. **Shams, A. M., L. J. Rose, L. Hodges, and M. J. Arduino.** 2007. Survival of *Burkholderia pseudomallei* on environmental surfaces. Appl. Environ. Microbiol. **73:**8001-8004.

30. **Shen, C. P., M. S. Phanikumar, T. T. Fong, I. Aslam, S. P. McElmurry, S. L. Molloy, and J. B. Rose.** 2008. Evaluating bacteriophage P22 as a tracer in a complex surface water system: The Grand River, Michigan. Environ. Sci. Technol. **42:**2426-2431.

31. **Silva, S., P. Teixeira, R. Oliveira, and J. Azeredo.** 2008. Adhesion to and viability of *Listeria monocytogenes* on food contact surfaces. J. Food Prot. **71:**1379-1385.

32. **Sinclair, R., S. A. Boone, D. Greenberg, P. Keim, and C. P. Gerba.** 2008. Persistence of category A select agents in the environment. Appl. Environ. Microbiol. **74:**555-563.

33. **Sinclair, R. G., J. B. Rose, S. A. Hashsham, C. P. Gerba, and C. N. Haas.** 2012. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. Appl. Environ. Microbiol. **78:**1969-1977.

34. **Sizun, J., M. W. N. Yu, and P. J. Talbot.** 2000. Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections. J. Hosp. Infect. **46:**55-60.

35. **Spicknall, I. H., J. S. Koopman, M. Nicas, J. M. Pujol, S. Li, and J. N. S. Eisenberg.** 2010. Informing optimal environmental influenza interventions: how the host, agent, and environment alter dominant routes of transmission. PLoS Comput. Biol. **6:**11.

36. **Taku, A., B. R. Gulati, P. B. Allwood, K. Palazzi, C. W. Hedberg, and S. M. Goyal.** 2002. Concentration and detection of caliciviruses from food contact surfaces. J. Food Prot. **65:**999-1004.

37. **Thomas, Y., G. Vogel, W. Wunderli, P. Suter, M. Witschi, D. Koch, C. Tapparel, and L. Kaiser.** 2008. Survival of influenza virus on banknotes. Appl. Environ. Microbiol. **74:**3002-3007.

38. **Tiwari, A., D. P. Patnayak, Y. Chander, M. Parsad, and S. M. Goyal.** 2006. Survival of two avian respiratory viruses on porous and nonporous surfaces. Avian Dis. **50:**284-287.

39. **U.S. Environmental Protection Agency.** 2001. Method 1602: male-specific (F+) and somatic coliphage in water by single agar layer (SAL) procedure. EPA 821-R-01-029. U.S. Environmental Protection Agency, Washington, DC.

40. **Vasickova, P., I. Pavlik, M. Verani, and A. Carducci.** 2010. Issues concerning survival of viruses on surfaces. Food Environ. Virol. **2:**24-34.

Chapter 4: Genetic characterization of microorganisms on highly touched and untouched fomites

4.1 Abstract

In the indoor environment, an important route of transmission of bacterial and viral disease is through the interaction with fomites. Touched fomites are mostly influenced by the interactions with individuals while untouched fomites are influenced by air movement. Understanding the bacterial communities on the fomites in an indoor environment may affect disease transmission models and quantitative microbial risk assessments. Therefore, in this study, the bacterial communities on highly touched and untouched fomites in a university setting were analyzed. Samples from touched and untouched fomites were collected from the common lounge, computer room, and cafeteria of six dormitories at the University of Michigan. Non-porous fomites of plastic, metal, and wood (e.g., computer mouse, door knob, and window sill, respectively) with surface areas ranging approximately 10 to 100 cm^2 were sampled using pre-moistened wipes. DNA was exacted from the samples and analyzed using 454 GS FLX Roche sequencing of the 16S rRNA genes. Results from 69 samples shows that the majority of the sequences found on both touched and untouched fomites were from three core phyla. Proteobacteria, Actinobacteria and Firmicutes represented an average of 81.4% and 83.8% of the bacteria community on touched and untouched fomites, respectively. The bacterial communities on touched and untouched fomites were statistically different. The bacterial communities on touched fomites were more diverse and had more fecal related bacteria present compared to untouched fomites. There were no correlations observed between sample date/time, locations, dormitory rooms, fomite materials or fomite types. The knowledge of the bacterial communities

on touched and untouched fomites can further explain the exposure pathway for fomites in the indoor environment.

4.2 Introduction

In 2010, the United States had 20.3 million people enrolled in higher education and of these approximately 2.5 million people lived in college residence halls (6, 43). In addition to personal living quarters, many of these residence halls offer shared amenities such as common lounges, computer rooms, study/meeting rooms, gyms, cafeterias, classrooms and offices. College students are generally at a higher risk of infectious diseases due to a shared living space, closer contact with other residents, variable hygiene habits and likeliness to engage in risk behavior (8, 30). Interactions with bacterial contaminated fomites in a setting where the population is working, eating and sleeping could lead to an added risk, especially when there is variable cleaning or infection control practices (3, 31). Recent outbreaks on university campuses have included meningitis, seasonal influenza, gastroenteritis, measles, mumps, *Streptococcus*, pertussis and Methicillin-resistant *Staphylococcus aureus* (MRSA) (8, 20, 30, 31).

Fomites can be a vehicle in the transmission for both enteric and respiratory pathogens in the indoor environment (2, 16, 26). Pathogens can survive on fomites for hours to months depending on the environmental conditions, such as type and concentration of the microorganism, temperature, relative humidity, and UV exposure (14, 16, 26, 41). The microbial diversity in the indoor environment is comprised of microorganisms from outdoor habitats (i.e., soil, water, and air) being transported indoors by humans, pets, animals or ventilation systems; microorganisms originating on indoor sources (i.e., fomites or water); and humans or pets

shedding of body secretions (i.e., blood, feces, urine, saliva and nasal fluid) (2, 21, 23). Touched fomites (e.g., study table, door knob, or computer mouse) are mostly influenced by the direct interactions with humans. Untouched fomites (e.g., top of a cabinet or bookshelf) are more influenced by air movement and less direct interaction with humans. Differences between the bacterial communities from touched and untouched fomites may be due to the cleaning frequencies, ventilation rates, and the continual inoculation and re-inoculation of fomites that are frequently touched (9, 21, 26). There are many studies that have shown pathogen contamination on fomites and the opportunity for transmission in daycare centers, schools, nursing homes, office buildings, residential homes, public areas, hospitals, and food preparation settings (16, 26). There have also been a few studies that sampled fomites in the university setting. These studies focused on detecting one organism (i.e., MRSA, *S. aureus or Stenotrophomonas maltophilia*) and the total coliforms or total heterotrophic bacteria on fomites in bathrooms, kitchens and computer rooms (3, 8, 31, 38).

When trying to better understand the diversity of the bacteria on fomites there are limitations to those methods, which include diluted target concentrations, environmental impurities, background inhibitors, and organisms in a viable but not cultivable state (15). High-throughput sequencing of the 16S rRNA gene allows us to examine the microbial communities on fomites to begin understanding the interactions between microorganisms, humans, and the indoor environment (13). When evaluating bacterial communities on hospital fomites, Oberauner *et al.* 2013 (34), was only able to detect 2.5% of the total bacterial diversity using standard cultivation when in comparison to the 16S rRNA pyrosequencing technique.

The pyrosequencing approach has provided insight into the diversity of bacterial communities on fomites in offices (17), hospitals (18, 34, 35), and residential households (9, 12, 19). In all of these studies, there was a core set of phyla present on the indoor fomites, which consisted of Proteobacteria, Firmicutes, Actinobacteria and in most cases Bacteroidetes. Distinct differences in the bacterial community structure were observed in comparison of different locations, rooms, and types of fomites (Table 4.1) (9, 12, 17-19, 34, 35). To our knowledge, only two studies have evaluated the bacterial communities on indoor university fomites (13, 23). Flores *et al.* 2011 (13) sampled public restroom surfaces and was able to cluster the fomite communities into three general groups: fomites associated with toilets, restroom floors, and routinely touched fomites (i.e., door handle, faucet knobs, and soap dispenser). Kembel *et al.*2014 (23) sampled fomites in restrooms, offices, and classrooms. The bacterial communities were most influenced by architectural design characteristics, building arrangement, human use and movement, and ventilation sources (Table 4.1) (23).

Table 4.1 Summary of studies analyzing the bacterial communities on fomites in the indoor environment using pyrosequencing.

	Jeon *et al.* (19)	**Flores *et al.*(12)**	**Dunn *et al.*(9)**	**Hewitt *et al.*(17)**	**Hewitt *et al.*(18)**
Location	N/A	Boulder, CO	Raleigh-Durham, NC	New York, NY; San Francisco, CA; Tucson, AZ	San Diego, CA
Category	Household	Household	Household	Office	Hospital
Room	Kitchen, bathroom	Kitchen	Kitchen, bathroom, bedroom, living room, outside		Neonatal intensive care units (NICU)
Fomite	Refrigerator, toilet	Counter top, faucet, sink, cabinet, microwave, refrigerator, freezer, oven, stove, wall, garbage can, floor	Cutting board, kitchen counter, refrigerator, toilet seat, pillowcase, door handle, tv screen, door trim (interior, exterior)	Chair, phone, computer mouse, keyboard, desktop	Baby bedside, door button, incubator, pyxus, sink, weigh cart
Date	N/A	September 2011	Autumn 2011	N/A	January 2009 (NICU1), February 2009 (NICU2)
Sampling method	Easy swab kit	Sterile cotton swab	Dual-tipped sterile BBL culture swabs	Dual-tipped sterile BBL culture swabs	Dual-tipped sterile BBL culture swabs
Sequencing method	454 GS Junior System	Illumina HiSeq2000	Illumina HiSeq or MiSeq	454 GS FLX System	454 GS FLX System
Area	25cm^2	N/A	N/A	13 cm^2	12 cm^2
No. sample	2/10 houses	82/4 houses	9/40 houses	5/90 offices (3 buildings)	13/NICU1, 17/NICU2 (2 hospitals)
Occupancy	4-5 ppl./house	N/A	Ppl., children, cats, dogs	Half offices inhabited by men or women	N/A

Table 4.1 (cont'd).

	Jeon *et al*. (19)	**Flores *et al.*(12)**	**Dunn *et al*.(9)**	**Hewitt *et al.*(17)**	**Hewitt *et al.*(18)**
Exposure	Untouched, touched	Untouched, touched	Untouched, touched	Touched	Untouched, touched
Core phyla	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria	Proteobacteria, Firmicutes, Actinobacteria	Proteobacteria, Firmicutes, Actinobacteria	N/A
Source	Skin, gut	Skin, food, water	Skin, oral cavity, gut, leaves, soil	Skin, nasal cavity, oral cavity, gut, soil	Gut, oral cavity, skin, urine, vagina, outdoor air, soil
Influence community	N/A	Communities were different between kitchens. Sample area had different communities (ie moist vs dry).	Communities were grouped into depositional environments, kitchen associated, and frequently touched surfaces. Communities were different between kitchens. Presence of dogs attributed mostly to variation in communities. Significant distinction between exterior and interior microbial communities.	Communities were correlated with location, especially NY and CA compared to AZ suggesting an effect to climate.	Communities were significantly different between buildings.
No influence community	Communities from refrigerator and toilet were similar.	Communities on fomite in the same kitchen were similar.	Occupants, presence of cats, presence of children, use of pesticides, presence of carpet, and the presence of allergies.	Offices inhabited by men or women. Different fomites.	NICU samples clustered with other fomites in offices, healthcare centers and restrooms.
Fomite hygiene	Assumption: more DNA was present in refrigerator vegetable drawer than toilets probably due to cleaning frequency.	Assumption: higher diversity was found on floors, exhaust fans, and freezer doors probably due to infrequent cleaning.	Assumption: surfaces that are regularly cleaned have lower levels of diversity than surfaces that are cleaned infrequently.	N/A	N/A

Table 4.1 (cont'd).

	Poza *et al.*(35)	**Oberauner *et al.*(34)**	**Kembel *et al.*(23)**	**Flores *et al.*(13)**
Location	Coruna, Spain	Graz, Austria	Eugene, OR	Boulder, CO
Category	Hospital	Hospital	University	University
Room	ICU and entrance hall	ICU	Lillis Hall: classroom, office, restroom	Restroom
Fomite	Computer screen, monitor, drawer, medical device, keyboard, door handle, refrigerator, microwave	Floor, medical device, workspace, bandage trolley, keyboard	Fomites above head level	Door handle, faucet handle, soap dispenser, toilet seat, toilet flush handle, floor
Date	June 2009 (3 consecutive days)	N/A	June 22-24, 2012	November 2010
Sampling method	Sterile lint	BiSKit, nylon flocked swab	Shop-Vac 9.4L Hang Up vacuum.	Sterile cotton swab
Sequencing method	454 GS FLX System	454 GS FLX System	Illumina MiSeq	454 GS Junior System
Area	N/A	1 m^2, 25 cm^2	2 m^2	N/A
No. sample	N/A	24/ICU (5 floor, 11 devices, 8 workplace)	155 fomites (4 floors in Lillis Hall)	10/12 bathrooms (6 male, 6 female in 2 buildings)
Occupancy	N/A	N/A	Low and high occupancy	N/A

Table 4.1 (cont'd).

	Poza *et al.*(35)	**Oberauner *et al.*(34)**	**Kembel *et al.*(23)**	**Flores *et al.*(13)**
Exposure	Touched	Touched	Untouched	Touched
Core phyla	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria, Cyanobacteria, Nitrospira	Proteobacteria, Firmicutes Deinococci	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria
Source	Skin, oral, gut, water	Skin, gut, water, soil	Human, soil, plants	Skin, gut, urine, vagina, oral, water, soil
Influence community	ICU and entrance hall bacterial communities were different. Entrance hall was more diverse than ICU.	Floor bacterial communities formed clusters distinct from medical devices.	Architectural design characteristics, building arrangement, human use and movement, and ventilation caused largest influence on communities. High occupant space (classrooms) and restrooms were associated with human microbiome. Low occupant space (offices) was associated with outdoor environments.	Communities were grouped into those found on toilet surfaces, restroom floors, and surfaces routinely touched with hands. Toilet flush handle had similar communities as the floor.
No influence community	N/A	Communities from medical devices were similar to workplaces.	N/A	Male and female restrooms were not statistically significant
Fomite hygiene	Sampling was done at 8AM before routine cleaning.	N/A	N/A	N/A

The microbial community structure for indoor environments and their impact on human health remains relatively unknown (7, 13, 17, 23, 37). Our hypothesis is that touched fomites, which have direct human interaction, will have a more diverse bacterial community and will have more genera associated with the human microbiome than the untouched fomites. Understanding the indoor environment, especially since humans spend approximately 90% of their lives indoors, will assist in determining the affect (good, bad or neutral) microorganisms have on human health (21, 22). Defining the bacterial community on touched and untouched fomites may indicate patterns and insight on the exposure routes in the indoor environment (25). This will lead to effective cleaning and infection control practices (21, 25).

4.3 Materials and methods

4.3.1 Fomite sample collection

Non-porous fomites (plastic, metal, and wood) were sampled in the common lounge, computer room and cafeteria from 6 dormitories at the University of Michigan. Samples were collected in the afternoon from the dormitories; East Quadrangle (EA) on February 19, 2007, Bursley (BU) on February 20, 2007, Stockwell (ST) on February 21, 2007, Couzen (CO) on March 6, 2007, Betsy Barbour-Helen Newberry (BN) on March 7, 2007, and Alice Lloyd (AL) on March 8, 2007. Fomite surface areas ranging from 10 to 100 cm^2 were sampled with pre-moistened Fellowes screen cleaning wipes (no. 99715; Fellowes, Itasca, IL), as described in Herzog *et al*. 2012 (16). Five touched and five untouched fomites were randomly collected in each location for a total of 180 samples. After sampling the fomite, the pre-moistened wipe was placed into a 50 ml tube containing 10 ml of phosphate buffered saline Tween-80 (PBST). The samples were extracted from the wipe by vortexing the tube for 1 min. The sample solution and wipe were

poured into a 60 ml syringe. The sample solution was pressed into the Amicon ultra-15 centrifugal filters (UFC910024, Millipore, Billerica, MA) for concentration. The original 50 ml tube was rinsed with 5 ml PBST and vortexed for 30 s. The rinsate was then added to the Amicon tube and centrifuged for 1 min at 3,000 x g. Concentrated samples were pipetted into a 1.5 ml eppendorf tube and stored in a -80 ^{o}C freezer.

4.3.2 DNA extraction, PCR and 454 sequencing.

DNA was extracted from all of the samples using the QIAampDNA mini kit (Qiagen, Valencia, CA). DNA was quantified using a Qubit fluorometer (Invitrogen, Grand Island, NY). Extracted DNA concentrations ranged from 0.066 to 3.74 ng/µg. PCR amplification was performed in triplicate on all samples. Samples were amplified using the Fast Start High Fidelity PCR system (Roche, Indianapolis, IN). PCR reactions were carried out in a total volume of 20 µl which included 1 µl of sample DNA (approximately 10 ng/µl), 0.4 µl of 10 mM dNTP, 3 µl of $MgCl_2$, 2 µl of 10x buffer, 0.3 µl of BSA, 0.3 µl of Taq DNA polymerase, 2 µl of Fusion Tag primer, 2 µl of 10mM reverse primer and 9 µl of dH_2O. Thirty cycles of PCR amplification were performed and included an initial denaturing step at 95 ^{o}C for 45 s, an annealing step at 57 ^{o}C for 45 s, and an extension step at 72 ^{o}C for 1 min. Prior to the first amplification cycle there was a denaturing step at 95 ^{o}C for 3 min. At the end of the 30 amplification cycles there was a final 72 ^{o}C extension for 4 min. PCR amplicon sizes were determined with 1% agarose gel (1xTAE) in three separate wells per samples. Gel bands within 270 to 300 bps were excised and DNA extraction was done using Qiagen gel extraction kit (Qiagen, Valencia, CA). For samples with a negative PCR results, a total of three separate attempts were made for amplification. Final eluted samples were purified using Qiagen PCR purification kit (Qiagen, Valencia, CA). Purified

samples were combined to final concentration of 0.5 ng/μl in a volume of 10 μl (per plate). Sixty-nine samples were given to the Research Technology Support Facility (RTSF) at Michigan State University for sequencing on the 454 GSFLX Titanium Sequencer. In addition, the Robert Britton Lab at Michigan State University ran 24 samples on the 454 GS Junior System. Detailed lists of the sequenced samples are found with the supplemental material in the appendix (Tables A3.1-A3.3).

4.3.3 Data analysis

Analysis of the sequences was through QIIME, software for a wide range of 16S rRNA microbial community analyses (5). Quality controls were maintained by using default parameters for high quality sequences (>200 bp in length, quality score >25, and exact match to barcode and primer) and clustered into OTUs at 97% sequence identity (13, 17). UCLUST generated the high quality clusters (10). Representative sequences for each OTU was aligned against Greengenes core dataset using PyNAST, used to efficiently align thousands of 16S rRNA genes (4). Taxonomy was assigned with the RDP-classifier (42). UniFrac analysis was used for the principal coordinated analysis (PCoA) to determine differences of the microbial communities (28). Phylogenetic diversity (PD) was calculated by the sum of the branches of the phylogenetic tree leading to sequences in a sample (11, 22). For PD, a cutoff of 400 sequences was made for an equal comparison of samples. Samples CO.CL.2, ST.CP.6, EA.CF.4, EA.CP.7, and BU.CL.1 only had 10 sequences and were not included in the PD comparison. For the samples sequenced on the 454 GS Junior System, the cutoff was 96 sequences; therefore, EA.CP.2 was not included in this analysis. The Student's t-test was used to determine the statistical difference between the diversity on touched and untouched fomites.

4.4 Results

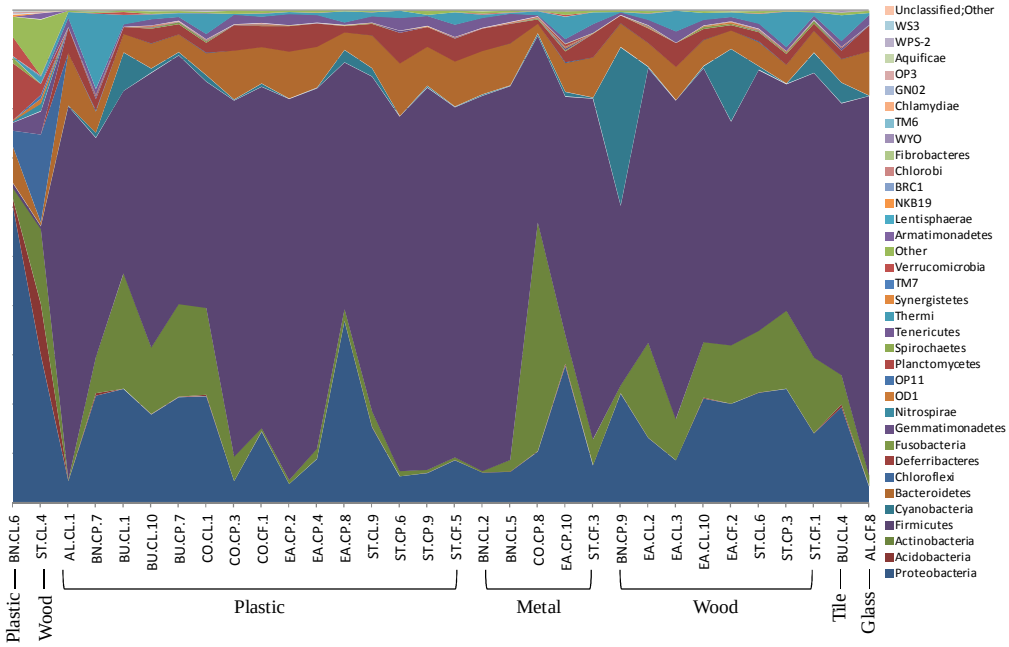
Touched and untouched fomites had a core set of phyla which included Proteobacteria, Actinobacteria and Firmicutes. The relative abundance of the core set of phyla represented an average of 81.4% and 83.8% of the bacterial community on touched and untouched fomites, respectively (Figure 4.1). However, the compositions of the community structure were different between touched and untouched fomites. The average relative abundance of Proteobacteria, Actinobacteria, Thermi, and Cynobacteria were higher on untouched fomites. For touched fomites Firmicutes, Bacteroidetes, Deferribacteres, and Tenericutes had a higher relative abundance compared to untouched fomites. It should be noted that the phylum Deferribacteres represents only one genus (*Mucispirillum*) and the average relative abundance on touched fomites was 3%. From this analysis there seems to be no patterns with the bacterial community and the fomite material (Figure 4.1).

The PCoA of the un-weighted and weighted UniFrac distances between sample exposures resulted in a clear clustering of touched and untouched fomites (Figure 4.2). When three of the fomites were sampled, it was assumed that they represented untouched fomites but the community structure fit with the touched fomites, depicted as green squares in figure 4.2. There were also fomites that were assumed to be touched at the time of sampling but the community analysis appeared to be untouched (Figure 4.2). PCoA plots were also analyzed to distinguish if there were any correlations between sample date/time, locations, dormitory rooms, fomite materials, and fomite types. There were no correlations with any of those parameters (Figures A3.1-A3.5). The PD of the bacterial communities from both fomite exposures were significantly

different (Student's t-test p=0.0005) (Figure 4.3). Touched fomites had a higher bacterial diversity than untouched fomites.

A general examination of the compositions of the genera (or lowest classification available) on touched and untouched fomites were compared, with a cutoff at an average relative abundance of 0.5% or higher. The total relative abundance of the genera were 79.6% and 74.9% for touched and untouched fomites, respectively (Figure 4.4). The main sources of the genera found on touched fomites were from the animal gut (50.6%), skin and oral (18.8%), plants (3.4%), water and soil (3.1%), and extremophiles (3.0%) (Figure 4.4a). On untouched fomites the sources of the genera were from skin and oral (39.8%), water and soil (14.4%), extremophiles (12.3%), plants (7.16%), and the animal gut (2.6%) (Figure 4.4b). Similar relative abundance, PCoA and PD results were observed from samples sequenced on the 454 GS Junior System and presented in the appendix (Figures A3.6-A3.14).

(a)



(b)

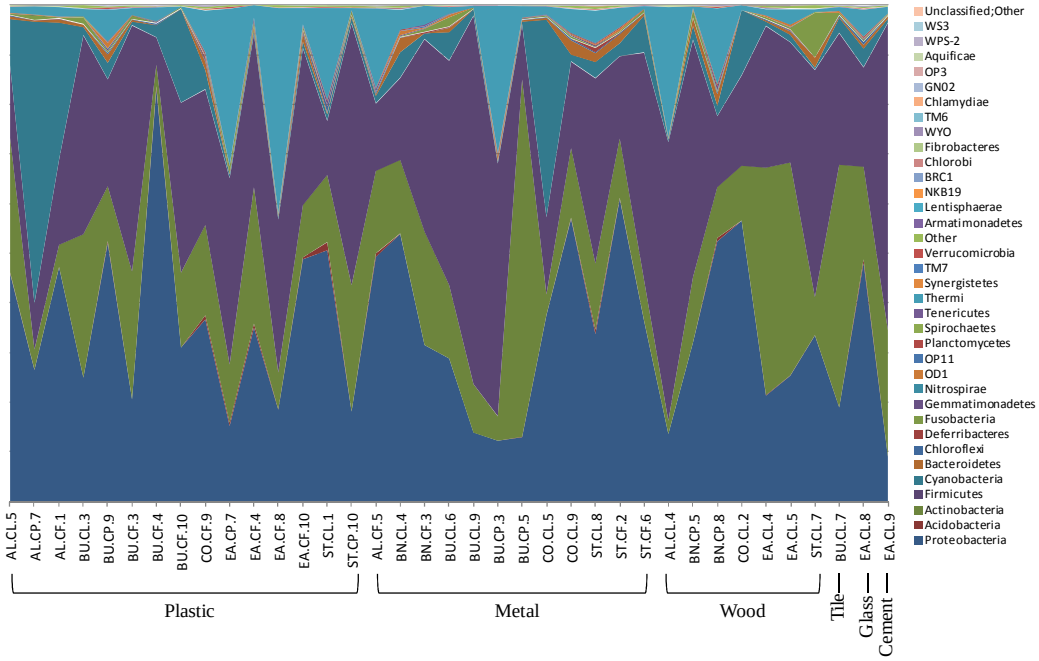


Figure 4.1 Relative abundance of bacterial communities for each sample on (a) touched fomites and (b) untouched fomites at the phylum classification level. The fomite material for each sample is labeled with plastic, metal, wood, tile, glass or cement.

(a) **(b)**

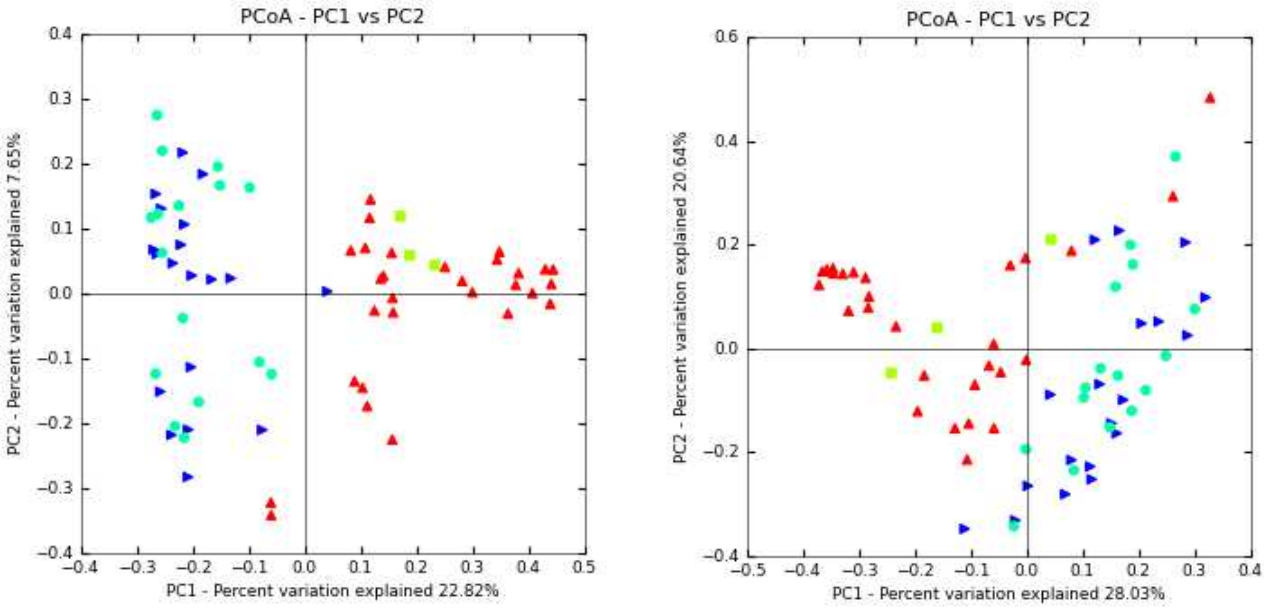


Figure 4.2 PCoA of the (a) un-weighted and (b) weighted UniFrac distances between sample exposures. Where ▲ are touched fomites, ■ are assumed to be from untouched fomites but community appears to be from touched fomites, ● are untouched fomites, and ▶ are assumed to be from touched fomites but community appears to be from untouched fomites.

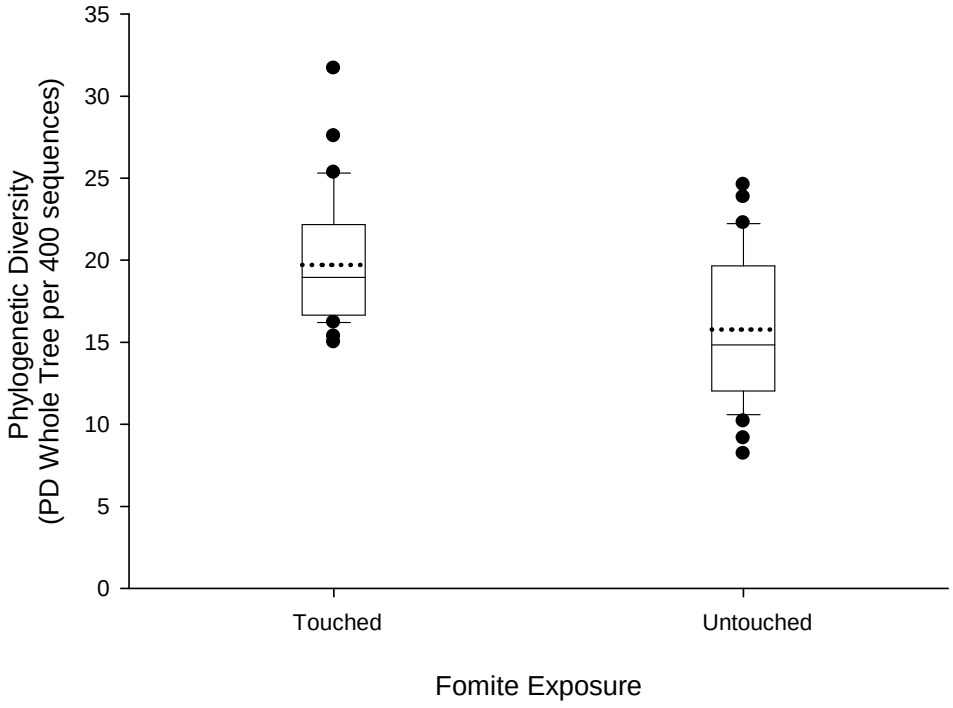


Figure 4.3 Phylogenetic diversity (PD whole tree per 400 sequences) of fomite exposures touched and untouched. The solid and dotted lines represent the median result and mean result, respectively. The solid circles represent the outlying samples. The box plot whiskers above and below the box indicate the 90^{th} and 10^{th} percentiles, respectively. Touched and untouched samples are significantly different (p=0.0005).

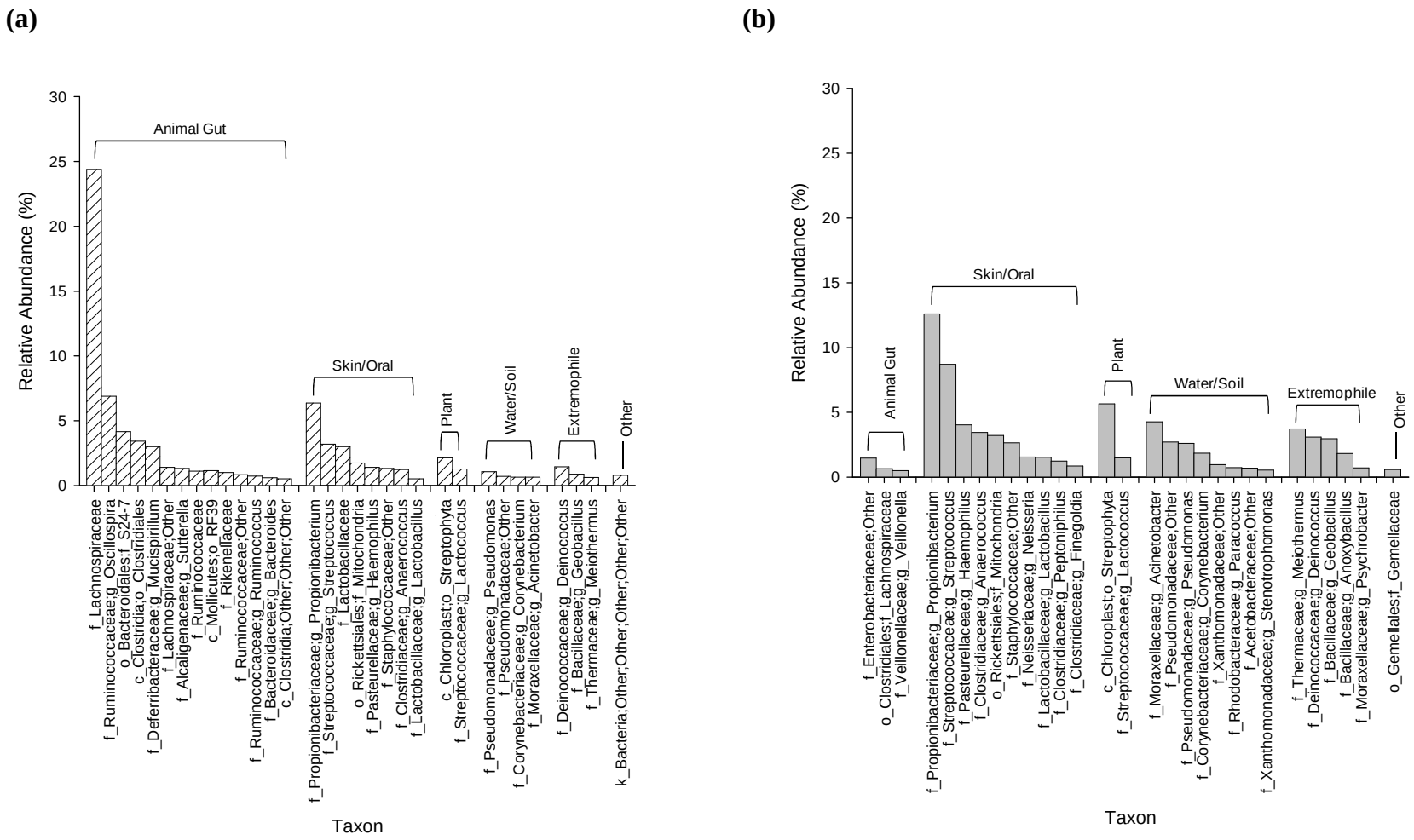


Figure 4.4 Characterization of organisms on (a) touched and (b) untouched fomites at the lowest classification available. Where the taxonomic rank is represented by c for class, o for order, f for family, and g for genus. The total relative abundance illustrated is 79.6% and 74.9% for touched and untouched fomites, respectively.

4.5 Discussion

Similar to the literature, the touched and untouched fomites had a common core set of phyla which included Proteobacteria, Actinobacteria and Firmicutes (9, 12, 17-19, 34, 35). The touched fomites had an additional dominate phyla of Bacteroidetes and Deferribacteres with an average relative abundances of 6.3% and 3.0%, respectively. Bacteroidetes was also observed as a dominate phylum in studies that analyzed touched fomites (Table 4.1). Additional dominate phyla for untouched fomites were Thermi (6.8%) and Cynobacteria (6.2%). By observing the bacterial community structure at the phylum level there seems to be a distinct difference between the two fomite exposures (Figure 4.1). Kembel *et al.* 2014 (23) discovered that human use and movement can influence the bacterial community structure. Each of the rooms in the dormitories that were sampled would have had a high occupancy for more than 14 hr per d. Most of the bacterial exposure of touched fomites comes from the direct interaction between humans and fomites while untouched fomite exposures are mainly influenced by air movement. Therefore, touched fomites would have a different community structure than untouched fomites.

The PCoA showed a distinct clustering of bacterial communities on touched and untouched fomites (Figure 4.2). While the majority of the samples were collected and identified as either touched or untouched, there were some samples that were assumed to have a certain exposure but the community analysis proved to be the opposite. For instance, a wood sofa hand rest-EA.CL.4, was assumed to be a touched fomite but the bacterial community appeared to be from an untouched fomite. All of the samples were collected at random and there were no observation in human behavior prior to sampling. Therefore, these samples could either be untouched fomites or it may be possible that these fomites were touched more frequently and the

microorganisms were transferred to human hands at a rate not equal to the microorganisms being deposited onto the fomites. Transfer efficiencies from non-porous fomites to fingers for *Escherichia coli*, *S. aureus*, *Bacillus thuringiensis*, MS2 coliphage, and poliovirus ranged from <0.04 to 57% at low relative humidity and 12.8 to 79.5% at high relative humidity (26). In a real-world scenario, the transfer efficiencies may be even higher than the reported ranges because unwashed hands have resulted in greater fomite to finger microbial transfer efficiency than washed hands (26). In addition, information regarding the fomite hygiene, such as how often the fomites were cleaned and which fomites were chosen, were not available. The use of disinfectant cleaning wipes can physically remove microorganism as well as chemically degrade the remaining microorganisms left on the fomite. The use of disinfectant cleaning wipes resulted in a range of 2.5 to 5 $\log_{10}$ reductions for *E. coli*, *S. aureus*, *B. thuringiensis* and poliovirus on nonporous fomites (27). Both limitations could explain why some fomites did not fit within the initial labeling.

The PCoA analysis between dormitory halls, rooms, fomite material and fomite type did not have any clear correlations on the bacterial communities (Figure A3.2-A3.5). Other studies have varied results on the correlation between different buildings, rooms, and fomites (Table 4.1). Many of the variations in the results could be due to the indoor environmental conditions. Natural environmental conditions such as seasonal variations and climate, relative humidity, temperature, and UV exposure are known to effect microbial community structure and survival (14, 16, 17, 21, 37). In addition, mechanical conditions can have an effect on bacterial communities. Frankel *et al.* 2012 (14) observed indoor bacterial concentration became more diluted with an increase in the air exchange rate. Similarly, Kembel *et al.* 2012 (22) found that

human pathogens were higher in rooms with lower flow rates and that mechanically ventilated rooms were less diverse than those ventilated with windows. Other important factors influencing bacterial communities were fomite hygiene, architectural design, building arrangements, and human use and movement (12, 23). Though all of these parameters effect the survival of bacterial communities, many of these parameters were rarely reported. Further investigation will be needed to better understand the interaction between the bacterial community structure and the influences of natural environmental conditions, mechanical conditions and human behavior.

The bacterial communities were more diverse on touched fomites than on untouched fomites (Figure 4.3). Frequently touched fomites are a reservoir for the transmission on pathogens, bacteria are picked up and/or deposit through hands direct contact with the fomites (25, 26). Higher bacterial diversity on touched fomites supports the idea that touched fomites have direct interactions with humans in comparison to untouched fomites. With the high occupancy of the university dormitories and the students' variable hygiene habits this could be a potential for disease outbreaks.

On touched fomites the majority of the community was of bacteria found in the human gut while the majority on untouched fomites was from bacteria found on skin and the oral cavity (Figure 4.4). The *Lachnospiraceae* family are a dominate group found in the gut community of humans and animals (29, 33, 36). *Lachnospiraceae*, especially concurrently with *Bacteroidales*, has been studied as an indicator for human fecal contamination in water (29, 32, 33). On touched fomites, the average relative abundance of *Lachnospiraceae* was 25.8% and *Bacteroidales* was 4.2% (Figure 4.4a). *Lachnospiraceae* was also present on untouched fomites but at a much lower

average relative abundance, 0.65%. To our knowledge, Deferribacteres *Mucispirillum* has only one species *M. schaedleri* studied in the literature (1, 24, 39, 40). *M. schaedleri* is found in the mammalian gastrointestinal tract (39). *Mucispirillum* was present on touched fomites at average relative abundance of 3%. *Propionibacterium* and *Streptococcus* were skin related genera and possible pathogens that were dominate on both touched and untouched fomites (Figure 4.4b) (18, 34). *Propionibacterium* had an average relative abundance of 6.4% and 12.6% for touched and untouched fomites, respectively. *Streptococcus* had an average relative abundance of 3.2% and 8.7% for touched and untouched fomites, respectively. All of these possible pathogen genera were found in every dormitory and room (common lounge, computer room and cafeteria). The limitation of the data collected on touched untouched fomites is the ability for quantification of the pathogens present at the species classification level to be able to determine the exposure concentration and potential risks. However, this data is able to identify possible exposure pathways and hazards. It was evident that for the indoor environment, exposure to potential pathogens occurs on touched fomites more commonly.

4.6 Conclusions

By characterizing the bacterial communities on touched an untouched fomites in dormitory halls helped to further understand the interactions between humans, bacteria and fomites. Humans clearly impacted touched fomites more than untouched fomites, which was evident by the higher diversity on touched fomites. Touch fomites also indicated an abundance of fecal indicators and genera associated with the human microbiome which appeared in every dormitory and room. Additional studies analyzing the various parameters (e.g., relative humidity, temperature, air flow rate, etc.) that effect microbial community structure would improve the understanding of

the indoor environment. The defined bacterial communities on touched and untouched fomites presented will give insight on probable exposure routes for effective fomite hygiene interventions to improve risks to human health.

APPENDIX

Table A3.1 Summary of all samples sequenced.

	Housing	Students	Number of positive samples (JR samples)
Dormitory			
Alice Lloyd (AL)	Co-ed	500	7 (2)
Couzen (CO)	Co-ed	525	8 (7)
Stockwell (ST)	Female	400	15 (2)
Betsy Barbour & Helen Newberry (BN)	Female	230	9 (2)
Bursley (BU)	Co-ed	1270	14 (3)
East Quadrangle (EA)	Co-ed	860	16 (7)
Room			
Common lounge (CL)			30 (6)
Computer lab (CP)			21 (7)
Cafeteria (CF)			18 (11)
Exposure			
Touched			32 (9)
Untouched			37 (15)
Fomite Material			
Plastic			30 (10)
Metal			17 (7)
Wood			15 (5)
Tile			2 (1)
Glass			2 (0)
Cement			1 (0)

Table A3.2 Sample description for those sequenced on the 454 GS FLX System.

Sample ID	Fomite	Fomite material	Exposure	Dormitory	Room	Final conc. (ng/µl)	Primer no.
EA-CL-4	Sofa hand rest	Wood	U2[a]	East Quad[e]	Common lounge	25.80	65
EA-CL-5	Chair hand rest	Wood	U2	East Quad	Common lounge	4.14	66
EA-CL-8	Cabinet top	Glass	U[b]	East Quad	Common lounge	1.87	67
EA-CL-10	Bookshelf	Wood	T[c]	East Quad	Common lounge	2.48	68
EA-CF-2	Chair	Wood	T	East Quad	Cafeteria	1.87	70
BU-CL-4	Study table	Tile	T	Bursley	Common lounge	3.11	71
EA-CP-4	Computer Mouse	Plastic	T	East Quad	Computer lab	9.30	72
ST-CL-9	Top fire alarm	Plastic	T2[d]	Stockwell	Common lounge	2.14	73
CO-CF-1	Microwave button	Plastic	T	Couzen	Cafeteria	4.17	74
BN-CL-2	Faucet	Metal	T	Betsy[f]	Common lounge	25.50	75
BN-CL-5	Lock	Metal	T	Betsy	Common lounge	22.60	77
AL-CF-1	Microwave	Plastic	U2	Alice Lloyd	Cafeteria	7.17	79
BU-CP-3	Doorknob	Metal	U2	Bursley	Computer lab	33.50	80
EA-CP-7	Computer stand	Plastic	U	East Quad	Computer lab	7.00	81
BN-CL-6	TV control	Plastic	T	Betsy	Common lounge	2.38	82
CO-CL-9	Bin top	Metal	U	Couzen	Common lounge	4.40	83
ST-CF-2	Salad bar utensil	Metal	U2	Stockwell	Cafeteria	6.10	84
BU-CL-6	Doorknob	Metal	U2	Bursley	Common lounge	8.10	85

a. U2-are samples that were assumed to be from touched fomites but the community appears to be from untouched fomites.
b. U-untouched fomites
c. T-touched fomites
d. T2-are samples that were assumed to be from untouched fomites but the community appears to be from touched fomites.
e. East Quad-East Quadrangle
f. Betsy-Betsy Barbour/Helen Newberry
g. N/R-not recorded

Table A3.2 (cont'd).

Sample ID	Fomite	Fomite material	Exposure	Dormitory	Room	Final conc. (ng/µl)	Primer no.
BU-CL-10	M card reader	Plastic	T2	Bursley	Common lounge	30.10	86
BU-CP-7	Top of computer	Plastic	T	Bursley	Computer lab	7.90	87
ST-CL-4	Windowsill	Wood	T	Stockwell	Common lounge	3.09	88
EA-CL-2	Study table	Wood	T	East Quad	Common lounge	11.00	89
EA-CP-2	Keyboard-space key	Plastic	T	East Quad	Computer lab	16.80	90
ST-CP-9	Top of telephone	Plastic	T	Stockwell	Computer lab	1.52	92
EA-CP-8	Computer hard drive	Plastic	T	East Quad	Computer lab	4.50	93
EA-CF-8	Tissue box	Plastic	U	East Quad	Cafeteria	29.00	95
AL-CL-1	Vending machine Button	Plastic	T	Alice Lloyd	Common lounge	7.70	96
AL-CL-5	Microwave buttons	Plastic	U2	Alice Lloyd	Common lounge	18.50	97
AL-CF-5	Ice cream dispenser	Metal	U2	Alice Lloyd	Cafeteria	19.60	99
AL-CL-4	Study table	Wood	U2	Alice Lloyd	Common lounge	3.95	100
AL-CF-8	Salad bar sneeze guard	Glass	T	Alice Lloyd	Cafeteria	3.18	101
BN-CL-4	Doorknob	Metal	U2	Betsy	Common lounge	12.30	102
BU-CP-9	Switch knob top	Plastic	U	Bursley	Computer lab	1.64	103
CO-CL-5	Water fountain button	Metal	U2	Couzen	Common lounge	12.90	104
BU-CF-4	Dining table	Plastic	U2	Bursley	Cafeteria	18.80	105
CO-CP-3	Study table	Plastic	T	Couzen	Computer lab	10.90	65
CO-CP-8	Top of scanner	Metal	T	Couzen	Computer lab	1.10	66
CO-CF-9	Top of fire alarm	Plastic	U	Couzen	Cafeteria	6.82	67

Table A3.2 (cont'd).

Sample ID	Fomite	Fomite material	Exposure	Dormitory	Room	Final conc. (ng/µl)	Primer no.
ST-CL-8	Top of waste bin	Metal	U	Stockwell	Common lounge	9.82	68
ST-CF-1	Dining table	Wood	T	Stockwell	Cafeteria	2.52	70
EA-CL-9	Windowsill	Cement	U	East Quad	Common lounge	10.61	71
ST-CL-6	Study table	Wood	T	Stockwell	Common lounge	3.45	72
EA-CF-10	Food cover	Plastic	U	East Quad	Cafeteria	9.04	73
ST-CF-6	Top of ice container	Metal	U	Stockwell	Cafeteria	2.81	74
ST-CL-7	Top of fire place	Wood	U	Stockwell	Common lounge	2.09	75
ST-CP-10	Top of air conditioner	Plastic	U	Stockwell	Computer lab	2.47	77
BU-CF-10	Cover for ice container	Plastic	U	Bursley	Cafeteria	11.40	79
BU-CL-3	ATM machine button	Plastic	U2	Bursley	Common lounge	1.93	80
CO-CL-1	TV remote control	Plastic	T	Couzen	Common lounge	6.20	81
BN-CP-8	Computer table (backside)	Wood	U	Betsy	Computer lab	4.84	83
BN-CP-9	Wall close to door	Wood	T2	Betsy	Computer lab	1.77	84
BN-CF-3	Milk dispenser	Metal	U2	Betsy	Cafeteria	3.47	85
ST-CL-1	Water fountain	Plastic	U2	Stockwell	Common lounge	5.45	86
ST-CP-3	Study table	Wood	T	Stockwell	Computer lab	3.60	89
EA-CL-3	Front desk	Wood	T	East Quad	Common lounge	13.70	90
ST-CP-6	Keyboard-enter and space bar	Plastic	T	Stockwell	Computer lab	7.91	91

Table A3.2 (cont'd).

Sample ID	Fomite	Fomite material	Exposure	Dormitory	Room	Final conc. (ng/µl)	Primer no.
EA-CP-10	Doorknob	Metal	T	East Quad	Computer lab	5.80	92
ST-CF-3	Salad bar deck	Metal	T	Stockwell	Cafeteria	1.52	93
ST-CF-5	N/R[g]	Plastic	T	Stockwell	Cafeteria	5.76	94
BU-CL-1	Public phone	Plastic	T	East Quad	Computer lab	6.67	95
EA-CF-4	Handle of rice cooker	Plastic	U2	East Quad	Cafeteria	20.80	96
CO-CL-2	Study table	Wood	U2	Couzen	Common lounge	1.36	97
BU-CL-9	Top of heater board	Metal	U	Bursley	Common lounge	3.05	99
BU-CL-7	Top of shelf	Tile	U	Bursley	Common lounge	10.70	100
BU-CP-5	Stapler	Metal & plastic	U2	Bursley	Computer lab	8.66	101
BN-CP-5	Study table	Wood	U2	Betsy	Computer lab	3.70	102
BN-CP-7	Top of printer	Plastic	T	Betsy	Computer lab	5.20	103
BU-CF-3	Salad bar spoon	Plastic	U2	Bursley	Cafeteria	2.60	104
AL-CP-7	Top of printer	Plastic	U2	Alice Lloyd	Computer lab	2.40	105

Table A3.3 Sample description for those sequenced on the 454 GS Junior System.

Sample ID	Fomite	Fomite material	Exposure	Dormitory	Room	Final conc. (ng/µl)	Primer No.
BN-CL-8	Water fountain handle	Plastic	U2	Betsy	Common lounge	1.31	65
BU-CF-7	Doorknob	Metal	T	Bursley	Cafeteria	4.77	67
AL-CL-2	Soda fountain button	Plastic	U2	Alice Lloyd	Common lounge	0.97	70
AL-CP-3	Counter workshop	Plastic	U2	Alice Lloyd	Computer lab	1.80	71
CO-CP-9	Top of shelf	Wood	U	Couzen	Computer lab	3.60	73
CO-CF-2	Dining table	Wood	U2	Couzen	Cafeteria	4.30	75
BN-CF-5	Salad dressing utensil	Plastic	U2	Betsy	Cafeteria	3.40	77
CO-CF-8	Windowsill	Wood	U	Couzen	Cafeteria	7.60	79
CO-CF-10	Floor	Tile	U	Couzen	Cafeteria	7.30	81
ST-CF-7	Top of microwave	Metal	U	Stockwell	Cafeteria	4.15	86
EA-CL-6	TV top	Plastic	T2	East Quad	Common lounge	9.15	91
EA-CP-5	Study table	Plastic	T	East Quad	Computer lab	27.50	92
EA-CF-9	Fire alarm	Plastic	T2	East Quad	Cafeteria	1.55	94
CO-CF-5	Doorknob	Metal	U2	Couzen	Cafeteria	8.71	95
EA-CF-5	Dining table	Wood	T	East Quad	Cafeteria	9.40	96
BN-CP-6	Computer screen	Metal	U2	Betsy	Computer lab	3.60	102
CO-CF-1*	Microwave	Plastic	U2	Couzen	Cafeteria	4.17	74
CO-CL-9*	Bin top	Metal	U	Couzen	Common lounge	4.40	83
ST-CF-2*	Salad bar utensil	Metal	T	Stockwell	Cafeteria	6.10	84
BU-CL-6*	Doorknob	Metal	U2	Bursley	Common lounge	8.10	85
BU-CP-7*	Top of computer	Plastic	U2	Bursley	Computer lab	7.90	87
EA-CL-2*	Study table	Wood	T	East Quad	Common lounge	11.00	89
EA-CP-2*	Keyboard-space key	Plastic	T	East Quad	Computer lab	16.80	90
EA-CP-8*	Computer hard drive	Plastic	T	East Quad	Computer lab	4.50	93

*Samples that were also run on the 454 GS FLX System

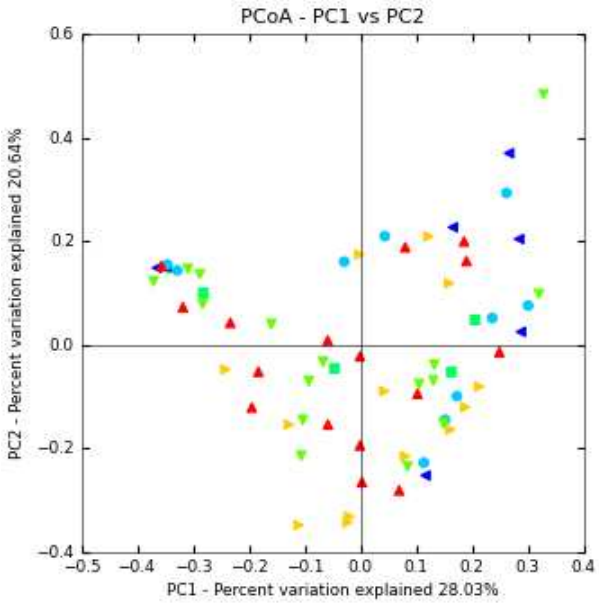


Figure A3.1 PCoA of the weighted UniFrac distances between sample date and time. Where the dates and sample time are represented by ▼ for 2/21/07 at 1:05 to 2:45P, ◀ for 3/8/07 at 1:45 to 3:10P, ● for 3/7/07 at 12:30 to 2:15P, ▲ for 2/19/07 at 1:30 to 4:00P, ▶ for 2/20/07 at 3:50 to 5:18P, and ■ for 3/6/07 at 1:20 to 3:05P.

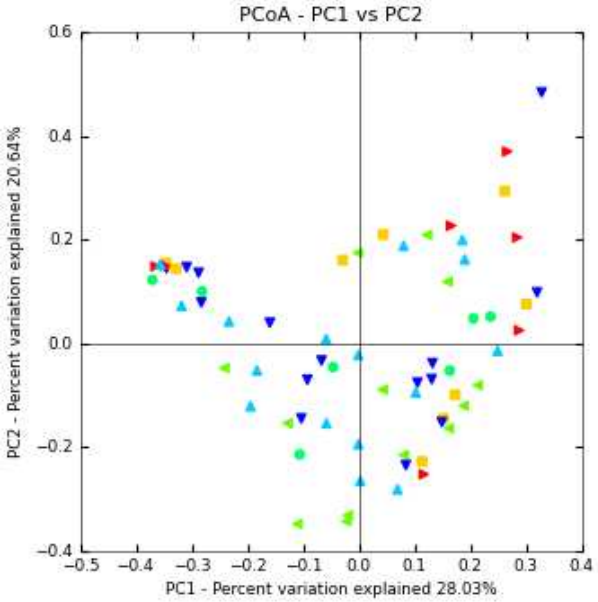


Figure A3.2 PCoA of the weighted UniFrac distances between sample locations. Where the dormitories are represented by ▼ for Stockwell, ► for Alice Lloyd, ■ for Betsy Barbour and Helen Newberry, ▲ for East Quadrangle, ◄ for Bursley, and ● for Couzen.

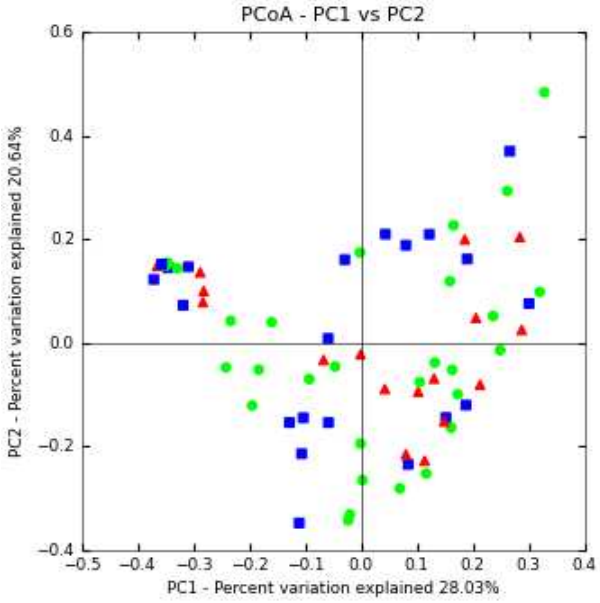


Figure A3.3 PCoA of the weighted UniFrac distances between dormitory rooms. Where ● are the common lounges, ■ are the computer labs, and ▲ are the cafeterias where samples are collected.

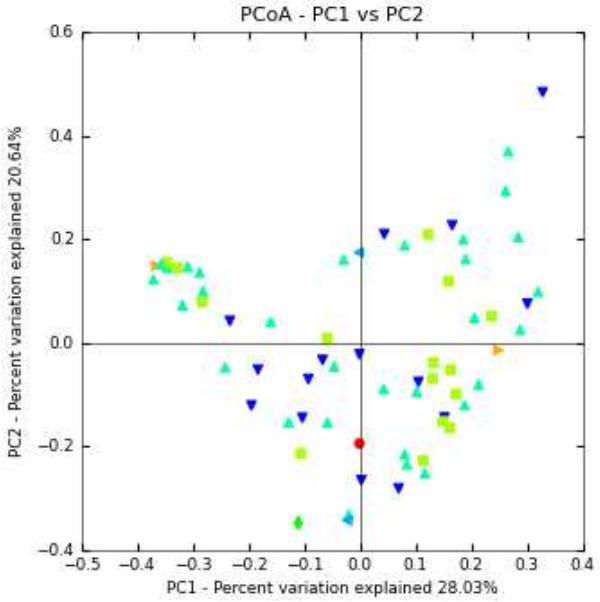


Figure A3.4 PCoA of the weighted UniFrac distances between fomite materials. Where ▼ are wood, ▲ are plastic, ■ are metal, ► are glass, ● are cement, and ◄ are tile.

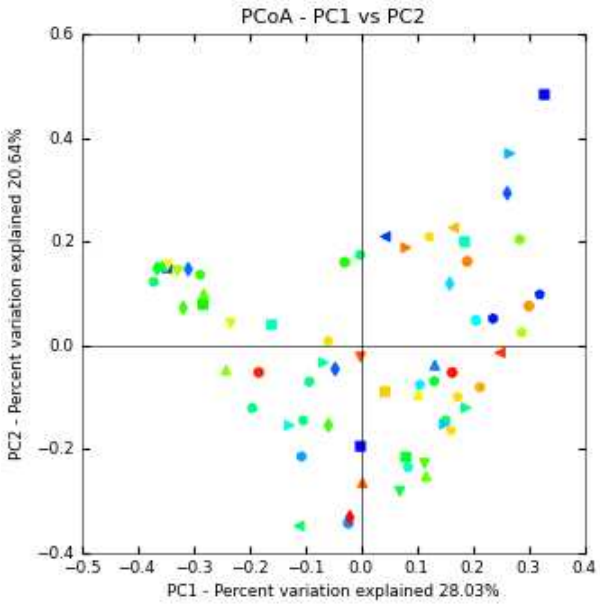
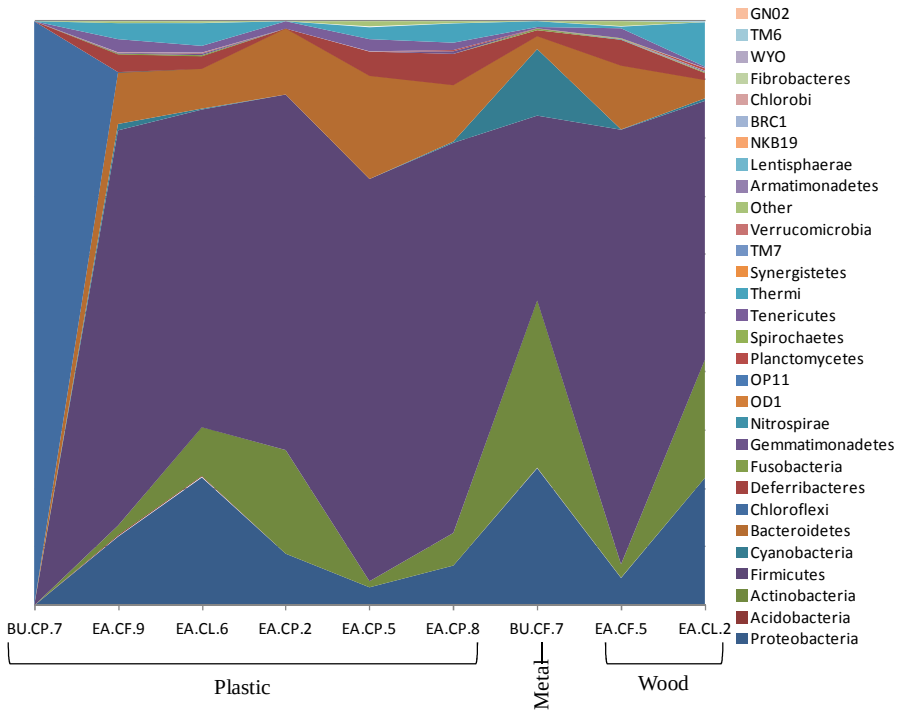


Figure A3.5 PCoA of the weighted UniFrac distances between fomite types.

(a)



(b)

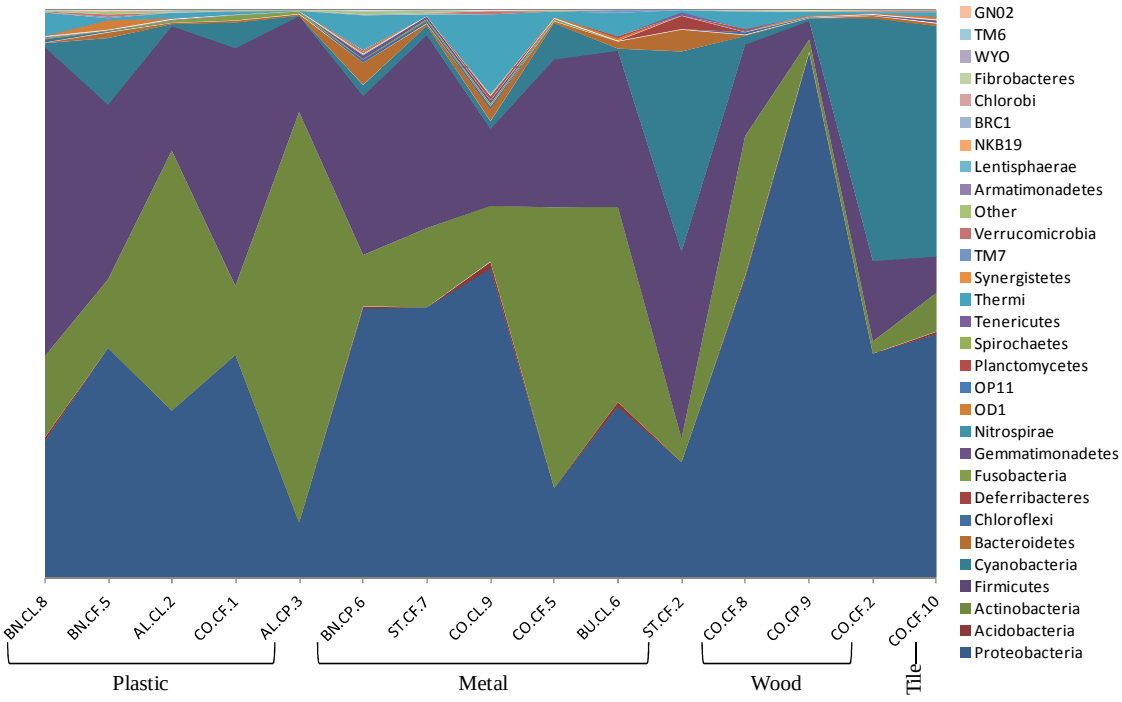
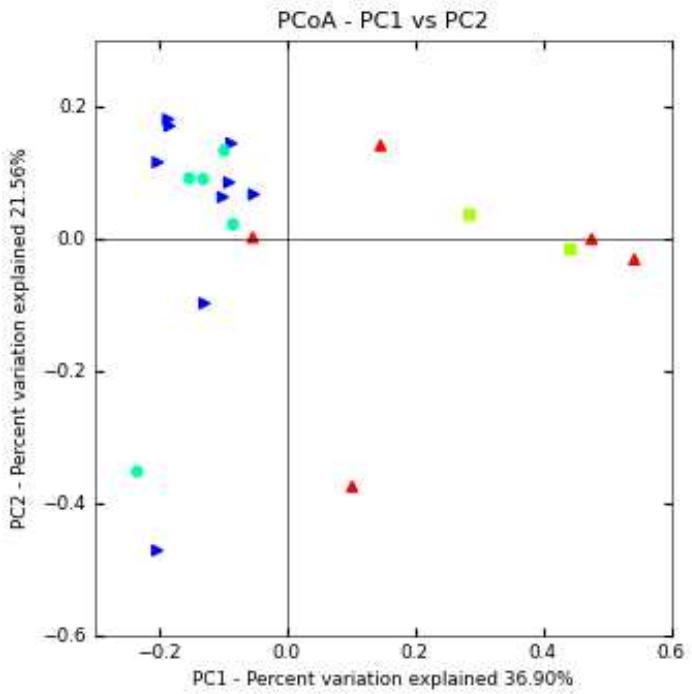


Figure A3.6 Relative abundance of bacterial communities for each sample on touched fomites (A) and untouched fomites (B) at the phylum classification level (sample sequenced on the 454 GS Junior System). The fomite material for each sample is labeled with plastic, metal, wood, or tile.

(a)



(b)

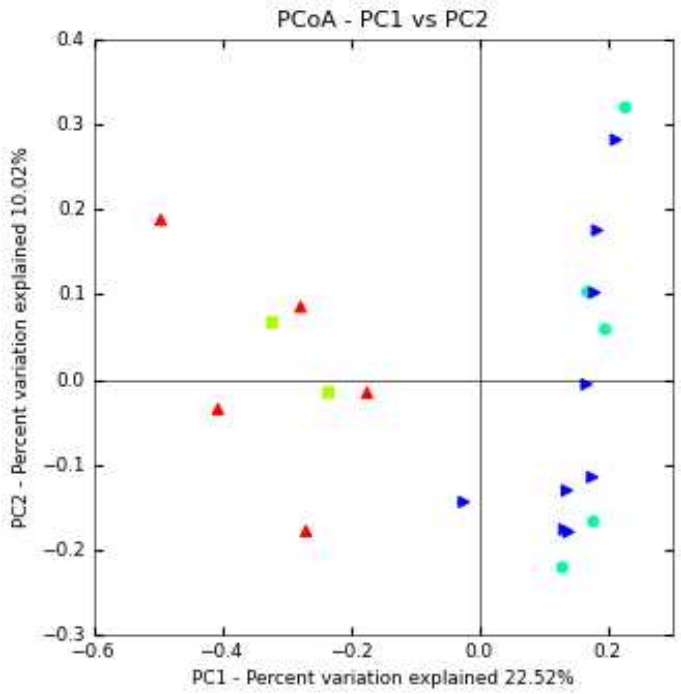


Figure A3.7 PCoA of the (a) weighted and (b) unweighted UniFrac distances between sample exposures (sample sequenced on the 454 GS Junior System). Where ▲ are touched fomites, ■ are assumed to be untouched fomites but community appears to be touched fomites, ● are untouched fomites, and ▶ are touched fomites.

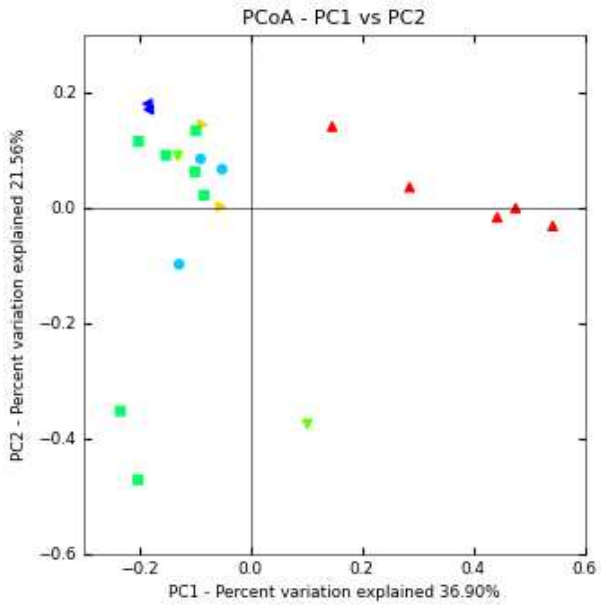


Figure A3.8 PCoA of the weighted UniFrac distances between sample date and time (sample sequenced on the 454 GS Junior System). Where the dates and sample time are represented by ▼ for 2/21/07 at 1:05 to 2:45P, ◀ for 3/8/07 at 1:45 to 3:10P, ● for 3/7/07 at 12:30 to 2:15P, ▲ for 2/19/07 at 1:30 to 4:00P, ▶ for 2/20/07 at 3:50 to 5:18P, and ■ for 3/6/07 at 1:20 to 3:05P.

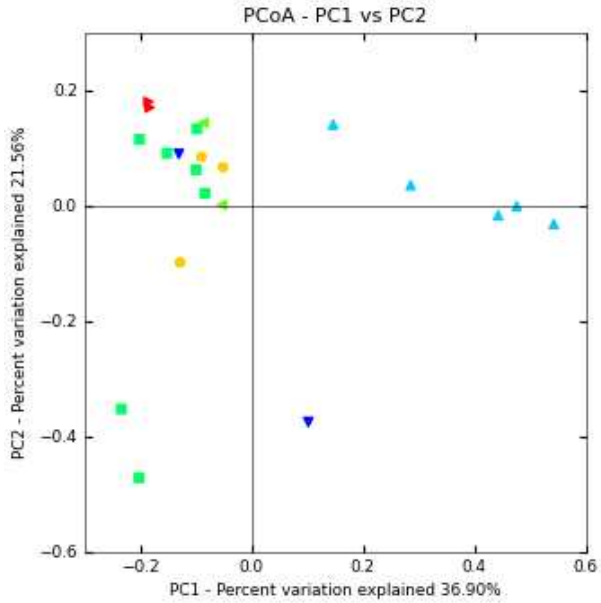


Figure A3.9 PCoA of the weighted UniFrac distances between sample locations (sample sequenced on the 454 GS Junior System). Where the dormitories are represented by ▼ for Stockwell, ► for Alice Lloyd, ● for Betsy Barbour and Helen Newberry, ▲ for East Quadrangle, ◄ for Bursley, and ■ for Couzen.

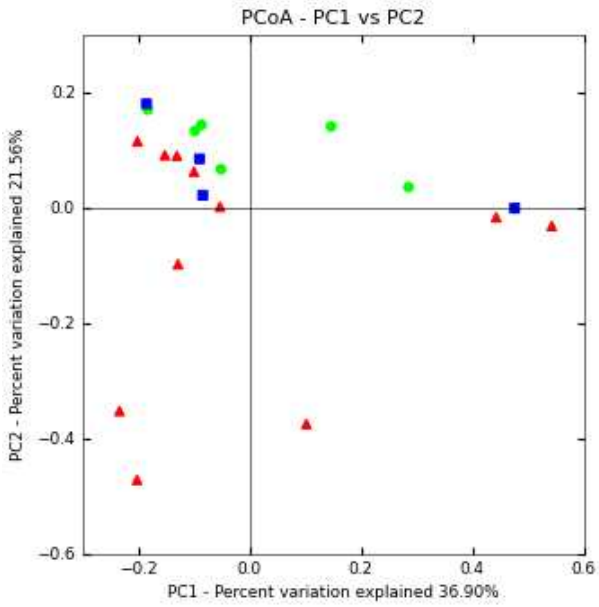


Figure A3.10 PCoA of the weighted UniFrac distances between dormitory rooms (sample sequenced on the 454 GS Junior System). Where ● are the common lounges, ■ are the computer labs, and ▲ are the cafeterias where samples are collected.

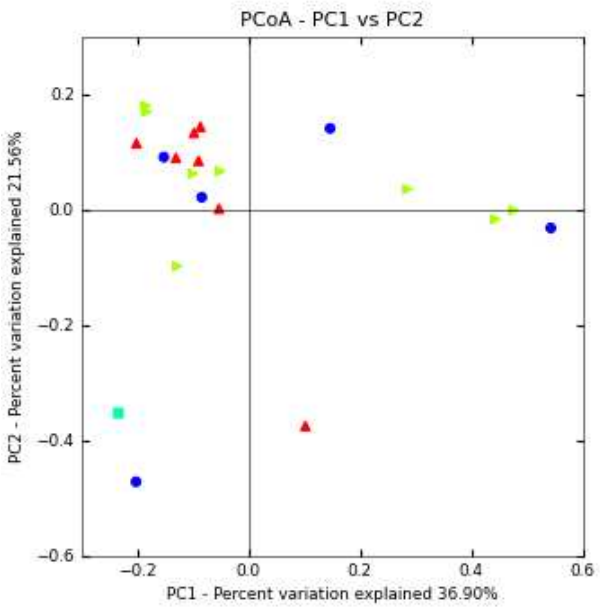


Figure A3.11 PCoA of the weighted UniFrac distances between fomite materials (sample sequenced on the 454 GS Junior System). Where ● are wood, ▶ are plastic, ▲ are metal, and ■ are tile.

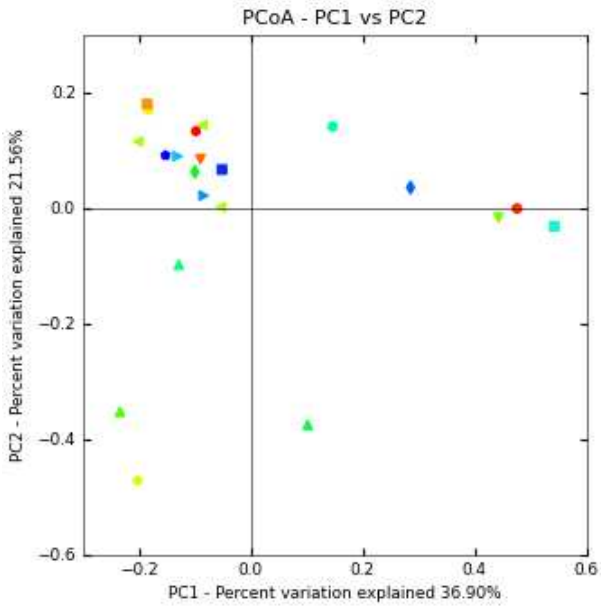


Figure A3.12 PCoA of the weighted UniFrac distances between fomite types (sample sequenced on the 454 GS Junior System).

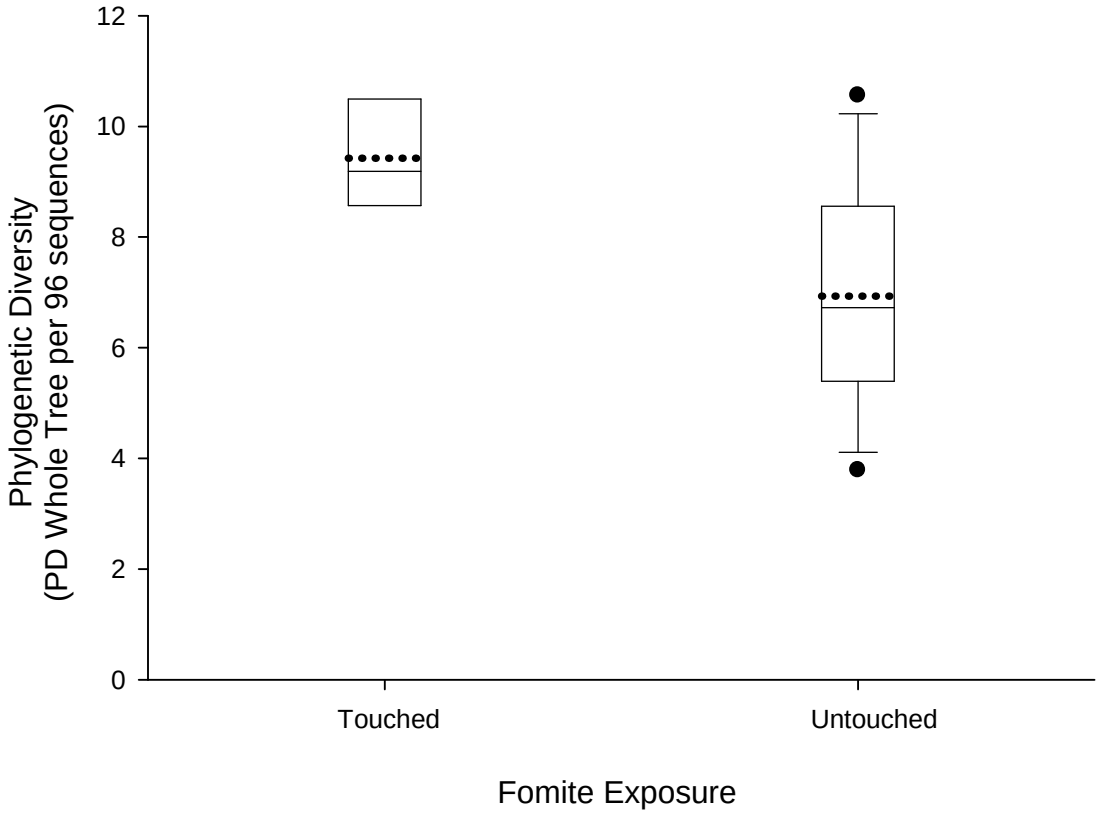


Figure A3.13 Phylogenetic diversity (PD whole tree per 96 sequences) of fomite exposures touched and untouched (sample sequenced on the 454 GS Junior System). The solid and dotted lines represent the median and mean, respectively. The solid circles represent the outlying samples. The box plot whiskers above and below the box indicate the 90^{th} and 10^{th} percentiles, respectively. Touched and untouched samples are significantly different (p=0.005), using the student t-test.

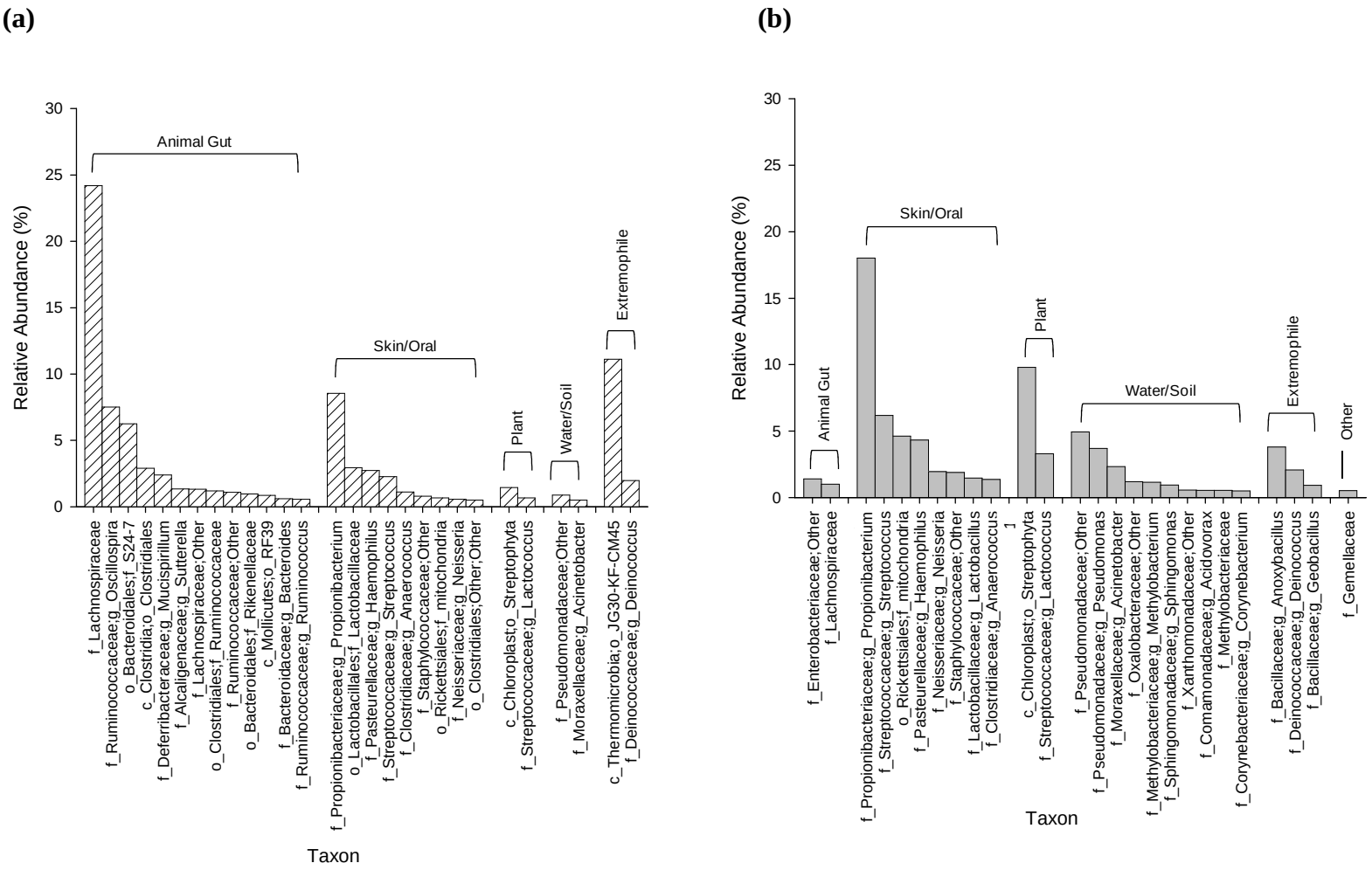


Figure A3.14 Characterization of organisms on (a) touched and (b) untouched fomites at the lowest classification available (sample sequenced on the 454 GS Junior System). Where the taxonomic rank is represented by c for class, o for order, f for family, and g for genus. The total relative abundance illustrated is 87.8% and 78.9% for touched and untouched fomites, respectively.

REFERENCES

REFERENCES

1. **Berry, D., C. Schwab, G. Milinovich, J. Reichert, K. Ben Mahfoudh, T. Decker, M. Engel, B. Hai, E. Hainzl, S. Heider, L. Kenner, M. Muller, I. Rauch, B. Strobl, M. Wagner, C. Schleper, T. Urich, and A. Loy.** 2012. Phylotype-level 16S rRNA analysis reveals new bacterial indicators of health state in acute murine colitis. ISME J. **6:**2091-2106.

2. **Boone, S. A., and C. P. Gerba.** 2007. Significance of fomites in the spread of respiratory and enteric viral disease. Appl. Environ. Microbiol. **73:**1687-1696.

3. **Brooke, J. S., J. W. Annand, A. Hammer, K. Dembkowski, and S. T. Shulman.** 2009. Investigation of bacterial pathogens on 70 frequently used environmental surfaces in a large urban US university. J. Environ. Health **71:**17-22.

4. **Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight.** 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics **26:**266-267.

5. **Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Tumbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight.** 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods **7:**335-336.

6. **Census Bureau, United States.** 2009. Characteristics of the group quarters population, p. S2601A, American Community Survey

7. **Corsi, R. L., Kinney, K.A., Levin, H.** 2012. Microbiomes of built environments: 2011 symposium highlights and workgroup recommendations. Indoor Air **22:**171-172.

8. **Decker, J. F., and R. M. Slawson.** 2012. An evaluation of behavioral health compliance and microbial risk factors on student populations within a high-density campus. J. Am. College Health **60:**584-595.

9. **Dunn, R. R., N. Fierer, J. B. Henley, J. W. Leff, and H. L. Menninger.** 2013. Home life: factors structuring the bacterial diversity found within and between homes. PLoS One **8**.

10. **Edgar, R. C.** 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics **26:**2460-2461.

11. **Faith, D. P., and A. M. Baker.** 2006. Phylogenetic diversity (PD) and biodiversity conservation: some bioinformatics challenges. Evol. Bioinform. **2:**121-128.

12. **Flores, G. E., S. T. Bates, J. G. Caporaso, C. L. Lauber, J. W. Leff, R. Knight, and N. Fierer.** 2013. Diversity, distribution and sources of bacteria in residential kitchens. Environ. Microbiol. **15:**588-596.

13. **Flores, G. E., S. T. Bates, D. Knights, C. L. Lauber, J. Stombaugh, R. Knight, and N. Fierer.** 2011. Microbial biogeography of public restroom surfaces. PLoS One **6**.

14. **Frankel, M., G. Beko, M. Timm, S. Gustavsen, E. W. Hansen, and A. M. Madsen.** 2012. Seasonal variations of indoor microbial exposures and their relation to temperature, relative humidity, and air exchange rate. Appl. Environ. Microbiol. **78:**8289-8297.

15. **Herzog, A. B., S. D. McLennan, A. K. Pandey, C. P. Gerba, C. N. Haas, J. B. Rose, and S. A. Hashsham.** 2009. Implications of limits of detection of various methods for *Bacillus anthracis* in computing risks to human health. Appl. Environ. Microbiol. **75:**6331-6339.

16. **Herzog, A. B., A. K. Pandey, D. Reyes-Gastelum, C. P. Gerba, J. B. Rose, and S. A. Hashsham.** 2012. Evaluation of sample recovery efficiency for Bacteriophage P22 on fomites. Appl. Environ. Microbiol. **78:**7915-7922.

17. **Hewitt, K. M., C. P. Gerba, S. L. Maxwell, and S. T. Kelley.** 2012. Office space bacterial abundance and diversity in three metropolitan areas. PLoS One **7:**1-7.

18. **Hewitt, K. M., F. L. Mannino, A. Gonzalez, J. H. Chase, J. G. Caporaso, R. Knight, and S. T. Kelley.** 2013. Bacterial diversity in two neonatal intensive care units (NICUs). PLoS One **8:**7.

19. **Jeon, Y. S., J. Chun, and B. S. Kim.** 2013. Identification of household bacterial community and analysis of species shared with human microbiome. Curr. Microbiol. **67:**557-563.

20. **Kak, V.** 2007. Infections in confined spaces: cruise ships, military barracks, and college dormitories. Infect. Dis. Clin. N. Am. **21:**773-784.

21. **Kelley, S. T., and J. A. Gilbert.** 2013. Studying the microbiology of the indoor environment. Genome Biol. **14**.

22. **Kembel, S. W., E. Jones, J. Kline, D. Northcutt, J. Stenson, A. M. Womack, B. J. M. Bohannan, G. Z. Brown, and J. L. Green.** 2012. Architectural design influences the diversity and structure of the built environment microbiome. ISME J **6:**1469-1479.

23. **Kembel, S. W., J. F. Meadow, T. K. O'Connor, G. Mhuireach, D. Northcutt, J. Kline, M. Moriyama, G. Z. Brown, B. J. M. Bohannan, and J. L. Green.** 2014. Architectural design drives the biogeography of indoor bacterial communities. PLoS One **9:**10.

24. **Li, R. W., S. T. Wu, W. Z. Li, K. Navarro, R. D. Couch, D. Hiil, and J. F. Urban.** 2012. Alterations in the porcine colon microbiota induced by the gastrointestinal nematode trichuris suis. Infect. Immun. **80:**2150-2157.

25. **Li, S., J. N. S. Eisenberg, I. H. Spicknall, and J. S. Koopman.** 2009. Dynamics and control of infections transmitted from person to person through the environment. Am. J. Epidemiol. **170:**257-265.

26. **Lopez, G. U., C. P. Gerba, A. H. Tamimi, M. Kitajima, S. L. Maxwell, and J. B. Rose.** 2013. Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. Appl. Environ. Microbiol. **79:**5728-5734.

27. **Lopez, G. U., M. Kitajima, A. Havas, C. P. Gerba, and K. A. Reynolds.** 2014. Evaluation of a disinfectant wipe intervention on fomite-to-finger microbial transfer. Appl. Environ. Microbiol. **80:**3113-3118.

28. **Lozupone, C., M. E. Lladser, D. Knights, J. Stombaugh, and R. Knight.** 2011. UniFrac: an effective distance metric for microbial community comparison. ISME J. **5:**169-172.

29. **McLellan, S. L., R. J. Newton, J. L. Vandewalle, O. C. Shanks, S. M. Huse, A. M. Eren, and M. L. Sogin.** 2013. Sewage reflects the distribution of human faecal Lachnospiraceae. Environ. Microbiol. **15:**2213-2227.

30. **Miko, B. A., B. Cohen, L. Conway, A. Gilman, S. L. Seward, and E. Larson.** 2012. Determinants of personal and household hygiene among college students in New York City, 2011. Am. J. Infect. Control **40:**940-945.

31. **Miko, B. A., B. Cohenz, K. Haxa, L. Conway, N. Kelly, D. Stare, C. Tropiano, A. Gilmans, S. L. Seward, and E. Larson.** 2013. Personal and household hygiene, environmental contamination, and health in undergraduate residence halls in New York City, 2011. PLoS One **8**.

32. **Newton, R. J., M. J. Bootsma, H. G. Morrison, M. L. Sogin, and S. L. McLellan.** 2013. A microbial signature approach to identify fecal pollution in the waters off an urbanized coast of Lake Michigan. Microbial Ecol. **65:**1011-1023.

33. **Newton, R. J., J. L. VandeWalle, M. A. Borchardt, M. H. Gorelick, and S. L. McLellan.** 2011. Lachnospiraceae and Bacteroidales aternative fecal indicators reveal chronic human sewage contamination in an urban harbor. Appl. Environ. Microbiol. **77:**6972-6981.

34. **Oberauner, L., C. Zachow, S. Lackner, C. Hogenauer, K. H. Smolle, and G. Berg.** 2013. The ignored diversity: complex bacterial communities in intensive care units revealed by 16S pyrosequencing. Sci. Reports **3**.

35. **Poza, M., C. Gayoso, M. J. Gomez, S. Rumbo-Feal, M. Tomas, J. Aranda, A. Fernandez, and G. Bou.** 2012. Exploring bacterial diversity in hospital environments by GS-FLX titanium pyrosequencing. PLoS One **7**.

36. **Reeves, A. E., M. J. Koenigsknecht, I. L. Bergin, and V. B. Young.** 2012. Suppression of *Clostridium difficile* in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family Lachnospiraceae. Infect. Immun. **80:**3786-3794.

37. **Rintala, H., M. Pitkaeranta, M. Toivola, L. Paulin, and A. Nevalainen.** 2008. Diversity and seasonal dynamics of bacterial community in indoor environment. BMC Microbiol. **8**.

38. **Roberts, M. C., O. O. Soge, D. No, S. E. Helgeson, and J. S. Meschke.** 2011. Characterization of *Methicillin-resistant Staphylococcus aureus* isolated from public surfaces on a university campus, student homes and local community. J. Appl. Microbiol. **110:**1531-1537.

39. **Robertson, B. R., J. L. O'Rourke, B. A. Neilan, P. Vandamme, S. L. W. On, J. G. Fox, and A. Lee.** 2005. *Mucispirillum schaedleri* gen. nov., sp nov., a spiral-shaped bacterium colonizing the mucus layer of the gastrointestinal tract of laboratory rodents. Intern J. Syst. Evol. Microbiol. **55:**1199-1204.

40. **Scupham, A. J., T. G. Patton, E. Bent, and D. O. Bayles.** 2008. Comparison of the cecal microbiota of domestic and wild turkeys. Microb. Ecol. **56:**322-331.

41. **Sinclair, R., S. A. Boone, D. Greenberg, P. Keim, and C. P. Gerba.** 2008. Persistence of category A select agents in the environment. Appl. Environ. Microbiol. **74:**555-563.

42. **Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole.** 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. **73:**5261-5267.

43. **Williams, J., De Vos B., Russell D.** 2013. 2010 Census group quarters enumeration assessment report. Decennial Statistical Studies Divsion

Chapter 5: Conclusions

The QMRA framework is a widely accepted formal process for estimating human health risks. QMRA has been a useful approach to a variety of scenarios in, but not limited to, public health, emergency response, environmental control measures, and decontamination efficacy. QMRA addresses probabilities of disease using mathematical models and integrated data sets that characterize microbial pathogen exposures through the environment. This framework has proven to be more sensitive than conventional epidemiological approaches for estimating human health risks (5, 2).

One of the critical steps to a QMRA is the exposure assessment. The exposure assessment identifies and determines the exposed population, the exposure pathway, environmental fate and transport, concentration, frequency, length of time of exposure and estimates the dose (or distribution) for an exposure (5, 2). The exposure assessment presents the most variability and uncertainty in the risk assessment due to the vast and dynamic nature of this data. Variability in the measured exposure data can be caused by differences in location, activity, human behavior, environmental conditions and the environmental matrix. This variation in the data will result in differences in exposure to a microbial pathogen (5, 2). Complete data sets and quantitative information for the exposure assessment are often not available, either lacking in information or non-existent. By simplifying assumptions to compensate for the lack of data may result in uncertainty about the exposure estimates. Availability and quality of data and information can reduce the amount of uncertainty. For better risk management decisions the variability and uncertainty in the exposure assessment should be characterized or limited (5, 2).

The overall objective of the presented work was to address knowledge gaps and concerns regarding microbial pathogens (bioterrorism and infectious disease) associated with public health risks at concentrations at the limit of detection in the indoor environment. The results collected in these three chapters can be applied for the enhancement of the exposure assessments for the indoor environment by addressing the data variability and uncertainty. The objectives of this work were addressed by reviewing the instrument and environmental limits of detection, calculating the risk estimates at the instrument and environmental limits of detection, evaluating the SRE variability to environmental factors at the limit of detection, and characterizing the bacterial communities on touched and untouched fomites.

In the exposure assessment, the limit of detection of any method plays a critical role in determining the method capabilities and assists in defining the exposure concentration. The environmental limit of detection is important because it takes into consideration the many steps to processing samples in an environmental matrix prior to detection. Whereas, the instrument limit of detection is evaluated with pure cultures and represents ideal conditions. A decontamination efficacy risk scenario for the indoor environment may have substantial variability and uncertainty due to working with microbial concentrations at or near the limit of detection.

Data assessment issues (i.e., quantitatively validating the methods and sampling procedures) were revealed during the decontamination process from the released *B. anthracis* spores in 2011 (3). Therefore, in chapter 2, a literature review was conducted on the instrument and

environmental limit of detection for methods detecting *B. anthracis*. The instrument limit of detection from 9 different methods ranged from 10 cells/ml to 10^8 cells/ml. Instrument limits of detection for real-time PCR and PCR were the most sensitive with median instrument limits of detection of 430 and 440 cells/ml, respectively. There were only 15 studies (out of the 71 articles on method limits of detection for *B. anthracis*) that reported the environmental limit of detection. Of those, there were only 4 articles on the detection of *B. anthracis* from fomites, even though; fomites are a critical exposure matrix in the transmission of pathogens in the indoor environment (1, 4). The most sensitive environmental limits of detection were 0.1 CFU/g soil, 17 CFU/L air, 1 CFU/L water, and 1 CFU/cm^2 fomite.

The potential risk to human health was then calculated using the limits of detection as the exposure concentration and assumptions based on the inhalation route in an indoor environment. There were enough articles published on the instrument limit of detection for PCR and real-time PCR for there to be a distribution of values for the exposure concentration parameter. However, the environmental limit of detection could not be evaluated as a distribution due to the lack of information. More environmental limit of detection studies should be conducted in order to further define the environmental exposure. Or to obtain this distribution experimentally evaluate the pathogen in an environmental matrix and processed through to detection. The median risk estimate at the instrument limit of detection and the environmental limit of detection were 0.0062 and 0.52, respectively.

The sensitivity analysis of the risk models indicated that the limit of detection model parameter had the most influence in determining the risk. Since SRE directly affects the limit of detection,

characterizing the SRE would result in a more sensitive and less variable detection method. Therefore, in chapter 3, the focus was to quantitatively evaluate the parameters that affect SRE from fomites (a relevant environmental matrix in the indoor environment) at the environmental limit of detection (fomite surface areas 100 and 1000 cm^2 at low concentrations 0.4 and 4 PFU/cm^2).

Similarly to *B. anthracis*, viral pathogens pose a potential risks to human health in the indoor environment but may have more persistence issues on fomites. In chapter 3 bacteriophage P22, a virus surrogate, was chosen over the robustness of *B. anthracis* spores to evaluate the variability in SRE as a function of fomite type, fomite surface area, sampling time, application media, relative humidity and wetting agent. Experimentally, the parameters that had the most effect on the SRE were sampling time, fomite surface area, wetting agent, and relative humidity. Sampling time affected the SRE the most, within 20 min less than 3% of bacteriophage P22 was recoverable. Fomite surface area of 100 cm^2 resulted in a higher SRE than 1000 cm^2 fomite surface area.

The low (<3%) recovery of bacteriophage P22 from fomites at 20 min prompted the comparison of the loss due to recovery versus inactivation. These results indicated that bacteriophage P22 was active (approximately 90% of sample concentration) on the fomite at 20 min and remained detectable (2 to 5%) 24 hr later. To enhance the standard recovery method for dry samples, a TSB wetting agent was applied with a disposable spreader to the fomite. This added step improved the SRE for all samples at 20 min. Relative humidity and temperature are critical parameters for virus survival on fomites. The effect of relative humidity on SRE was

investigated and it was observed that the SRE was higher when relative humidity was greater than 28%.

Chapter 3 highlighted some of the parameters that must be considered when recovering viruses on fomites near the environmental limit of detection. In addition, it revealed that there was still substantial risk to human health after samples had dried onto the fomite. The survival and SRE are known to be organism-specific. Future work should focus on to quantitatively evaluating the parameters that affect SRE from fomites of select model organisms for pathogenic gram-negative, gram-positive and spore-forming bacteria.

To further define the indoor exposure pathway, samples were collected in university dormitories to determine the bacterial communities on fomites for an *in situ* case scenario. The use of cultivation method was necessary in chapter 3 to be able to evaluate recovery and survival of active bacteriophage P22 on fomites. However, in chapter 4, the use of high-throughput sequencing of the 16s rRNA gene provided the ability to examine bacterial communities at a greater depth than culture-dependent methods, which underestimates the bacterial community structure. The bacterial community structure on touched and untouched fomites were defined, as well, as any effect from the dormitory halls, rooms, fomite material, and fomite type had on the community.

Three core phyla (Proteobacteria, Actinobacteria, and Firmicutes) were present on touched and untouched fomites at different relative abundances. Touched fomites had a more diverse bacterial community compared to untouched fomites. Fecal related bacteria (such as

Lachnospiraceae and Mucispirillum) were at a higher relative abundance on touch fomites than untouched fomites. There were no correlations of bacterial community structure and the parameters of dormitory halls, rooms, fomite material, and fomite type.

The work presented in chapter 4 did not quantify the amount of bacteria present or employ the improved recovery method, but instead examined the potential hazards on touched fomites (where exposure interaction between fomite, pathogen and human take place) compared to untouched fomites. The knowledge of bacterial communities on touched and untouched fomites is further evidence of the role fomites have on the transmission of infectious diseases in the indoor environment. Additional studies should focus on the possible effects of the parameters such as human behavior, fomite hygiene protocols, sampling time and environmental conditions (temperature, relative humidity, etc.) on the bacterial community. While the use of high-throughput sequencing of the 16s rRNA gene provided multiple indicators, a limitation to this technique was the inability to identify the microorganism at the species level. The next step of this research would be to develop and use molecular techniques based these results from fomites samples in the indoor environment to identify specific pathogen that are responsible for the potential risk to human health.

The results from these chapters aimed to define parameter for the exposure assessment of microbial hazards at the environmental limit of detection for the indoor environment. The impact of these results is the establishment of environmental monitoring strategies and clean up goals. As well as, reliably assist in identifying with certainty the risk to human health.

REFERENCES

REFERENCES

1. **Boone, S. A., and C. P. Gerba.** 2007. Significance of fomites in the spread of respiratory and enteric viral disease. Appl. Environ. Microbiol. **73:**1687-1696.

2. **Haas, C. N., J. B. Rose, and C. P. Gerba.** 1999. Quantitative microbial risk assessment. John Wiley, New York.

3. **Sanderson, W. T., M. J. Hein, L. Taylor, B. D. Curwin, G. M. Kinnes, T. A. Seitz, T. Popovic, H. T. Holmes, M. E. Kellum, S. K. McAllister, D. N. Whaley, E. A. Tupin, T. Walker, J. A. Freed, D. S. Small, B. Klusaritz, and J. H. Bridges.** 2002. Surface sampling methods for Bacillus anthracis spore contamination. Emerg. Infect. Dis. **8:**1145-1151.

4. **Sinclair, R., S. A. Boone, D. Greenberg, P. Keim, and C. P. Gerba.** 2008. Persistence of category A select agents in the environment. Appl. Environ. Microbiol. **74:**555-563.

5. **U.S. Environmental Protection Agency and U.S. Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS).** 2012. Microbial risk assessment guideline: pathogenic microorganisms with focus on food and water. EPA/100/J-12/001; USDA/FSIS/2012-001.