

ENVIRONMENTAL CONTAMINATION FROM GLOVE DISPOSAL PRACTICES

by

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A Thesis Submitted to the Faculty of the

MEL AND ENID ZUCKERMAN COLLEGE OF PUBLIC HEALTH

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

WITH A MAJOR IN ENVIRONMENTAL HEALTH SCIENCES

In the Graduate College

THE UNIVERSITY OF ARIZONA

2018

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## **ACKNOWLEDGEMENTS**

I would like to thank my Committee: Dr. Marc Verhougstraete, Dr. Kelly Reynolds, and Dr. Robert Canales for consistent encouragement and support throughout my education here at MEZCOPH. Your passion for teaching and public health inspired me every day to strive to be more than I thought I could be. This was a very tough journey for me, but you never wavered in your support or teachable moments.

I would also like to thank all the MEZCOPH faculty and staff for their support and assistance from my many questions and requests. Special thanks to Dr. Stephanie Griffin, Dr. Jonathan Sexton, and Sally Littau for the knowledge you taught me about the laboratory and industrial hygiene techniques. A special thank you to Chris Tisch, Michael Tearne, Amy Glicken, Tanya Nemece, Ryley Tegler, Karen O'Shaughnessy, Ashton Norman, and Sharon Van Skiver for a plethora of assistance and quick answers.

Thank you to my laboratory mates for your support, encouragement, and laughter every day and night. Your willingness to assist and learn were always welcomed and greatly appreciated. Thank you for also teaching me.

Thank you to my foundation of support through not only this journey but all the past hills and valleys of life. I could not have done this without any of you, for you are who I thank God for every single moment of my life. Thank you will never be enough, to my fiancé and family: Travis, Mom, Harris, Dad, Rosalinda, Mark, Heather, and Twyla. Mom — Thank you for always believing I would be a tap dancing scientist. We did it!

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## **ABSTRACT**

**Purpose:** Personal Protective Equipment (PPE) provides a barrier between health professionals and pathogens. Misconceptions related to PPE and its role in environmental contamination, may lead to risky behaviors and/or perceptions in healthcare professionals due to broken barriers of protection. Evidence suggests that doffing and disposal of used PPE can lead to environmental contamination. The purpose of this study was to ascertain the potential for environmental contamination when medical gloves are flung, tossed, or thrown; while using a harmless PR772 bacteriophage and fluorescent dye tracers. The objectives of this study were to 1) measure the overall spread of bacteriophage and fluorescent dye from glove disposal to the surrounding environment; 2) determine the contamination along the glove flight path and the distance from the health professional; and 3) compare the occurrence of bacteriophage and fluorescent dye in the vicinity of glove disposal.

**Methods:** Fifteen Health Professionals flung, tossed, or threw PR772 and fluorescent dye contaminated gloves into a wastebasket, located 1.22 m away. Twenty designated sample areas were set up along the glove flight path, along a wall behind the wastebasket and outside the flight path that represented equipment within a patient room. Following each glove disposal trial, designated Sample Areas were: 1) visually inspected with a blacklight to quantify the fluorescent dye stains and 2) swabbed with a 3M Letheen Broth sponge to quantify PR772.

**Results:** The mean of PR772 contamination from all sample areas was 4.22 log<sub>10</sub> PFU/mL. The area closest to the participant (<0.30 m) had the highest PR772 concentrations (mean = 2.61 log<sub>10</sub> PFU/mL; range -0.3 to 6.32 log<sub>10</sub> PFU/mL). The

sample areas within the first 0.61 m of the health professional were statistically higher ( $p < 0.05$ ) than  $\geq 0.61$  m for PR772 and all sample areas,  $< 0.61$  m, were positive for both tracers. Based on the fluorescent dye's ability to predict the presence absence of viral tracers, it was found to be an appropriate surrogate when used as a teaching tool for PPE disposal scenarios.

**Conclusion:** Among medical personnel, gloves are used every workday and have the potential to contaminate the surrounding surfaces during improper disposal practices. Therefore, proper disposal techniques are required to minimize pathogen transmission. Due to limited education/training, and non-compliance with glove disposal recommendations, health professionals flinging gloves into the wastebasket can contribute significant pathogen contamination within 0.61 m around themselves, with a possibility of contaminating up to 1.52 m. Establishing industry-wide policies, adequate training and education to health professionals on appropriate glove disposal can reduce the spread of microbial contaminants and reduce exposure risks to patients and personnel.

## INTRODUCTION

Personal Protective Equipment (PPE) is used every day within the healthcare industry to protect health professionals from occupational exposures to pathogens. PPE provides a barrier between health professionals and pathogens. Improper use or disposal of PPE increases exposure risks in patients and personnel. The Centers of Disease Control and Prevention (CDC) and the World Health Organization (WHO) have published guidelines for PPE doffing aimed at minimizing pathogen spread. Observations of health professionals' behavior and perceptions towards proper use and disposal of PPE is not always consistent with published guidelines. Previous research has focused on doffing (removing) PPE but few studies have considered the act of disposing PPE in the spread of environmental contamination (Bell et al., 2015; Casanova et al., 2008; Guo et al., 2014; Hallihan et al., 2015; Kang et al., 2017; Lai et al., 2011; M E Tomas et al., 2015).

### *Environmental Contamination*

Environmental contamination is a concern in the healthcare industry. Environmental surfaces contaminated with pathogens are known to play a role in pathogen transmission and healthcare acquired infections (Weber et al., 2013). Studies have documented survivability and spread of pathogens post terminal cleaning where *Clostridium difficile* (*C. difficile*) was found on 53% of surfaces in the room following the discharge of an infected patient; Methicillin-Resistant *Staphylococcus aureus*' (MRSA) viability range on surfaces lasted between 31-318 days; and Vancomycin-Resistant *Enterococci* (VRE) survives on surfaces with very little decrease over a 7 day

period (Deshpande et al., 2017; Kramer et al., 2006; Lankford et al., 2006; Wagenvoort et al., 2000; Weber et al., 2016).

Infectious patients are the main source of environmental contamination, within the healthcare industry, due to microbial shedding (Atmar et al., 2008; Barbut and Petit, 2001; Otter et al., 2013). Health professionals also play a role in disease spread when they contaminate their gloves after touching the patient or contaminated objects and do not practice proper hand hygiene (Boyce et al., 2007, 1997; Kramer et al., 2006). Of nurses that had no direct contact with an MRSA infected patient but touched equipment in the room, 42% tested positive for MRSA on their gloves (Boyce et al., 1997). Another study resulted in 53% of health professionals' hands contaminated with pathogenic bacteria from occupied patient rooms while 24% of health professionals working from cleaned patient rooms contaminated their hands (Bhalla et al., 2004). Patient direct and indirect contact leads to the spread of environmental contamination.

#### *Floor Contamination*

Recently studies have placed an emphasis on floors as a vital role in pathogen transmission, but floors have been documented in the past decades as an environmental surface contaminated with pathogens. Over a five year study on floor bacteria, colony counts ranged between 3.3-5.2 CFU/10 cm<sup>2</sup> in operating room areas considered clean, while other areas ranged between 44.8-487.4 CFU/10 cm<sup>2</sup> (Suzuki et al., 1984). Out of the 32% of the surfaces contaminated in the rooms of MRSA infected patients, floors were the among the highest (Boyce et al., 1997).

In the past, floor studies mostly focused on the type of flooring and pathogen survivability. Vinyl tiling often has a textured surface area, where microbes can become

trapped in these crevices which may protect from desiccation, and potentially enabling them to colonize (Coughenour et al., 2011). *Staphylococcus aureus* (*S. aureus*) survived on vinyl tiles for at least 40 days with MRSA strains producing the most biofilms within 48 hours (Zarpellon et al., 2015). This study was done within high humidity and high temperatures (Zarpellon et al., 2015), enhancing bacterial growth possibilities. VRE recovered on vinyl composition tile was too numerous to count showing little decrease over a 7 day period (Lankford et al., 2006). Also, vertical surfaces, such as walls, have smaller bacterial colonies than horizontal surfaces, with an average of 1.6 CFU/10 cm<sup>2</sup> among the 35 samples taken in operating rooms (Suzuki et al., 1984). Learning about potentials for floor and wall contamination will enhance our understanding of pathogen niches and movement throughout healthcare facilities.

In two recent floor studies by Koganti and Deshpande, 2016 and 2017 respectively, investigated the role of floors in pathogen transmission (Deshpande et al., 2017; Koganti et al., 2016). In this study, over a 3-day period, high touch objects were cultured to recover MS2 either  $\leq 3$  feet from the bed and  $>3$  feet from the bed. Day 1 showed 58.2% ( $2.3 \pm 0.2$  PFU) of all the surfaces were positive for MS2  $\leq 3$  feet from bed and 39.7% ( $1.2 \pm 0.2$  PFU)  $>3$  feet of the bed, in all inoculated patient rooms. Within these inoculated patient rooms, viruses were recovered from 50% ( $1.5 \pm 0.5$  PFU) of sites including personal items such as cell phones, wheelchairs, books and clothing (Koganti et al., 2016). Day 2, 100% ( $1.9 \pm 0.1$  PFU) of the adjacent rooms to the inoculated patient rooms tested positive for MS2 on the floors as well as 40% ( $0.9 \pm 0.1$  PFU) of the designated high touch surfaces (Koganti et al., 2016). In another study 41 out of 100 hospital rooms surveyed had between 1-4 high touch objects in contact with the floor

(Deshpande et al., 2017). These objects in contact with the floor were cell phone chargers, canes, clothing, call buttons, blood pressure cuffs, bed linens, towels, and pillows. From the 31 gloves or hands swabbed, 18% had MRSA, 6% had VRE, and 3% had *C. difficile* (Deshpande et al., 2017). This shows indirect pathogen transfer between floors and hands by fomites in contact with the floor (Deshpande et al., 2017).

This recent research implicated floors as a reservoir for pathogens; therefore, learning how floors become contaminated is critical to prevent the spread of pathogens in hospitals (Deshpande et al., 2017; Koganti et al., 2016). Transfer efficiency rates from non-porous fomites to gloves was found to be between 28%-42% for bacteria and 33%-66% for viruses (Rusin P, Maxwell S, 2002). During an Ebola Virus Disease (EVD) PPE doffing and disposal study, PPE was in contact with the floor 4% of the time during disposal practices with health professionals (Hallihan et al., 2015). This can lead to pathogen transmission and increased exposure potentials for health professionals and patients.

### *Healthcare Associated Infections*

Pathogenic microorganisms that can spread directly or indirectly from human to human are defined as infectious diseases (The World Health Organization, 2017). Infectious diseases, community-acquired exempt, that cause infection three days after admission to a healthcare facility are known as Healthcare Associated Infections (HAI) (CDC, 2015a). According to a 2011 HAI surveillance survey across 10 states, 1 out of every 25 inpatients contract at least 1 HAI every day in United States' acute care hospitals (Magill et al., 2014). The most common pathogens were *C. difficile*, MRSA, and VRE (Magill et al., 2014). In 2012, HAIs total annual cost for the healthcare industry

in funds, labor, and resources resulted in a \$9.8 billion burden (Dik et al., 2016; Zimlichman et al., 2013). *C. difficile* accounted for 30.3% of HAI cases in the United States with a \$1.5 billion price tag (Zimlichman et al., 2013). MRSA cost the U.S. healthcare industry \$1.4 billion from surgical site and central bloodline HAIs (Zimlichman et al., 2013). With the growing financial burden, research is needed to investigate and provide solutions to the spread of HAIs.

### *Clostridium difficile*

*C. difficile* is a spore-forming, gram-positive bacillus that produces toxins: enterotoxin A, cytotoxin B, toxin A-/B+, and binary toxin CDT (CDC, 2010; Deneve et al., 2009; Landelle et al., 2014; Poxton et al., 2001; Riegler et al., 1995). Signs and symptoms range from mild to life-threatening infections such as: diarrhea, fever, loss of appetite, nausea, abdominal pain, and pseudomembranous colitis (Barbut and Petit, 2001; CDC, 2010; Moncrief and Wilkins, 2000). Testing for *C. difficile* is usually done by PCR (CDC, 2010). It is spread through the fecal-oral route from contaminated objects (CDC, 2010). *C. difficile* spores, which are resistant to drying, temperatures above 160°F, and chemicals, can persist up to 5 months on environmental surfaces (Boyce, 2007; Kim et al., 1981; Kramer et al., 2006; Rodriguez-Palacios and Lejeune, 2011).

Due to *C. difficile*'s resiliency, it is difficult to ensure that environmental surfaces are not harboring the spores after an infectious *C. difficile* patient vacates the room. *C. difficile* recovery from either isolation and non-isolation patient rooms was not significantly different ( $P = 0.6$ ) (Deshpande et al., 2017). There was also no statistically significant difference between cleaning while a patient was still staying in the room compared to after patient discharge cleaning. There was a 44% presence of *C. difficile*

while a patient was in the room and a 53% *C. difficile* presence after discharge cleaning (Deshpande et al., 2017). Due to *C. difficile*'s risk impact and extensive survivability on surfaces even in environmentally stressful conditions, it is important to develop effective interventions to break the transmission cycle within a *C. difficile* patient room.

#### *Methicillin-Resistant Staphylococcus aureus*

MRSA is a gram-positive bacteria that became antibiotic resistant over time in result to uncontrolled antibiotic consumption (El-baz et al., 2017; Wagenvoort et al., 2000). MRSA varies in its survivability because of its variety of hospital acquired strains but also its geographical strains (El-baz et al., 2017; Wagenvoort et al., 2000). MRSA survives well (31-318 days) over a range of environmental conditions including 24-47% relative humidity, 68-72°F, and 31-318 days of viability (Wagenvoort et al., 2000). According to a hospital study, outbreak strains survive up to 3 months longer than sporadic strains and in higher titer quantities (Wagenvoort et al., 2000).

MRSA studies have shown variability most likely due to the variations in laboratory conditions such as relative humidity and/or temperature. MRSA survived the longest on vinyl and plastic, possibly due to crevices used as shelter from desiccation. Yet MRSA's best survivability showed a significant difference ( $p < 0.001$ ) on vinyl and plastic when there was a biological protectant, such as human proteins, that helps against desiccation and adherence (Coughenour et al., 2011). *S. aureus* has been shown to survive for 28 days on vinyl composite tile and increase  $1.00 \pm 0.7$  log within 2 days (Gupta et al., 2017). Yet, the *Staphylococcus* species survivability depends heavily on temperature, as temperature increased, the growth decreased (McEldowney and Fletcher,

1988). Relative humidity had an effect on *S. aureus* growth, as relative humidity increased the growth decreased (McEldowney and Fletcher, 1988).

A study of MRSA patient's nurses following completion of their morning rounds and who did or did not touch the patient, found that 58% had contaminated gloves vs 42%, respectively (Boyce et al., 1997). If a patient with MRSA was in a room prior to the new patient, that patient has a 1 in 94 chance of acquiring MRSA (Huang et al., 2006). With MRSA's ability to survive in varying conditions, it is essential to follow strict PPE and cleaning procedures.

#### *Vancomycin-Resistant Enterococcus*

Enterococci are gram-positive bacteria that reside in the human intestines but are also found in soil, water, and food due to its resiliency (CDC, 2015a). They can survive in temperatures ranging from 50 – 113°F. VRE is resistant to the antibiotic, Vancomycin, which treats infections by enterococci (CDC, 1993; Facklam and Collins, 1989). Infections happen mostly in those that are immunocompromised; hospitalized and had antibiotic treatments, surgical procedures, or are previously colonized with VRE (Facklam and Collins, 1989).

There is a 1 in 59 chance of acquiring VRE if the patient occupying the room prior had VRE (Huang et al., 2006). Another study found that even after a thorough cleaning, the chance is seven times higher of acquiring VRE if admitted into a previous VRE positive patient's room (Hayden et al., 2006). This 4 period study (each period lasting between 57-82 days) did result in decreased VRE acquisitions with applied interventions (environmental cleaning education and hand hygiene intervention) but duration until acquisition was not significant ( $p = 0.45$ ) and still resulted in 10.4 VRE

cases per 1000 patients per day (Hayden et al., 2006). From another study, 39% of health professionals' gloves tested positive for VRE after doffing, and VRE was found on 29% of their hands (Tenorio et al., 2001). Contamination happens because of its survivability on environmental surfaces and on health professionals' gloves which can spread to other fomites and other areas outside the infected patient's room.

### *Ebola Outbreak*

During a 2014 EVD outbreak, 21 Dallas, Texas health professionals were exposed to bodily fluids of an Ebola positive patient. Despite wearing PPE, two health professionals contracted EVD (Chevalier et al., 2014). This event led to additional scrutiny and research of appropriate PPE levels, PPE donning/doffing policies, and PPE compliance in the healthcare industry. The CDC and the WHO suggested modification of several PPE protocols during and after the Ebola 2014 pandemic (CDC, n.d.; Chevalier et al., 2014; Kang et al., 2017). However, even the most astute PPE use while dealing with highly infectious disease patients cannot eliminate all risk for professional health workers.

The first recorded EVD outbreak in West Africa was in 1976 (Breman et al., 1978; Deng et al., 1978; Johnson et al., 1978). The largest EVD outbreak happened in 2014 affecting several countries but the three most distressed were Sierra Leone, Guinea and Liberia (CDC, 2016a). The 2014 EVD outbreak had 28,639 reported cases and 11,316 deaths (CDC, 2016a). Sierra Leone lost half of its country's work force, while Liberia's healthcare professionals were reduced by 8% and 1% in Guinea due to EVD deaths, infections, caring for those in the household or those fleeing from the disease (CDC, 2016a; UNDP, 2014). The World Bank estimated that the 2014 EVD outbreak

cost \$2.2 billion to the gross domestic product (GDP) of these three countries combined (CDC, 2016a). The United States Government was estimated to have spent \$2.4 billion in response to the 2014 EVD outbreak; allocating to the CDC, emergency response activities, resources and aid (CDC, 2016a; USAID, 2016). This outbreak highlighted the importance of health screening security to reduce the spread during future events, not only for those countries directly affected but also for those who contributed personnel, resources, and other aid.

### *Ebola Virus Characteristics*

EVD is a non-segmented, enveloped, single stranded negative RNA virus in the *Filoviridae* family (Johnson et al., 1977; Kiley et al., 1980). It is between 30-1500 nm in length and about 80 nm wide (Johnson et al., 1977). EVD is a zoonotic pathogen that also effects non-human primates such as monkeys, gorillas, and chimpanzees (Breman et al., 1978; Deng et al., 1978; Johnson et al., 1977; Rajak et al., 2015). There are four human infectious species called Zaire, Sudan, Tai Forest, Bundibugyo and only one primate infectious species: Reston (Murray, 2015). The natural reservoir is uncertain but fruit bats are suspected to be the primary sources based on evidence of antibodies detected in their blood (Swanepoel et al., 1996; Towner et al., 2009). The ID<sub>50</sub> of Ebola is 1-10 organisms (Piercy et al., 2010). The patient is most infectious in the latter days of illness (median duration of illness from onset of fever is 10 days), at death and after death due to the high EVD titer of cell lysis (Dowell et al., 1999). Ebola can persist on steel and plastic for more than a week (Cook et al., 2015; Piercy et al., 2010).

### *EVD Transmission, Symptoms, and Detection*

Ebola's routes of transmission are person-to-person contact through the non-intact skin or mucous membranes with blood or bodily fluids such as urine, saliva, sweat, feces, vomit, breast milk and semen (Liu and et al., 2015; Rodriguez et al., 1999). Exposure can result from contact with contaminated objects with infected blood or bodily fluids, infected fruit bats, or non-human primates (Liu and et al., 2015; Rodriguez et al., 1999). The low infectious dose, long-term survival tendencies and numerous means of infection highlight the importance of PPE during care of infected and recently deceased person.

Recently, there was confirmation of Ebola being contracted sexually in Liberia, 199 days after the survivor had symptoms (Christie et al., 2015). The CDC and the WHO reverted advice of condom use for at least 3 months, to male survivors, to continually use condoms for all sexual activities until further studies define Ebola's persistence in semen (Christie et al., 2015).

Common signs and symptoms of EVD occur within a 2-21 day incubation period which includes: sudden fever, headache, myalgia, pain in the large joints, and back pain (Feldmann and Geisbert, 2011; Liu and et al., 2015). Although there are many images of EVD patients hemorrhaging from their eyes, nose and nailbeds; it is an uncommon sign (Funk and Kumar, 2015; Weber et al., 2016). Patients contracting a high viral load ( $>10^7$  copies/ml) usually leading to death start showing signs and symptoms after two days with abdominal pain, nausea, vomiting and diarrhea possibly followed by a skin rash after 5-7 days (Feldmann and Geisbert, 2011; Liu and et al., 2015). Infected patients commonly die from septic shock and blood clots throughout the body leading to multi-organ system failure (Feldmann and Geisbert, 2011; Liu and et al., 2015). EVD survivors'

commonalities are a low viral load ( $<10^4$  copies/ml), longer incubation period, increased specific antibodies, immediate supportive care and/or experimental therapies (Feldmann and Geisbert, 2011; Liu and et al., 2015; Murray, 2015).

EVD confirmation in a suspected patient is usually completed by quantitative Polymerase Chain Reaction (qPCR) (Sanchez et al., 2001). EVD is also confirmed by antibody-capture enzyme-linked immunosorbent assay and electron microscopy (Pattyn et al., 1977; Zaki and Goldsmith, 1999). EVD can be detected within 3-10 days after the show of symptoms. If tested before this time frame, results may show false negatives (Sanchez et al., 2001). The CDC established a specialized laboratory in Sierra Leone, and mobile testing laboratories, to detect EVD as soon as possible, therefore reducing time and exposure to others in the community involved. Since the 2014 EVD outbreak and because of Ebola's rapid spread to nearby countries, specialized laboratories were also established in Guinea, and Liberia (CDC, 2015b).

#### *EVD Treatment and Protection*

At the time of this thesis development, the only treatment options available for EVD positive patients were experimental therapies and vaccines (Feldmann and Geisbert, 2011; Liu and et al., 2015; Murray, 2015; Weber et al., 2016). Some of these experimental therapies are ZMapp, a combination of antibodies; convalescent serum therapy; RNA interference therapy and many others but not all have 100% success rates for humans (Feldmann and Geisbert, 2011; Murray, 2015). Therefore, some researchers believe that combinations of these therapies may be needed for EVD survival (Feldmann and Geisbert, 2011). During the 2014 Ebola outbreak, risk of mortality, in the beginning, was greater than 70% and decreased to 40% due to supportive care such as oral hydration

or administration of parenteral fluids during early onset of symptoms when there was known EVD contact (Agua-Aqum et al., 2016; Lamontagne et al., 2017).

From the 2014 Ebola outbreak, it is known that several health professionals, within Africa, Europe, and the U.S., contracted the disease even when wearing PPE (CDC, 2016b; Chevalier et al., 2014; Murray, 2015). Doffing PPE became a researched issue due to these self-contamination cases of EVD (Murray, 2015). After the Dallas Ebola episode, evaluating potential exposures, infection control practices and devising large scale training sessions became some of the necessities for jurisdictions around the United States (Chevalier et al., 2014). This focus also led to ensure health professionals are trained on these revised PPE donning and doffing protocols, to decrease self-contamination when doffing.

#### *Personal Protective Equipment*

PPE is known to reduce the exposure of highly infectious diseases to health professionals (Verbeek et al., 2016). Previous health professional surveys identified gloves are used 88%-93% of the time, during possible patient contact, compared to other PPE such as gowns, masks, and goggles (Girou et al., 2004; Mitchell et al., 2013; Wilson et al., 2017). Indicating that gloves are imperative to protect health professionals, as well as patients.

Numerous studies have reviewed how PPE is used, doffed or contaminate surfaces within the healthcare environment. According to a study, there was more environmental contamination when doffing gloves (52.9%) than with gowns (37.8%) (Myreen E. Tomas et al., 2015). Incorrect doffing was observed between 13-39.5% (Mitchell et al., 2013; Myreen E. Tomas et al., 2015) of the time by health professionals

and there was no significant difference between health professional fields, including physicians, nurses and allied health care personnel ( $p=0.26$ ) (Myreen E. Tomas et al., 2015). Since gloves are the most commonly used PPE, it is important to ensure that personnel are trained in proper doffing and disposal.

PPE becomes contaminated from direct and indirect contact with an infectious patient. Several studies indicate that even when health professionals do not touch the patient but have contact with high touch objects within the infected patient's room, their gloves are contaminated with that pathogen (Bhalla et al., 2004; Boyce et al., 1997; Tenorio et al., 2001). There was a significant difference in the environmental contamination when health professionals used their own doffing method compared to CDC PPE doffing methods ( $p<0.001$ ) (Guo et al., 2014). CDC recommended PPE doffing methods are located on the CDC website under HAI or Ebola sections in the form of videos, courses, PowerPoint presentations or pdf postings detailing the steps to remove gloves, gowns, face masks/goggles, masks, respirators, etc. (CDC, 2014, n.d.).

Exposure to pathogens in the healthcare settings can be reduced by applying scientific evidence, hazard communication, PPE donning and doffing training, appropriate disposal procedures and cleaning protocols. However, a lack of information on the disposal of soiled PPE and the potential to contaminate the surrounding environment limits the efficacy of such interventions in reducing HAIs (CDC, n.d.; Kuzu et al., 2005; Loveday et al., 2014; Tenorio et al., 2001; Whitby et al., 2007; World Health Organization, 2009).

*Public Health Professional's Behavior and Perceptions of PPE*

According to the WHO, transmission of pathogens could be the result of inappropriate glove use which includes doffing inappropriately (World Health Organization, 2009). Even when health professionals reportedly know the importance of appropriate PPE use, there is a separation from training and practice due to limitations within the environment that they work. Several studies have identified barriers to appropriate PPE use, in the healthcare industry, which include a lack of training, constantly changing industry PPE protocols, difficulty with PPE use (e.g. donning gloves when hands are swollen and moist), insufficient access to products, the lack of pathogen transmission knowledge, and improper PPE disposal practices (Barr et al., 2017; Flores and Pevalin, 2006; Kang et al., 2017; Liu and et al., 2015; Loveday et al., 2014; Weber et al., 2016).

Fluctuations in proper glove use also happens due to misunderstanding, incorrect perceptions and emotions such as self-preservation when dealing with what health professionals considered “dirty tasks” (Wilson et al., 2017). Glove use increased among healthcare professionals when a task was considered dirty or high risk and the need to protect oneself from contamination (Kuzu et al., 2005). Glove misuse incidents occur 39% of the time when healthcare professionals touched surfaces or objects outside of the patient zone before and after touching the patient (Loveday et al., 2014). These items included high touch objects (e.g. clinical equipment, bedside table, etc.) as stated in the floor studies (Loveday et al., 2014). Another study suggests that glove doffing procedures are not clear to healthcare professionals and that Infection Prevention and Control (IPC) policies should be clearer and appropriate for work reality (Wilson et al., 2017). Another

study confirms that IPC policies are misunderstood by health professionals from those that read them but less than half of the health professionals surveyed actually read the IPC policies (O'Boyle Williams et al., 1994). Yet, if health professionals had more training, they were more likely to follow universal protocols which leads to a decrease in environmental contamination (O'Boyle Williams et al., 1994; Verbeek et al., 2016).

#### *Surrogates for HAI Associated Pathogens*

Surrogate studies allow scientists to measure microbial transport, exposure, and infection risks with minimal risk of infection (Aranha-Creado and Brandwein, 1996; Sinclair et al., 2012). The definition of surrogate is an organism used to study the fate of a pathogen in a specific environment (Sinclair et al., 2012). Surrogates are used mostly for the safety of researchers and the public. Other advantages for using surrogates are that they are innocuous, able to grow high titers, detectable from inexpensive bacteriophage assays within several hours of post inoculation, and unlike pathogenic viruses do not need specialized testing facilities (Aranha-Creado and Brandwein, 1996). A framework was developed to identify the best surrogate for the associated pathogen include: defining the pathogen hazard, describing the organism's ideal environment, and assessing the surrogate's survival, movement, and disinfection characteristics (Sinclair et al., 2012).

Establishing an EVD surrogate for the initial section of this experiment, entailed determining how EVD is transported during the CDC doffing protocol after treating a highly infectious patient. Employing this framework, bacteriophage PR772 was chosen due to its functional morphology, prioritizing needs and disinfection (Sinclair et al., 2012).

### *Bacteriophage PR772*

PR772 is from the *Tectiviridae* family and an unenveloped, linear double stranded DNA bacteriophage (Turgeon et al., 2014). Its size is ~80 nm in an icosahedral shape with a lipid inner membrane (Lute et al., 2004). Most Biosafety Level (BSL) 1 surrogates are much smaller than PR772 and in comparison to EVD's ~80 nm width (Aranha-Creado and Brandwein, 1996; Johnson et al., 1977). The phage's functional morphology was closely related to EVD in size and with its lipid layer to resemble an envelope.

Prioritizing needs and disinfection were the most decisive aspects when using this framework's steps to select PR772. Out of all the *Tectiviridae* family surrogates, PR772 is the only one that has a BSL 1 host, *Escherichia coli* (HER 1221) with best growth conditions in tryptic soy broth (TSB) at 37°C (98.6°F) (Gallandat and Lantagne, 2017). Other advantages to PR772 is the ability to obtain high titers ( $10^8$  - $10^{10}$ /ml) and demonstrated stability needed in laboratory experiments for recovery (Aranha-Creado and Brandwein, 1996; Lute et al., 2004). High titer bacteriophages concentrations are necessary evaluate contamination within a healthcare setting and the effectiveness of interventions. (Casanova et al., 2009). Plaque assays have been used to quantify bacteriophages as the "gold standard" method which allows for low-cost production, viable bacteriophage enumeration, and no specialized equipment (Anderson et al., 2011). PR772 and EVD have similar sensitivity to 67% ethanol, however, PR772 is more rapidly inactivated compared to EVD treatment with chlorine bleach (Cook et al., 2015; Gallandat and Lantagne, 2017).

Controversy has revolved around the best surrogate for EVD due to its envelope, helical shape, genetics and size. Bacteriophage MS2 was thought to be the best surrogate

because of its resistance to chlorine and single stranded RNA genome, but recent studies have shown otherwise (Gallandat and Lantagne, 2017). PR772 and  $\Phi 6$  are considered the best EVD surrogates but using multiple surrogates would best represent EVD under variable environmental conditions.

After being aerosolized, five different bacteriophages were tested for infectivity and suitability as surrogates for airborne viruses (Turgeon et al., 2014). MS2 was more resistant to aerosolization and sampling than PR772 and  $\Phi 6$  (Turgeon et al., 2014). This study showed there was no significant difference with air samplers and qPCR monitoring results comparing  $\Phi 6$ , PR772 and MS2 or a significant difference when using plaque assays between  $\Phi 6$  and PR772 (Turgeon et al., 2014). MS2 was shown to be more resistant to desiccation and damage from aerosolization and the air samplers than the other bacteriophages (Turgeon et al., 2014). Yet, this surrogate proved to be too conservative, and was found to be more disinfectant resistant than EVD than previously thought (Gallandat and Lantagne, 2017; Sassi et al., 2018)

More recent studies found that  $\Phi 6$  was the most effective and appropriate surrogate for EVD (Gallandat and Lantagne, 2017; Sassi et al., 2018). This was  $\Phi 6$ 's enveloped, lipid-based, and RNA characteristics that showed similarities with digestion survivability and chlorine inactivation constant values (Gallandat and Lantagne, 2017; Sassi et al., 2018). Although both of these studies show  $\Phi 6$  as the better surrogate for EVD, they also suggest that multiple surrogates should be used for EVD due to relative humidity, temperature and surface chemistry affecting the surrogates' inactivation mechanisms (Gallandat and Lantagne, 2017; Sassi et al., 2018). A PPE study, which is similar to our initial full PPE donning and doffing experiment, did not detect any  $\Phi 6$  in

the results (Casanova et al., 2016). From our donning and doffing pre-trials, PR772 was recovered from the PPE. Therefore, bacteriophage PR772 was chosen as the EVD surrogate due to its functional morphology, prioritizing needs, disinfection and results from similar studies.

### *Fluorescent dye*

Fluorescent dyes can be used as a non-microbial tracer that gives instant qualitative results, as it fluoresces under long wave ultraviolet light. Fluorescent dye has been used as a teaching tool to allow health professionals and researchers to visually see contamination throughout the environment (M E Tomas et al., 2015). Several PPE studies used fluorescent dye for its safety, visual representation, and instantaneous results (Alhmidi et al., 2016; Bell et al., 2015; Casanova et al., 2008; Guo et al., 2014; Kang et al., 2017; Lai et al., 2011). Studies have shown that there was no significant difference in overall percentage of contamination between bacteriophage MS2 and fluorescent dye (Alhmidi et al., 2016; Bell et al., 2015; Myreen E. Tomas et al., 2015). Fluorescent dye does not differ significantly from bacteriophage results when examining possible self-contamination events between different PPE ensembles or methods and thus it can be a useful teaching aid (Bell et al., 2015). However, using fluorescent dyes to assess low level exposures is limited to concentrations visible to the naked eye (Bell et al., 2015)

### *Conclusion*

HAIs and highly infectious diseases are a public health concern for all healthcare facilities. Since environmental contamination is known to contribute to pathogen transmission throughout healthcare facilities, it is imperative to learn if there are other contamination possibilities contributing to the healthcare environment. A knowledge gap

is the repercussions from inappropriate PPE disposal which come from a health professional's behavior in disposing of their PPE. If contaminated PPE is not removed and discarded safely to reduce exposure, then the health professional and anyone in the area may be at risk of contamination (Fischer et al., 2014).

### *Purpose and Objectives*

The purpose of this study is to ascertain any environmental contamination when gloves are inappropriately disposed of while using PR772 bacteriophage and fluorescent dye tracers. Inappropriate glove disposal entails flinging, tossing, or throwing, rather than appropriately placing used gloves into a wastebasket. The objectives of this study were to 1) measure the overall spread of bacteriophage and fluorescent dye from glove disposal to the surrounding environment; 2) determine the contamination along the glove flight path and the distance from the health professional; and 3) to evaluate use of fluorescent dye to serve as a teaching tool for PPE disposal scenarios in lieu of bacteriophage.

## **METHODS**

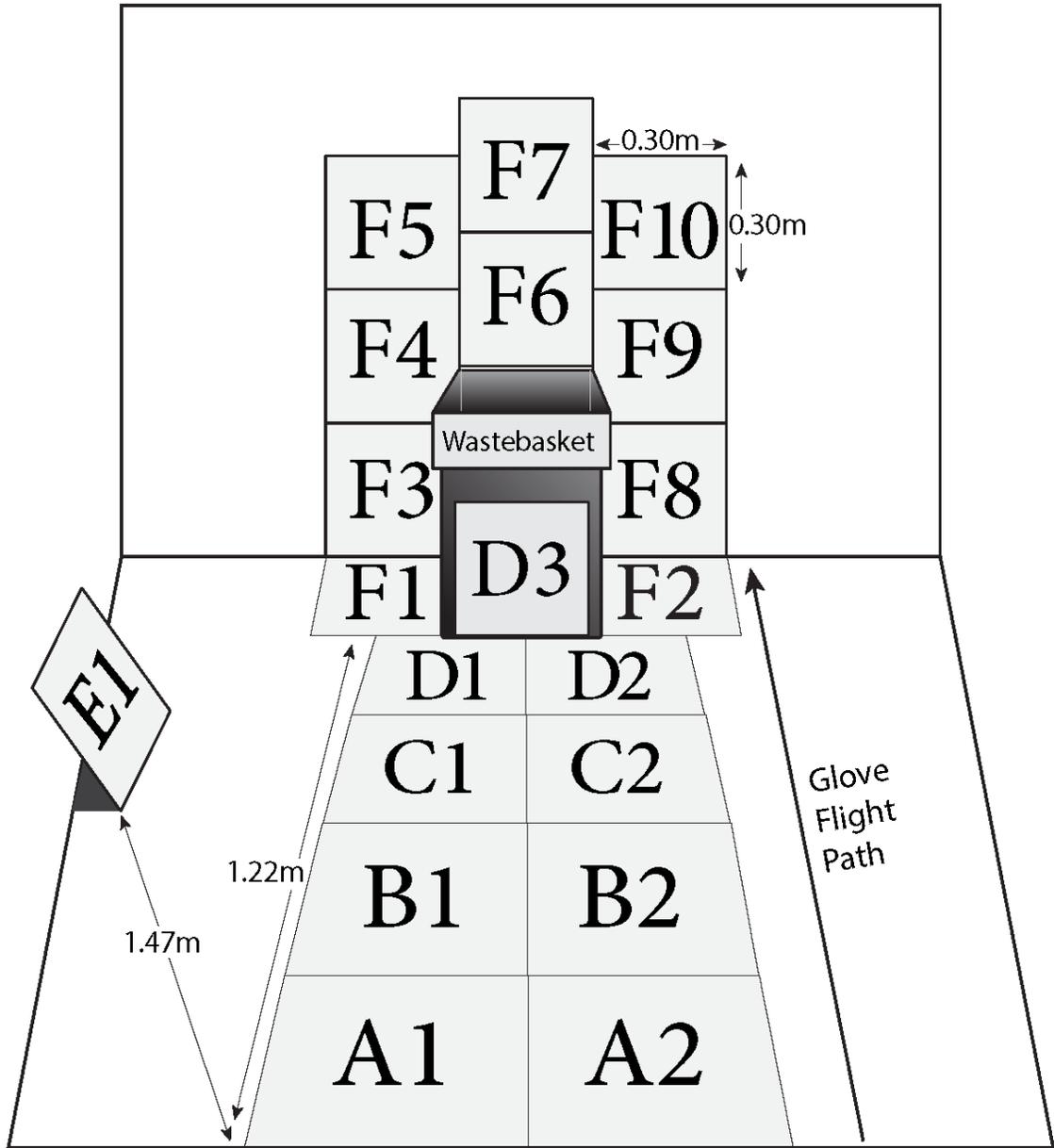
Study protocols were approved by the University of Arizona Institutional Review Board. Study participants were recruited from public health professional fields to ensure a basic knowledge of doffing Personal Protective Equipment (PPE). There were 15 health professionals with experience in a range of fields, such as clinical, hospital, laboratory or fire/EMT occupations. The survey and glove disposal portions of the study were performed at the Environment, Exposure Science, and Risk Assessment Center (ESRAC) laboratory at the University of Arizona (Tucson, Arizona).

### *Public Health Professionals Survey*

Surveys were used to obtain the participants' background knowledge of PPE protocols and practiced disposal methods. The Qualtrics system (Qualtrics, Provo, UT) was used to collect participant information on their years of experience, field experiences, PPE proficiency, CDC Ebola protocol familiarity, soiled PPE disposal method, contaminated glove disposal method, and if they ever witnessed coworkers dispose of PPE inappropriately. This study was under the umbrella of a larger study to verify CDC Ebola PPE doffing protocols for Emergency Responders, which the Public Health Professionals survey was also used for. The survey was given to the health professionals before starting each trial.

### *Glove Disposal Experiment*

Health professionals sanitized their hands with alcohol-based hand rub (ABHR) (Purell hand gel, GOJO Industries, Inc., Akron, OH) following manufacturers recommendations before donning nitrile gloves. Then 1.0 mL of PR772 bacteriophage (concentration = 9.20 to 9.57 log<sub>10</sub> PFU/ml with a mean of 9.37 log<sub>10</sub> PFU/ml), followed by 1.0 mL of fluorescent dye (GloGerm, Moab, UT) was applied to both gloved palms, similar to a previously published study (Casanova and Weaver, 2015). Health professionals rubbed their hands together for 15 seconds to spread both phage and fluorescent dye all over gloves. No mandatory drying time was required. They were instructed to doff and dispose of their gloves as they would during work but restricted to stand 1.22 m away from the wastebasket (Figure 1).



**Figure 1: Trial set up of sample areas.** Sample area =  $0.30 \text{ m}^2$  Areas in increments of 0.30 m: A  $\leq 0.30 \text{ m}$ ; B = 0.30 m – 0.61 m; C = 0.61 m – 0.91 m; D = 0.91 m - 1.22 m; E = 1.47 m to left of participant; F = 1.22 m - 1.52 m. D3 = 1.22 m, attached to front of wastebasket.

After glove disposal, the room was darkened, and a black light was used to observe the presence/absence of fluorescence on sample areas. Fluorescent stains were also observed and noted on the health professionals' hands and forearms but not

recorded. At the conclusion of the study, participants were given the opportunity to perform hand hygiene with ABHR or soap and water.

Every sample area, 0.30 cm<sup>2</sup>, was swabbed with a Lethen Broth sponge-stick (3M, St. Paul, MN), then held on ice until processed that same day. Sampled areas were cleaned, after every single trial, by completely wetting each tile with 70% ethanol, for a 30 second contact time followed by a second spray towel wiping and drying. After trials, sampled areas were inspected for any residual fluorescent stains. If stains were still visible a clean sample area tile it was substituted for the next participant. Sample area tiles were cleaned with soap and water to remove fluorescent stains to be used again.

Temperature and humidity were recorded, after health professional flung gloves, for all trials with a mean of 22.8±0.3°C and 17.5±2.5%, respectively. Bacteriophage PR772 (HER 221) (ATTC: BAA-769-B1) was used as a nonpathogenic virus in this study as well as a conjoined study on PPE use while managing a potential EVD patient. At the time of this experiment, PR772 was thought to best represent EVD due to its large size (~80 nm), lipid-protein membrane and disinfection susceptibility being similar to EVD (Gallandat and Lantagne, 2017; Lute et al., 2004; Turgeon et al., 2014). PR772 is especially useful due to its ability to attain high plaque titers, ease of culturing, and Biosafety Level 1 host (*E. coli* strain) (Lute et al., 2004).

#### *Laboratory Methods*

Bacteriophage PR772 (HER 221) (ATTC: BAA-769-B1) and its host *Escherichia coli* strain K12J53-1 (ATCC: BAA-769) was acquired from the American Type Culture Collection (ATTC). Propagation for bacteriophage PR772 (HER 221) (ATTC: BAA-769-B1) and *Escherichia coli* strain K12J53-1 (ATCC: BAA-769) was performed according

to American Type Culture Collection (ATCC) propagation and preparation methods (American Type Culture Collection, 2014, n.d.). To recover the bacteriophage, the host culture must be actively growing for 18-24 hours. The host culture was propagated by adding 0.5 mL of Tryptic Soy Broth (TSB) to rehydrate the pellet followed by vortex mixing. The host (1 mL) was aseptically transferred onto Tryptic Soy Agar (TSA) plates, allowed to dry then streak plated; incubated at 37°C overnight, then repeated streak plating method with host lawn and incubated again overnight.

After a healthy lawn of the *E. coli* host was propagated, the host culture was prepared by obtaining a loop full of *E. coli* host and added to 125 mL of TSB. The host culture was agitated and incubated at 37°C for 6 hours. Then bacteriophage PR772 was allowed to thaw for 1 hour in a cryovial until 0.5 mL of TSB was added and then vortexed for 30 seconds. According to the Environmental Protection Agency (EPA) Method 1601 double layer assay (DAL) with some modifications, 0.5 mL of *E. coli* strain K12J53-1 and 0.1 mL bacteriophage PR772 dilutions, with sterilized Phosphate Buffered Saline (PBS), were added to 5 mL of top agar (Environmental Protection Agency, 2001). Then swirled and poured onto pre-warmed TSA plates (dilutions of -2, -3, -4, -5, -6, -7, -8, -9, -10, -11), in duplicates and allowed to solidify. After 18 hours of incubation at 37°C, 5.0 mL of TSB was added to the higher but countable bacteriophage clearings of the TSA plates (labeled -7, -8 dilutions) and swirled, to loosen the bacteriophage, every 15 minutes for 2 hours. After the 2 hours of agitation, the solution from these 4 plates was pipetted from all plates into a sterile, vial container (50 mL) and centrifuged at 2500 rpm for 10 minutes. The supernatant was pipetted out and passed through a 0.22 Millipore filter (Merck, Germany). This filtrate was then titered. Bacteriophage PR772

propagation was titered to  $10^9$  concentration per mL then 1.0 mL aliquots of PR772 filtrate was stored in cryovials at  $-80^{\circ}\text{C}$  until use on trial day, which was titered again to confirm  $10^9$  concentration.

Each sponge-stick was agitated by hand within its transport bag for 30 seconds then pipetted, measured, recorded and transferred into sterile, vial sample containers. Each sample container was labeled with sample area identification code, amount eluted, date and initials of researcher. Again, EPA Method 1601 DAL method with modifications of 0.5 mL of *E. coli* strain K12J53-1 and 0.1 mL bacteriophage PR772 dilutions, with sterilized Phosphate Buffered Saline (PBS), were added to 5 mL of top agar (Environmental Protection Agency, 2001). Serial PBS vials were prepared at -1 and -2 dilutions. Duplicate assays were completed for all samples. Samples were incubated at  $37^{\circ}\text{C}$  for 20 hours. After incubation, clearings in the host lawn were counted as plaque forming units (PFU). All serial dilutions were averaged and then calculated to PFU/mL. A negative control for laboratory integrity was verified by exposing a sponge-stick to the glove flinging laboratory room's environment by opening the sponge-stick container and holding sponge-stick in air for 30 seconds above glove flight path. This negative control was expected to have no clearings within the host lawn, ensuring that PR772 was not aerosolized. Positive host culture (*E. coli* strain K12J53) controls were performed to ensure no contamination from other bacteria or PR772. The positive host culture controls all had lawn growth of *E. coli* strain K12J53 which was expected.

#### *Data Analysis*

Descriptive statistics of the data were performed using Microsoft Excel (Microsoft Corporation version 2016). R computing program (R Core Team, 2017) was

used to perform one-sample t-test of the PR772 contamination between  $\geq 0.61$  m and  $<0.61$  m and its correlation. McNemar's test was analyzed by R computing program for the comparison of fluorescent dye and PR772 bacteriophage.

## **RESULTS**

### *Public Health Professionals survey*

This project included the use of a survey amongst health professionals and a laboratory experiment to determine contamination from inappropriate glove disposal. The survey results show that 12 out of the 15 health professionals participating in the glove disposal experiment had more than 5 years of PPE skills (Table 1). Over 50% of health professionals reported seeing a peer perform improper PPE disposal in the previous 12 months (Table 1). Three disposal methods were observed: underhanded throw into/towards the wastebasket (toss); overhanded throw into/towards the wastebasket (throw); pulling on the gloves to stretch and launch into/towards the wastebasket (fling). The survey of 15 health professionals revealed that 0% used the flinging method to dispose of PPE in real life. When placed 1.22 m away from the wastebasket, 33% of participants during the glove disposal experiment were observed flinging their gloves into the wastebasket. Furthermore, during the glove disposal experiment, 8 out of 15 (53%) participants successfully flung both gloves into the wastebasket without landing on the floor.

**Table 1: Health Professionals' Survey Results in Glove Experiment**

Number of Health Professionals (n=15)		
Current occupational experience with Personal Protective Equipment (PPE)		
EMT	2	
Allied Health	1	
Medical Center	6	
Laboratory	6	
Years of experience with (PPE)		
5+	12	
3-5	1	
1-2	1	
<1	1	
Peers seen not following PPE disposal protocol		
Too Many Times to Count	2	
Frequently	1	
Often	5	
Seldom	6	
Never	1	
Personal glove disposal method	Survey Results	Experiment Results
Throw	1	2*
Toss	6	8**
Fling	0	5***
Place	8	Not Applicable

Success of both gloves into wastebasket from throw \*100%; toss \*\*50%; fling \*\*\*40%

### *Glove fling and Bacteriophage PR772*

The laboratory glove disposal experiment involved seeding donned nitrile gloves with bacteriophage. Concentrations of the seeded PR772 bacteriophage ranged from 9.20 to 9.57 log<sub>10</sub> PFU/mL with a mean of 9.37 log<sub>10</sub> PFU/mL. Following disposal of gloves, concentrations in all sample areas ranged from below detection limit (-0.3 log<sub>10</sub> PFU/mL) to 6.32 log<sub>10</sub> PFU/mL (mean of 4.22 log<sub>10</sub> PFU/mL). The area closest to the participant (Figure 1, Area A, <0.30 m) had the highest PR772 concentrations (mean = 2.61 log<sub>10</sub> PFU/mL; range -0.3 to 6.32 log<sub>10</sub> PFU/mL). Up to 80% of the areas within the first 0.61 m of the health professional tallied positive for bacteriophage (Table 2). The lowest PR772 concentrations (0.48 log<sub>10</sub> PFU/mL) were measured on the wastebasket (Figure 1, Area D3, 1.22 m). Within the 0.91 m - 1.22 m area (Figure 1, Areas C & D), 32% of the flings resulted in contamination with one fling equaling 5.82 log<sub>10</sub> PFU/mL. This is possibly due to used gloves hitting the wastebasket edge and unsuccessful disposals into the wastebasket. PR772 concentrations averaged 0.34 ± 1.12 log<sub>10</sub> PFU/mL on Area E1, left and outside of the glove flight path (Table 2).

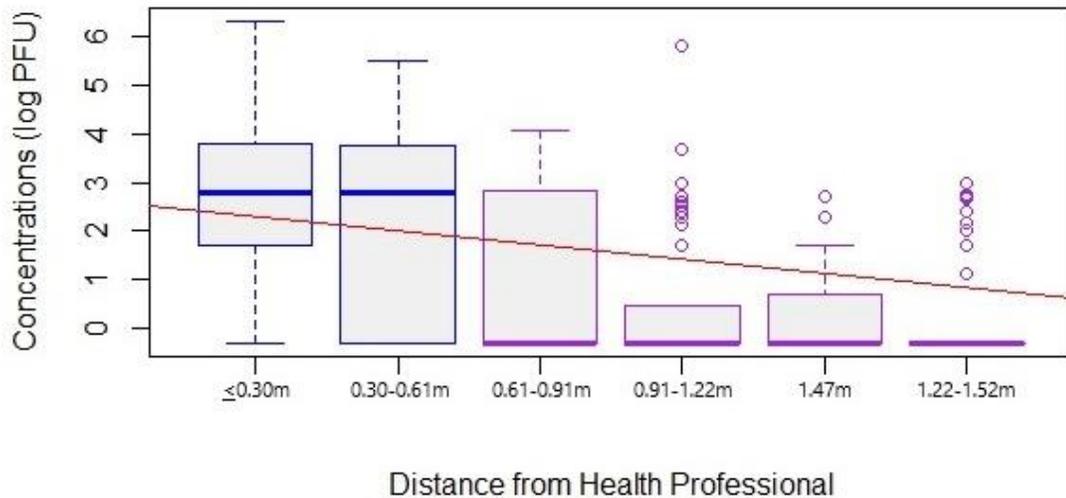
**Table 2: Contamination from Glove Disposal Along the Glove Flight Path**

Distance from health professional (meters)	Bacteriophage				Fluorescent Dye	
	Mean $\pm$ SD ( $\log_{10}$ PFU/ml)	Median ( $\log_{10}$ PFU/ml)	Range ( $\log_{10}$ PFU/ml) Min Max	Positive Occurrence (%)	Positive Occurrence (%)	
$\leq 0.30$ (A)	2.61 $\pm$ 1.87	2.79	-0.3 6.32	80	87	
0.30 - 0.61 (B)	2.32 $\pm$ 1.96	2.78	-0.3 5.51	70	77	
0.61-0.91 (C)	1.02 $\pm$ 1.68	-0.3	-0.3 4.09	40	43	
0.91-1.22 (D)	0.48 $\pm$ 1.44	-0.3	-0.3 5.82	29	31	
left at 1.47 (E)	0.34 $\pm$ 1.12	-0.3	-0.3 2.70	27	0	
1.22-1.52 (F)	0.04 $\pm$ 0.83	-0.3	<-0.3 3.00	14	4	

Distance Areas starting from Health Professional:  $A \leq 0.30$  m,  $0.30 \text{ m} < B \leq 0.61$  m,  $0.61 \text{ m} < C \leq 0.91$  m,  $0.91 \text{ m} < D \leq 1.22$ ,  $E = 1.47$  m,  $1.22 \text{ m} < F \leq 1.52$  m. Glove flight path consisted of an area of two side by side  $0.30 \text{ m}^2$  tiles in front of the health professional along with a  $1.22 \text{ m}$  path to the wastebasket. One  $0.30 \text{ m}^2$  tile was placed  $1.47 \text{ m}$  left of health professional, to display contamination outside glove flight path. Plaque Forming Units (PFU) per milliliters (mL)

A significant negative correlation ( $p < 0.05$ ) was measured between PR772 concentrations and the increasing distances from the health professional (Figure 2). Observing the central tendencies within distances  $< 0.61$  m, PR772 median concentrations were  $2.79 \log_{10}$  PFU/mL at  $< 0.30$  m and  $2.78 \log_{10}$  PFU/mL at  $0.30$  m -  $0.61$  m (Figure 2). The PR772 mean concentrations for these same distances are  $2.61 \pm 1.87 \log_{10}$  PFU/mL at  $0 - 0.30$  m and  $2.32 \pm 1.96$  within  $0.30$  m -  $0.61$  m (Table 2). Yet, as distances increase farther than  $0.61$  m from the health professional, the means tended to decrease from  $2.61 \pm 1.87 \log_{10}$  PFU/mL ( $\leq 0.30$  m) to  $0.04 \pm 0.83 \log_{10}$  PFU/mL ( $1.22 - 1.52$  m) (Table 2). PR772 concentrations were significantly greater at distances  $\leq 0.61$  m than at distances  $> 0.61$  m away from the health professional ( $p < 0.05$ ). Although PR772 concentrations tended to decrease with distance, eight samples collected within the  $0.91$  m -  $1.52$  m range (Areas C, D, & F) had PR772 concentrations that exceeded the mean concentrations in the  $< 0.31$  m areas (Table 2).

## Bacteriophage Comparison between Distances



**Figure 2: Bacteriophage Concentrations (log<sub>10</sub> PFU/mL) by Distances from Participant Disposing of Gloves Toward Waste Basket**  
Compare medians of contamination for areas ≤ 0.61 m (blue) to areas >0.61 m (purple) from the health professional disposed of their gloves, seeded with bacteriophage PR772 by flinging/tossing/throwing into the wastebasket 1.22 m away.  
\*\*\* p < 0.05 Plaque Forming Units (PFU) per milliliters (mL)

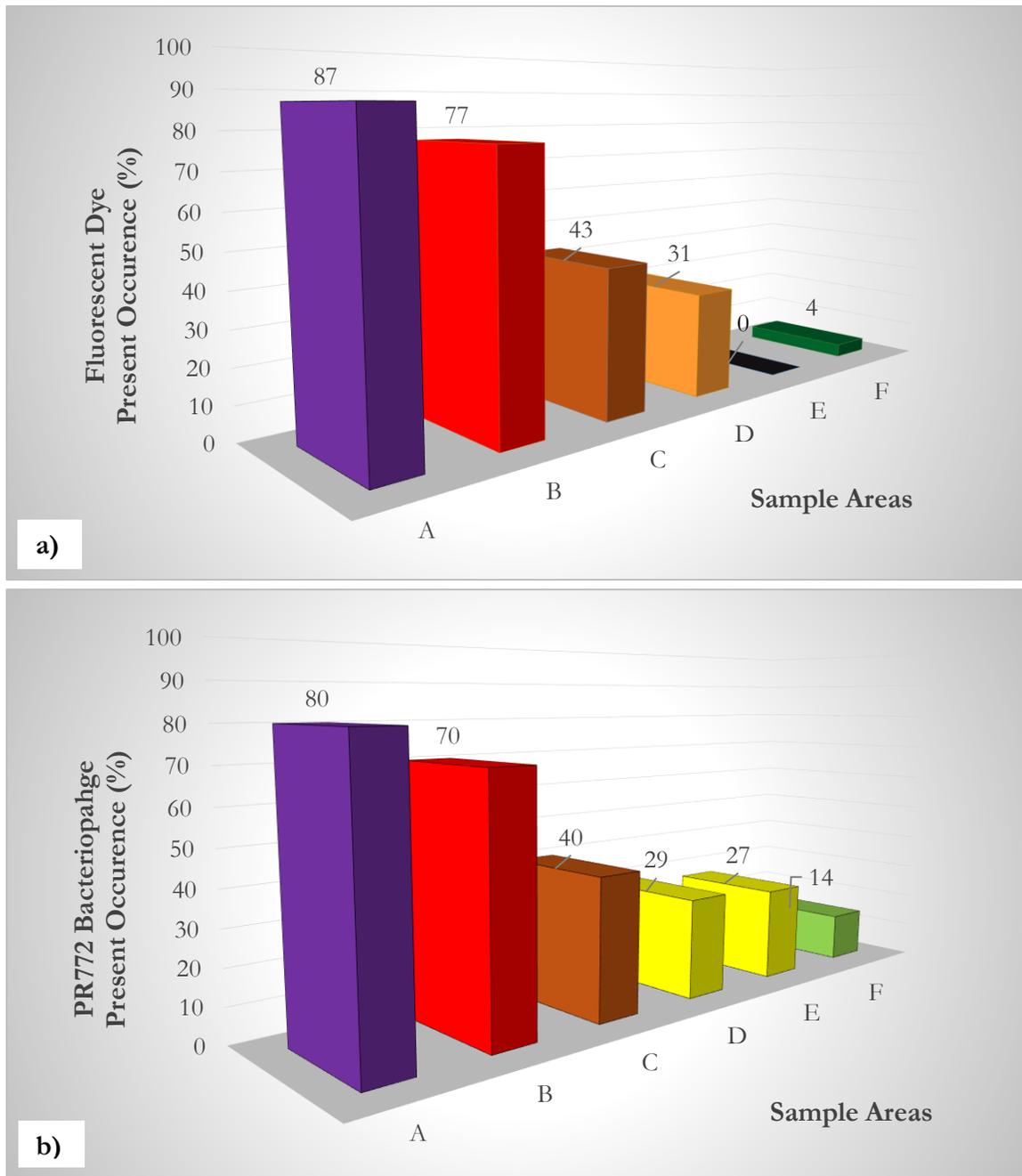
### *Glove fling and Fluorescent dye*

Fluorescent dye was also added to the donned gloves along with the PR772. The fluorescent dye was detected all along the glove flight path except F1-F5 and F8-F9 which happen to be areas along the wall behind the wastebasket (Figure 1). Fluorescent dye was not detected on area E1, left and outside the glove flight path. It should be noted that fluorescent dye stains were detected outside the designated sample areas, within a 0.61 m circumference of the health professional, but PR772 was not measured at these spots.

### *Glove fling and Fluorescent dye and Bacteriophage Comparison*

After all trials, F4 had neither fluorescent dye nor PR772. F4 was located along the wall just top left of the wastebasket (Figure 1). Areas between 1.22 m – 1.52 m recorded the lowest positive occurrences for both bacteriophage and fluorescent dye, 14% and 4% respectively (Table 2). Area E1, symbolizing surfaces or equipment outside glove flight path, recorded 27% positive occurrence for PR772 and 0% for fluorescent (Table 2). A decrease in both fluorescent dye and bacteriophage positive percent occurrence by area was measured with increased distance from the health professional.

The fluorescent dye stains (Figure 3a) was compared to the PR772 positive occurrences (Figure 3b) on the sample areas from all 15 health professionals. Similarities between positive fluorescence and PR772 bacteriophage detection occurred mostly within Areas A, B, C, D which happen to be along the glove fling flight path. Most of the sample areas sections were equal in occurrence or amid a difference of 7% for fluorescent dye and PR772, especially along the glove flight path's left half (tiles A1, B1, C1, D1, Figure 1). Differences of the fluorescent dye and bacteriophage occur more frequently within areas E and F. Fluorescent dye occurs on 4 out of 10 area F tiles (1.22 m – 1.52 m) and PR772 occurs on 8 out of 10 'F' tiles (Figure 3a), with a PR772 concentration mean of 0.04 log<sub>10</sub> PFU/mL (Table 2). No positive fluorescent dye occurred on area E (Figure 3a) yet PR772 contaminated area E, 4 out of 15 times (Figure 3b).



**Figure 3: Tracer Positive Present Occurrence by Sample Area and Distance from Health Professional**

a) Fluorescent Dye Positive Present Occurrence by Sample Area and Distance from Health Professional. b) Bacteriophage PR772 Positive Present Occurrence by Sample Area and Distance from Health Professional. Distance of Sample Area from Health Professional Disposing Gloves: **A**:  $\leq 0.30$  m; **B**:  $0.30 \text{ m} < \text{B} \leq 0.61$  m; **C**:  $0.61 \text{ m} < \text{C} \leq 0.91$  m; **D**:  $0.91 \text{ m} < \text{D} \leq 1.22$  m; **E**:  $1.47$  m outside glove flight path; **F**:  $1.22 \text{ m} < \text{F} \leq 1.52$  m. Sample Area IDs relate to placement within Figure 1

Approximately 83.0% of the 299 sampled areas (F18 sample area is missing due to loss of bacteriophage sample during laboratory analysis) were positive for both fluorescent dye and PR772 bacteriophage contamination, 6.4% were positive for only bacteriophage, and 10.7% positive only for fluorescent dye (Table 3). McNemar’s test concluded no significant difference between bacteriophage and fluorescent dye contamination ( $p = 0.0687$ ), indicating the fluorescent dye was a reasonable surrogate for bacteriophage during the PPE disposal experiments.

**Table 3: Comparison of Bacteriophage & Fluorescent Contamination on all Sample Areas**

<b>Fluorescent Contamination</b>	<b>Bacteriophage Contamination</b>	
	<b>Positive</b>	<b>Negative</b>
<b>Positive</b>	63	19
<b>Negative</b>	32	185

\*total tiles = 299 due to missing data for sample area F18. Not a statistically significant difference ( $p = 0.0687$ ) between the contamination of bacteriophage and fluorescent on tiles when gloves disposed of inappropriately from 1.52 m away from wastebasket.

## DISCUSSION

Gloves are the most utilized type of PPE in healthcare (Girou et al., 2004; Mitchell et al., 2013). However, neither the WHO or CDC have detailed PPE disposal protocols for used gloves; only doffing methods. One study detected pathogenic bacteria on 86% of tested healthcare professional’s gloves (Girou et al., 2004). Some of these gloves (~4%) end up on the floor during disposal practices (Hallihan et al., 2015) and some of the microorganisms on the gloves will contaminate the surrounding environment

and the floor, as the current study demonstrated. Therefore, when gloves contaminate floors and surfaces, it may lead to microbial spread throughout the healthcare facility, due to objects such as pens, bags, and cellphones falling/placed on contaminated floors and surfaces then transferred to high touched surfaces outside the room.

This is the first study to my knowledge that explores inappropriate glove disposal practices and the contributions to environmental microbial contamination. PR772 bacteriophage and a fluorescent dye were used 1) to measure the overall spread of bacteriophage and fluorescent dye from glove disposal to the surrounding environment, 2) to determine the contamination along the glove flight path and the distance from the health professional, and 3) to evaluate fluorescent dye's ability to serve as a teaching tool for PPE disposal scenarios in lieu of bacteriophage.

#### *Environmental Contamination from Glove Fling*

The primary objective of this study was to confirm environmental contamination from inappropriate glove disposal. Existing literature evaluates contamination from various PPE doffing protocols such as gloves, gowns, aprons but lack information on disposal contamination (Alhmidi et al., 2016; Bell et al., 2015; Casanova et al., 2008; Guo et al., 2014; Kang et al., 2017; Lai et al., 2011; Mitchell et al., 2013; M E Tomas et al., 2015). In the current study, environmental contamination stemming from flinging seeded gloves towards a wastebasket was found throughout all sampled areas (except area F4) with a maximum concentration of 6.32 log<sub>10</sub> PFU/mL (Table 2). On area E1 (left and outside the glove flight path), there was a 27% positive PR772 bacteriophage occurrence but a 0% fluorescent dye occurrence. In addition, fluorescent dye was observed outside the sample areas, during the glove disposal experiment, when showing

health professionals the possible contamination from their actions. Most of the fluorescence was detected within a 0.61 m circumference of the health professional. Additionally, fluorescent dye was found on health professionals' wrists, fingers, and forearms. The presence of both tracers on surfaces and skin, within the vicinity of flung gloves, indicates the potential for contaminated gloves to spread microorganisms through an environment. To reduce surface contamination in healthcare settings, facilities should adopt and enforce surface cleaning practices and CDC-recommended glove doffing methods which should include a further developed and detailed used glove disposal protocol.

#### *Contamination According to Distance*

Another objective of this study was to determine the distance of contamination along the glove flight path. There was an expectation that contamination would be along the flight path and higher contamination around the wastebasket but unexpectedly the higher contamination was around the health professional.

With the majority of PR772 and fluorescent dye detection within 0.61 m of the health professional, this finding coincides with previous studies (Guo et al., 2014; Lai et al., 2011). These studies state that the environmental contamination within a 0.61 m circumference of the health professional results from the health professional's personal devised way of doffing gloves rather than following CDC doffing methods (Guo et al., 2014; Lai et al., 2011). The current study found a significant difference ( $p < 0.05$ ) in the bacteriophage concentration values between distances  $\leq 0.61$  m and  $> 0.61$  m.

Environmental contamination detected along the glove flight path and doffing areas represents a risk for microbial exposure to anyone, including patients and environmental

cleaning services staff, present in the nearby area. This risk should be considered when defining doffing and disposal policies and patient care.

Significant PR772 concentrations were detected in areas  $\leq 0.61$  m from the health professional. Although in areas  $>0.61$  m up to the wall at 1.52 m (e.g. sample areas C, D, F), some PR772 swabs collected high concentrations that reached  $5.82 \log_{10}$  PFU/mL. This is a concern since many opportunistic pathogens and highly infectious diseases are infectious at or below these levels (Dowell et al., 1999). The PR772 concentrations on surfaces, in this study, represent a potential hazard, not only to people within the glove disposal area but also those outside the immediate area who may come into contact with contaminated surfaces or objects (Bhalla et al., 2004; Boyce et al., 1997; Dowell et al., 1999). Contaminated objects, initially impacted from disposal or objects that fell or placed on contaminated surfaces can be moved to other areas, therefore transferring pathogens throughout a healthcare facility and possibly exposing those who are not wearing PPE because they are not in an area required to wear PPE, such as a nurse's station.

Area E1 represented surfaces and equipment outside the glove flight path to indicate possible contamination to areas beyond the direct line of glove disposal. The current study identified PR772 presence in this area 27% of the time following glove flings. Based on this information, environmental contamination can reach objects and surfaces such as medical equipment, bedrails, tables, and floors outside the glove flight path which may become a reservoir for pathogens (Bhalla et al., 2004; Boyce et al., 1997).

PR772 and fluorescent dye contamination results from this study indicate that microbial contamination from health professional glove doffing process is possible beyond the already known distance (0.61 m) (Guo et al., 2014; Lai et al., 2011). These tracers reached a distance up to 1.52 m when inappropriately disposing of gloves. Based on this spatial analysis, environmental cleaning protocols should include doffing areas, especially floors and all stationary equipment used on patients. Gloves should never be flung, tossed or thrown but gently placed into a wastebasket. Sanitation wipes should be easily accessible to doffing area to wipe down any portable equipment if left in the room during doffing and also if gloves accidentally land on the floor.

#### *Glove Disposal Contaminating Floors*

Results from the current study highlighted the spread of microorganisms from seeded gloves to the area surrounding wastebaskets, including the floor. Recent floor studies demonstrated that hospital floors are “under-appreciated reservoirs for pathogens” and considered a potential principal transmission site for HAIs (Boyce et al., 1997; Deshpande et al., 2017; Gupta et al., 2017; Koganti et al., 2016; Suzuki et al., 1984). With nearly 50% of health professionals flinging gloves unsuccessfully into the wastebasket in the current study, floor contamination should receive additional consideration in HAI associated pathogen transmission process. Another study identified that 41 out of 100 hospital rooms had between 1-4 high touch objects (e.g. cell phone chargers, canes, clothing, call buttons, blood pressure cuffs, bed linens, towels, and pillows) that touched the floor (Deshpande et al., 2017). Other studies show direct and indirect pathogen transfer between floors and hands by fomites in contact with the floor contamination (Deshpande et al., 2017; Koganti et al., 2016; Suzuki et al., 1984). This

current study found that inappropriate glove disposal can be one of the many activities leading to contaminated environments and floors; leading to the spread of HAI associated pathogens throughout the healthcare environment.

#### *Fluorescent Dye and Bacteriophage Comparison*

The last objective was to determine if fluorescent dye could be a surrogate for PR772 bacteriophage since it is larger than MS2. Comparison between MS2 and fluorescent dye has been researched when studying PPE doffing (Casanova et al., 2008; Myreen E. Tomas et al., 2015). The current study suggests that fluorescent dye is an appropriate surrogate when demonstrating the contamination from inappropriate glove disposal to assist in behavior awareness. Fluorescent dye results were not significantly different from bacteriophage contamination in this scenario which can assist in health professionals understanding how pathogens can contaminate their surroundings, similar to previous studies (Alhmidi et al., 2016; Casanova et al., 2008; Tamimi et al., 2014; Myreen E. Tomas et al., 2015). This teaching tool can give rapidly visualized results: educating and training health professionals on pathogen transmission while instilling the understanding and need to follow PPE disposal protocols (Bell et al., 2015; Guo et al., 2014; Lai et al., 2011; M E Tomas et al., 2015).

#### *Public Health Professional Behavior and PPE Compliance*

Glove disposal protocols should be adopted in everyday situations to keep transmission of HAI associated pathogens minimal and ensure protection for all within healthcare facilities. During the glove disposal experiment, one health professional stated that “flinging gloves into the wastebasket is a cool trick to show the patients and they love it.” Inappropriately discarding used gloves calls attention to all health professionals’

behavior and awareness of the possible pathogen contamination and transmission due to limited policies and their personal disposal procedures.

From a paramedic survey, self-reported discrepancies were found during hand hygiene practices (Barr et al., 2017). Although paramedics state knowing the importance of hand hygiene; there is practice gap reported that over 50% of paramedics often do not perform hand hygiene immediately before or after touching a patient or their surroundings (Barr et al., 2017). This current study showed a recall bias; there was a discrepancy between the health professionals' survey statement on their glove disposal method—0% stated not flinging gloves—and their actions when disposing the gloves during the experiment—33% flung their gloves. It was found that 53% of those health professionals flinging gloves successfully into the wastebasket, executed without awkwardly doffing the gloves; suggesting previous experience with such methods.

Behavior studies state that mixed policies from institutions, hospitals and/or government and a lack of in-depth training lead to a relaxed atmosphere between coworkers on compliance with PPE protocols (Carthey et al., 2011; O'Boyle Williams et al., 1994; Pirincci and Altun, 2016; Wilson et al., 2017). From this survey, over 50% of health professionals reported observing their peers improperly disposing of PPE. This survey result may be due to, as some studies have shown, a disconnect of definitive CDC PPE disposal policies, disposal training, and practicing said policies for health professionals which leads to additional pathogenic transmission and self-contamination (Aftab et al., 2016; O'Boyle Williams et al., 1994; Pirincci and Altun, 2016; Wilson et al., 2017). However, there is evidence that, with more training, health professionals will comply with policies (O'Boyle Williams et al., 1994). Behavior and compliance must

integrate in practice to lower potential pathogen transmission within the healthcare industry.

#### *Glove Disposal Suggestions*

Glove disposal protocols should entail the following steps: 1) ensure wastebasket is at least 1.52 m away from equipment, 2) doff gloves over the wastebasket, 3) doff away from patients and those within the room, 4) place (don't fling) used gloves into the wastebasket, and 5) areas outside the 0.61 m disposal site should be included in routine cleaning practices. Infection control policies should be reevaluated to include cleaning protocols for equipment and surfaces farther than 0.61 m since analysis (Guo et al., 2014). Studies have shown reduction in environmental contamination when increasing cleaning of surfaces and floors (Gallimore et al., 2008; Martinez et al., 2003). Further training of CDC-recommended doffing methods and a proper disposal procedure (gently placing gloves in the wastebasket) posting above patient room wastebaskets could also minimize the environmental contamination.

#### *Future Studies and Limitations*

Future research studies should elaborate on these results by comparing the inappropriate glove disposals with appropriately, gently placing gloves into the wastebasket. Future studies should also be undertaken utilizing several bacteriophage surrogates to highlight the possible movement of multiple pathogens, as the current study was initially focused on EVD and an associated surrogate (i.e. PR772).

A limitation was the assumption that all health professionals participating knew the CDC protocol for removing used gloves (CDC, 2014). The significant difference of contamination between the two distances of  $\leq 0.61$  m and  $>0.61$  m might be due to the

differences in CDC doffing methods and personally devised doffing methods rather than the disposal method of flinging gloves into the wastebasket (CDC, 2014). Studies have shown that CDC-recommended PPE removal methods significantly decreased environmental contamination than personal doffing methods, after a video demonstrated CDC removal methods was shown to participants (Guo et al., 2014; Lai et al., 2011).

## **CONCLUSION**

Doffing full PPE for highly infectious diseases (e.g. gown/coverall, apron, double gloves, face mask, hood, boot covers, face shield/goggles), such as EVD, is important to protect health professionals during the rare events. However, highly infectious disease scenarios are rare in US hospitals while gloves are used every workday and have the potential to contaminate the surrounding surfaces during improper doffing and disposal practices. Therefore, proper doffing and disposal techniques are required to minimize pathogen transmission from solid gloves. Lack of reoccurring education on pathogen transmission and training on PPE use might encourage flinging gloves into the wastebasket then pathogens could significantly contaminate 0.61 m around the health professionals' environment, with a possibility of contaminating up to 1.52 m. Inappropriate disposal of used gloves can lead to floor and environmental surface contamination and possible transmission, infecting health professionals and other patients. Establishing industry-wide policies, adequate training and education to health professionals on appropriate glove disposal can ensure a healthier environment.

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