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Inactivation of Bacteriophage $\Phi 6$ on Tyvek Suit Surfaces by Chemical Disinfection

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Inactivation of Bacteriophage $\Phi 6$ on Tyvek Suit Surfaces by Chemical Disinfection

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A thesis submitted to the faculty of Georgia State University in partial fulfillment of the requirements for the Master of Public Health degree in the Division of Environmental Health

Georgia State University
2015

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ABSTRACT

Travis Brown: Inactivation of Bacteriophage $\Phi 6$ on Tyvek Suit Surfaces by Chemical Disinfection
(Under the direction of Lisa M. Casanova)

The 2014 West Africa Ebola outbreak saw a substantial number of healthcare workers (HCWs) being infected, despite the use of personal protective equipment (PPE). PPE is intended to protect HCWs when caring for patients with Ebola virus disease (EVD), but PPE may play a role in the spread of Ebola in healthcare environments. Before the removal of PPE, chemical disinfection may prevent the transfer of pathogens to HCWs, but the efficacy of common disinfectants against enveloped viruses, such as Ebola, on PPE surfaces is relatively unknown. The purpose of this study is to assess the efficacy of two common disinfectants, chlorine bleach (Clorox® bleach) and quaternary ammonium (Micro-Chem Plus®), used in healthcare settings for inactivation of enveloped viruses on PPE. The virucidal activity of the two disinfectants were tested against bacteriophage $\Phi 6$, an enveloped, non-pathogenic surrogate for enveloped viruses, on Tyvek suit surfaces. Virus was dried onto Tyvek suit surface, exposed to the disinfectants at use-dilution for a contact time of one minute, and the surviving virus was quantified using a double agar layer (DAL) assay. The Clorox® bleach and Micro-Chem Plus® produced a $>3.21 \log_{10}$ reduction and $>4.33 \log_{10}$ reduction, respectively, in $\Phi 6$ infectivity. The results of this study suggest that chlorine bleach and quaternary ammonium are effective in the inactivation of enveloped viruses on Tyvek suit surfaces. Chemical disinfection of PPE should be considered as a viable method to reduce the spread of pathogenic, enveloped viruses to HCWs, patients, and other environmental surfaces in healthcare settings.

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LIST OF ABBREVIATIONS

Φ6	Bacteriophage Phi6
BE	Beef Extract
DAL	Double Agar Layer
DI	Deionized
EVD	Ebola Virus Disease
HCW	Health Care Worker
mL	Milliliter
μL	Microliter
PBS	Phosphate Buffer Solution
PFU	Plaque Forming Units
PPE	Personal Protective Equipment
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

1. INTRODUCTION

1.1. Background

Personal protective equipment (PPE) is intended to protect health care workers (HCWs) from pathogen exposure, but PPE can also serve as a vector for pathogen transmission to the wearer, patient, and the surrounding environment (Casanova, Sobsey, Rutala & Sobsey, 2008). During the 2014 West Africa Ebola outbreak, the nosocomial transmission of Ebola virus to HCWs, despite the use of PPE, raised questions regarding the role of PPE in the transmission of viral pathogens. Previous studies show that enveloped viruses can survive on PPE for at least 4 hours and can transfer to HCWs skin and clothing during the PPE removal process (L. Casanova, Rutala, Weber, & Sobsey, 2010).

Infectious virus can be transferred by a contaminated surface, so the interruption of viral transfer with chemical disinfection is vital to the control of nosocomial outbreaks (Boone & Gerba, 2007). Disinfection of PPE could serve as an important infection control measure in healthcare settings, but the efficacy of common disinfectants against enveloped viruses on PPE surfaces is unknown. Disinfectant efficacy on PPE must be evaluated to assist in the selection of an appropriate germicide. If a germicide is effective, the disinfection of PPE could become an integral part in the PPE removal process and serve as an emergency response method for HCWs in need of extraction.

The use of surrogate virus in disinfectant efficacy studies is recommended when investigating a highly infectious virus like Ebola (Steinmann, 2004). Bacteriophages are often ideal, advantageous surrogate viruses to use in virus survival and disinfectant efficacy studies (L. M. Casanova & Waka, 2013; Hoelzer, Fanaselle, Pouillot, Van Doren, & Dennis, 2013), mainly due to their ease of propagation and assay and lack of pathogenicity (Sinclair, Rose, Hashsham,

Gerba, & Haas, 2012). A recent non-pathogenic, surrogate virus candidate for enveloped viruses with human pathogenicity is bacteriophage $\Phi 6$ (Adcock et al., 2009). Bacteriophage $\Phi 6$ is a lipid enveloped RNA virus that is a member of the Cystoviridae family and a phage of *Pseudomonas* bacteria (Laurinavicius, Kakela, Bamford, & Somerharju, 2004). The lipid envelope of bacteriophage $\Phi 6$ makes it a potential model for numerous pathogenic lipid enveloped viruses including Ebola, influenza, and coronaviruses Severe Acute Respiratory Syndrome (SARS), and Middle Eastern Respiratory Syndrome (MERS). There have been no studies to date that use bacteriophage $\Phi 6$ as a surrogate virus in disinfectant efficacy experiments.

The use of bacteriophage $\Phi 6$ for studying disinfectant efficacy can increase our understanding of enveloped virus persistence after disinfection and the degree of which PPE contributes to nosocomial infection. Therefore, this study was done with the following aims:

- Evaluate the disinfectant efficacy of sodium hypochlorite and quaternary ammonium on Tyvek suit surfaces by the inactivation of bacteriophage $\Phi 6$.
- Determine which disinfectant is most effective for inactivating enveloped viruses on Tyvek suit surfaces

1.2. Study Objectives

The study objectives are:

- To determine the efficacy of inactivation of enveloped viruses using chemical disinfectants.
 - Quantify reduction of bacteriophage $\Phi 6$ on a Tyvek suit surface using sodium hypochlorite and quaternary ammonium compounds
- To suggest a recommendation for an effective disinfectant against enveloped viruses on personal protective equipment surfaces.
- To identify the advantages and disadvantages of using bacteriophage $\Phi 6$ as a surrogate for enveloped viruses.

2. LITERATURE REVIEW

2.1. Viral nosocomial infections

Nosocomial infections, or hospital-acquired infections, are infections that spread in healthcare settings (Aitken & Jeffries, 2001). Nosocomial infections are a common cause of morbidity in United States hospitals, with the most recent prevalence survey calculating 721,800 nosocomial infections in 2011 (Magill et al., 2014). With emerging and re-emerging viral infections, such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Ebola virus, viral nosocomial infections are becoming of increasing concern (Edmond, Diekema, & Perencevich, 2014; Pedrosa & Cardoso, 2011). It is difficult to quantify rates of viral nosocomial infections due to improper surveillance techniques and differential laboratory diagnostics in hospitals (Sattar, 2004), but it is estimated that 5-32% of all nosocomial infections can be attributed to viruses (Valenti, Menegus, Hall, Pincus, & Douglas, 1980)

Transmission of viruses can occur through a variety of pathways, including direct and indirect contact, airborne droplets, and vehicles, such as food, water, medical equipment, and a variety of other environmental surfaces (Boone & Gerbia, 2007). Healthcare settings, such as hospitals and nursing homes, in which human-to-human contact is frequent, provides optimal conditions for an infectious outbreak (Aitken & Jeffries, 2001). Viral nosocomial infections are of great concern for both patients and healthcare workers (HCWs) given the following two factors: the large prevalence of pathogenic organisms and a large prevalence of immunocompromised individuals in one contained setting (Pedrosa & Cardoso, 2011).

Fomites are porous and nonporous surfaces or objects that can harbor pathogenic microorganisms and act as a vehicle in pathogen transmission (Boone & Gerbia, 2007). Environmental surfaces in healthcare settings play a significant role in the transmission of

viruses (Dancer, 2009). Despite disinfection, viruses have the ability to survive on a variety of hospital surfaces for extended periods and times and can transfer to hands of HCWs (Otter, Yezli, & French, 2011). Nosocomial viral transmission to an affected patient mainly occurs through the hands of HCWs, but contaminated objects, surfaces, and air is both directly and indirectly involved in transmission as well (Weber, Rutala, Miller, Huslage, & Sickbert-Bennett, 2010). Environmental surfaces in hospitals that can harbor viruses include patient care items, dialysis machines, medication, bathroom surfaces, patient room surfaces, and personal protective equipment (L. Casanova, Alfano-Sobsey, Rutala, & Sobsey, 2008; Cozad & Jones, 2003)

2.2. Survival and Transfer of Viruses on Personal Protective Equipment

Personal protective equipment (PPE) is used to prevent the transmission of pathogens to healthcare or laboratory personnel (Edmond et al., 2014). Proper and consistent use of PPE is essential to reducing nosocomial transmission and protecting patients and HCWs (Zellmer, Van Hoof, & Safdar, 2015), but improper use of PPE may play a part in the transmission of viral nosocomial infections. The West Africa Ebola outbreak saw a large number of HCWs contracting Ebola, which was attributable to two factors: insufficient supply of PPE and variation in the PPE donning and doffing procedures (Fischer, Hynes, & Perl, 2014). The West Africa Ebola outbreak in 2014 and the SARS outbreak in 2002 raised concerns that HCWs could contaminate their skin and clothes during PPE doffing, which results in self-inoculation and spread of virus to patients, other HCWs, and fomites (L. Casanova et al., 2008)

Enveloped viruses are thought to not survive as long as naked viruses on environmental surfaces, but enveloped viruses can survive on surfaces long enough to spread infection, particularly in healthcare settings (Russell, Hugo, & Ayliffe, 1999). Virus survival data suggests that enveloped viruses can remain infectious on a variety of PPE materials for at least 4 hours

(L. Casanova et al., 2010). Considering the risk of infection involved with PPE, doffing of PPE without contaminating skin or clothing is vital to the prevention of viral transmission to the wearer. Contaminated PPE can spread pathogens to other surfaces in the environment that can become a vector for pathogen transmission. Transmission of virus to HCW skin and clothing is frequent even when following proper CDC protocol (L. Casanova et al., 2008), and the majority of HCWs do not remove PPE in the correct order and fail to properly dispose of contaminated PPE (Zellmer et al., 2015). In response to the Ebola outbreak of 2014, the CDC announced new recommendations for type of PPE used when caring for patients with Ebola virus disease and detailed guidelines for the donning and doffing processes (CDC, 2014). It is unknown if the new guidelines are effective in preventing wearer contamination.

2.3. Interruption of Viral Transmission with Chemical Disinfection

Environmental surfaces that are contaminated can easily transfer virus, so disinfection is vital to the interruption transmission and is an important infection control measure (Boone & Gerbia, 2007). Disinfection is the process of eliminating pathogenic microorganisms, excluding bacterial spores, from an inanimate surface (Rutala, 1996). Proper disinfection of a surface relies on a variety of factors including the disinfectant-surface system, disinfectant concentration, and contact time (Rutala, Barbee, Aguiar, Sobsey, & Weber, 2000). Improper disinfection practices can lead to nosocomial infection outbreaks (Otter et al., 2011)

Enveloped viruses are more sensitive to chemical disinfection than naked viruses, because of their larger size and lipophilic nature (Russell et al., 1999). Inactivation of naked viruses by chemical disinfection is assumed to involve damage to structural and functional proteins of the virion and its nucleic acids, but inactivation of enveloped viruses may only depend on damage to the lipid envelope, making them more susceptible to disinfection (Klein &

Deforest, 1983; Sattar, Springthorpe, Karim, & Loro, 1989). However, enveloped viruses may be less susceptible to disinfectant inactivation than is realized, due to variations in natural or stimulated environmental conditions (Sattar et al., 1989).

Interruption of virus transfer from contaminated to clean surfaces can be achieved with a proper disinfectant (Sattar, 2004), with an ideal disinfectant having a broad spectrum of biocidal activity. There are a variety of disinfectant classes with virucidal activity, including halogens and cationic surfactants (Russell et al., 1999). The selection of a proper disinfectant is vital, because use of an ineffective disinfectant product with a good surfactant can free virus from surface without inactivating it, and thus making the virus more readily available for transfer (Sattar, 2004). Sodium hypochlorite, a halogen class disinfectant, is a broad-spectrum disinfectant that is commonly used in conditions where hazardous agents are present, but its disinfectant effectiveness and cleaning ability depends upon the concentration of available chlorine and pH of the solution (Fukuzaki, 2006). Sodium hypochlorite inactivates virions through structural alteration, alteration of viral markers, and alteration of the viral genome (Maillard, Hann, Baubet, & Perrin, 1998; Russell et al., 1999). Quaternary ammonium compounds, another commonly used, broad-spectrum disinfectant, are cationic surfactants that are most active against lipophilic, or enveloped, viruses, whose primary inactivation mechanism is through alteration of the viral envelope (Jimenez & Chiang, 2006).

2.4. Testing for Disinfectant Efficacy

Previous studies investigating the virucidal activities of chemical germicides primarily use two different methods: suspension testing and quantitative carrier testing. Numerous studies have been done using suspension tests (Rutala et al., 2000), but it is suggested that disinfection studies need to be standardized by using the carrier-based method with virus being dried onto a

specific surface (Sattar, Springthorpe, Adegbunrin, Zafer, & Busa, 2003). The carrier-based test method involves the inoculum in question being exposed to disinfectant after being dried onto a model surface. Unlike the suspension test method, the carrier method closely mimics field conditions for surface contamination and virus transmission in healthcare settings (Bellamy, 1995). Pathogens in nature are more likely to be found absorbed and/or embedded into a surface, so the carrier-based test represents a more realistic, “worst-case-scenario” field situation, with the virus being dried onto the surface and more difficult to decontaminate (Sattar et al., 2003).

Surrogate viruses for disinfectant efficacy experiments are recommended for when the virus in question is highly infectious (Steinmann, 2004). Bacteriophages are often ideal, advantageous surrogate viruses to use in virus survival and disinfectant efficacy studies (L. M. Casanova & Waka, 2013; Hoelzer et al., 2013), mainly due to their ease of propagation and assay and lack of pathogenicity (Sinclair et al., 2012). A recent surrogate virus candidate for enveloped viruses with human pathogenicity is bacteriophage $\Phi 6$ (Adcock et al., 2009). Bacteriophage $\Phi 6$ is a lipid enveloped RNA virus that is a member of the Cystoviridae family and a phage of *Pseudomonas* bacteria (Laurinavicius et al., 2004). The lipid envelope of bacteriophage $\Phi 6$ permits it to be modeled after numerous pathogenic lipid enveloped viruses including Ebola, influenza, Severe Acute Respiratory Syndrome (SARS), and Middle Eastern Respiratory Syndrome (MERS).

3. MATERIALS AND METHODS

3.1. Preparation of Hard Water

All disinfectant dilutions were done in 400ppm hard water. The hard water was prepared from the following two stock solutions: 14.01g NaHCO₃ in 250mL Dracor water (Solution 1) and 7.06g MgCl₂ and 18.50g CaCl₂ in 250mL Dracor water (Solution 2). Solution A was filtered using a 0.22µm pore size filter, and solution B was autoclaved at 121°C for 20 minutes.

The hard water was prepared by adding 12mL of solution 1 to a 1000mL volumetric flask, adding 600mL of Dracor water, adding 12mL of solution 2, and diluting to the 1000mL mark with Dracor water. The solution was then transferred to a sterile 4L container and diluted with 2000mL Dracor water. The final solution was then adjusted to the appropriate pH (7.6-8.0) by adding 1N HCl dropwise.

3.2. Chemical Disinfectants

The disinfectants sodium hypochlorite (Clorox® bleach) and quaternary ammonium (Micro-Chem®) were evaluated in terms of their effectiveness in the inactivation of bacteriophage φ6 dried onto Tyvek suit surfaces. Both disinfectants required dilution and were prepared on the same day of experimentation using hard water as the diluent. Table 3.2.1 summarizes the disinfectant type, active ingredient, and use dilution. The bleach underwent a 1:10 dilution (0.825% hypochlorite) and the Micro-Chem Plus® a 1:64 dilution. Fresh chlorine bleach was purchased for each experiment to account for the loss of free chlorine concentration over time.

Table 3.2.1. Disinfectants Tested for Reduction in Infectivity

Disinfectant	Disinfectant Type	Active Ingredient	Use-dilution
Clorox® Bleach	Halogen	8.25% sodium hypochlorite	1:10
Micro-Chem Plus®	Quaternary ammonium	2.25% alkyl (60% C14, 30% C16, 5% C12, 5% C18) dimethyl benzyl ammonium chlorides & 2.25% alkyl (68% C12, 32% C14) dimethyl ethylbenzyl ammonium chlorides	1:64

3.3. Neutralizing Solutions

Neutralizing solutions were used to halt the disinfection process of the germicides. This ensured a specific contact-time with the virus by chemically altering the germicide to make it inactive. The neutralizing solutions for this study were 0.1% sodium thiosulfate for chlorine bleach and 3% glycine for the quaternary ammonium. The selection of these neutralizing solutions is based upon previous research (Rutala, Peacock, Gergen, Sobsey, & Weber, 2006).

Table 3.3.1 outlines the neutralizing solutions used for each germicide

Table 3.3.1. Neutralizing Solutions

Disinfectant	Neutralizing Solution
Micro-Chem®	3% glycine
Clorox® bleach	0.1% thiosulfate

3.4. Testing for Disinfectant Toxicity to Bacterial Host Cells

The disinfectant-neutralizer residues can interfere with the ability to form a lawn of bacteria needed for the plaque assay, thus the toxic effects on bacterial host cells can influence the quantification of surviving virus after disinfection. The *Pseudomonas syringae* host cells were exposed to the sodium hypochlorite/thiosulfate and quaternary ammonium/glycine combinations to test for any toxic effects that might interfere with the quantification of virus on the host cell lawn.

P. syringae cell monolayers were exposed to disinfectant-neutralizer and visually compared to controls that were not exposed to disinfectant-neutralizer on a light box. Bleach and

quaternary ammonium solutions were diluted in 400ppm hard water to simulate the hardness of water that may come out of the tap. For each disinfectant, 50 μ L of disinfectant, 950 μ L of the corresponding neutralizer, and 1mL of host were prepared in 3 glass test tubes. Each disinfectant-neutralizer-host test tube was added to a tube of top agar and then poured onto a TSA plate. For the control trial, only host and top agar were poured onto TSA plates. The disinfectant-neutralizer plates were visually inspected for toxic effects and compared to the control plates after 24 hours of incubation.

3.5. Testing for Disinfectant Interference with Viral Infection

Exposure of *P. syringae* host cells to disinfectant-neutralizer residues can interfere with the ability of the bacteriophage to infect the cells. If there is disinfectant interference present, the reduction in number of plaques can be attributed to the disinfectant, when it is actually damage to the host cells. To test for disinfectant interference, two parallel titers were done. The first titer mixed *P. syringae* host cells with the disinfectant/neutralizer combination, and the second titer, or the control, was a titer assay of the bacteriophage stock. The two titers were compared to examine differences among the two. If the disinfectant/ neutralizer titer is significantly lower than the control titer, this indicates that the disinfectant/neutralizer is interfered with the ability of the bacteriophage to infect the host cells.

Disinfectants were diluted in 400 ppm to appropriate concentration. Two sets of virus dilutions were made for the disinfectant/neutralizer titer and the control titer. A total of 10 host tubes for each disinfectant tested were prepared with 50 μ L of disinfectant, 950 μ L of the corresponding neutralizer, and 1mL of *P. syringae* host. A double agar layer (DAL) assay was performed using the host tubes, and the control titer was done by a standard DAL.

3.6. Recovery of Bacteriophage Φ 6 from Tyvek Suit Surface

The efficiency of recovering bacteriophage $\Phi 6$ from a Tyvek suit surface was determined using 1.5% beef extract as the eluent. Two time trials were done at 0 and 60 minutes. The $\Phi 6$ stock solution ($\sim 10^8$ PFU/mL) was thawed and 100 μ L was pipetted into 900 μ L of PBS solution. Six Tyvek suit carriers were autoclaved and 10 μ L of $\Phi 6$ in PBS solution was placed on each carrier. For the time 0 trial, three of the carriers were immediately placed in 50mL centrifuge tubes with 5mL of 1.5% beef extract and shaken for 20 minutes. The eluent from each centrifuge tube was assayed along with 5 dilutions. The same procedure was done for the other carriers after 60 minutes of contact time with $\Phi 6$ solution. A titer assay of the $\Phi 6$ virus stock was also done to calculate concentration of stock.

3.7. Disc-based Quantitative Carrier Test Method for Virus Disinfection

A total of 6 replicate experiments were done for each disinfectant. For each experiment, three control carriers (no disinfectant applied) and three test carriers (disinfectant applied) were assayed. The carriers were approximately 1 cm² coupons cut from a Tyvek suit. The protocol used for the carrier test method for virus disinfection was adapted from methods used by Sattar et al (2003).

The carriers were placed in separate wells of a Pyrex 9-well glass plate. The $\Phi 6$ stock was diluted in PBS to a concentration of approximately 10^7 PFU/mL, and 20 μ L was applied to each carrier for a target concentration of 10^5 PFU/mL on each carrier. The virus was left on the surface of the carrier until it completely dried (3 hours). After the virus dried, 50 μ L of disinfectant was placed on the three test carriers for one minute, and then 950 μ L of corresponding neutralizing solution was placed on each. For the control carriers, 50 μ L of TSB was applied for one minute, and 950 μ L of TSB afterwards. To elute virus from surface, 150 μ L of 15% beef extract solution (pH 7.5) was added to each carrier well. The carriers gently shook

for 20 minutes. The eluent from each carrier was assayed along with 5 dilutions and negative controls. For the negative controls, host cells were only exposed to TSB and disinfectant-neutralizer.

To quantify the reduction in $\Phi 6$ infectivity by chemical disinfection, the concentration of virus on the test carriers after disinfection was calculated and compared to the concentration of virus on the control carriers. Reduction was expressed as N_t/N_0 , where N_0 is the concentration of virus recovered from the control carriers and N_t is the concentration of virus recovered from the disinfectant carriers after contact time t .

3.8. Statistical Analyses

The data was stored, organized and analyzed using Microsoft Excel 2013. Before statistical analyses, plaque assay data underwent logarithmic transformation to produce a normal distribution. All statistical analysis was performed with a level of significance at $\alpha=0.05$. For the percent recovery, disinfectant interference, and disinfectant efficacy experiments, mean values were reported along with 95% confidence intervals. GraphPad Prism version 6 was used to create graphs and figures.

4. RESULTS

4.1. Test for Viral Elution off Tyvek Surface

The efficiency of eluting $\Phi 6$ off the Tyvek suit surface was evaluated by drying virus on Tyvek carrier and exposing the carrier to the 1.5% beef extract eluting solution. For the three replicate experiments done, three carriers were exposed to virus and immediately put in the beef extract solution (t=0 minutes), and three carriers were exposed to virus, allowed to dry after an hour, and then put in beef extract solution (t=60 minutes). A total of nine carriers at each time were examined. Viral titer of the stock solution was determined as a reference of how much virus was applied to the carrier surface. The viral elution efficiency was quantified by calculating the percent recovery and \log_{10} reduction in infectious titer. The results can be seen in Table 4.1.1.

The carriers that were exposed to virus and immediately placed in 1.5% beef extract solution and had a percent recovery of 20.2% (95% CI [19.3, 21.5]), or a 0.69 \log_{10} (95% CI [0.66, 0.72]) reduction in infectious titer. The carriers that were exposed to virus for 60 minutes before being placed in the 1.5% beef extract solution had a lower percent recovery of 14.9% (95% CI [14.4, 15.3]), or a 0.83 \log_{10} (95% CI[0.81, 0.84]).

Table 4.1.1 Percent Recovery and Log Reduction of $\Phi 6$

Trial (n=9)	Percent Recovery (95% CI)	Log Reduction (95% CI)
0 Minutes	20.2 (19.3, 21.5)	0.69 (0.66, 0.72)
60 Minutes	14.9 (14.4, 15.3)	0.83 (0.81, 0.84)

4.2. Test for Disinfectant Toxicity to Bacterial Host Cells

The toxic effects of the disinfectant/neutralizer combination were observed through visual inspection of host cell monolayers using a light box. The exposure of 1:10 Clorox® bleach and 0.1% sodium thiosulfate residues to *P. syringae* host cells did not interfere with the ability to form a lawn of bacteria needed for the DAL assay. The same result was seen for the 1:64 Micro-

Chem® and 3% glycine residues. No toxic effects were observed, and the host cells were able to form a lawn of bacteria needed for the DAL assay.

4.3. Test for Disinfectant Interference with Φ6 Infectivity

To determine if disinfectants were interfering with the ability of Φ6 to infect host cells, titer assays of the virus stock were performed using host cells that were exposed to disinfectant/neutralizer combinations and host cells that were unexposed (controls). The viral titers of the Clorox® bleach and Micro-Chem® trials were then compared to the unexposed controls. If the 95% confidence intervals of the disinfectant/neutralizer samples overlapped with the control sample, this suggests that there is no interference with Φ6 infectivity. The results can be seen in Table 4.3.1.

Both the Clorox® bleach and Micro-Chem® viral titers produced 95% confidence intervals that did not overlap with the control. The Clorox® bleach produced a significantly higher viral titer at 10.15 PFU/mL (95% CI [10.13, 10.18]) than the control at 10.04 PFU/mL (95% CI [10.00, 10.07]), while the Micro-Chem® produced a significantly lower viral titer at 9.48 PFU/mL (95% CI [9.38, 9.56]), suggesting a loss of 0.5 log₁₀ when using Micro-Chem®.

Table 4.3.1 Disinfectant/Neutralizer Interference with Φ6 Infectivity

Sample (n=3)	Viral titer (log₁₀PFU/mL)	95% Confidence Interval
Chlorine bleach	10.15	(10.13, 10.18)
Micro-Chem	9.48	(9.38, 9.56)
Control	10.04	(10.00, 10.07)

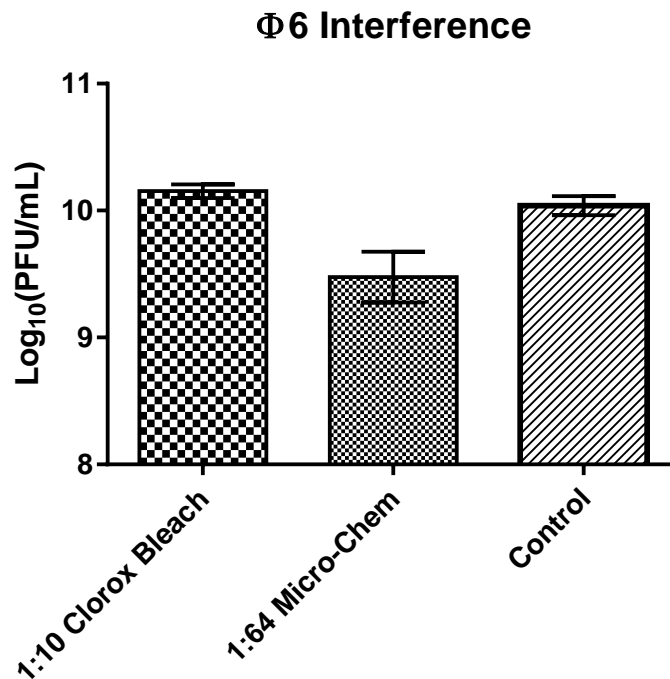


Figure 4.3.1. Disinfectant Interference with Φ6 Infectivity

4.4 Test for Disinfectant Efficacy against Φ6 on Tyvek Suit Surface

The log₁₀ reductions of Φ6 infectivity on Tyvek suit surfaces by Clorox® bleach and Micro-Chem Plus® can be seen in Table 4.4.1. The log₁₀ reductions in each disinfectant were calculated by two independent exposure trials, with a total of 18 carriers evaluated for each disinfectant. For both the Clorox® bleach and Micro-Chem Plus® trials, no plaques were present after disinfectant exposure. However, the calculations were done assuming that 1 plaque was present in the original sample, reflecting a detection limit of 1PFU/carrier. The calculated mean log₁₀ reductions indicate the detection limit of the technique used. The Clorox® bleach and Micro-Chem Plus® produced a >3.21 log₁₀ reduction and a >4.33 log₁₀ reduction in Φ6 infectivity at one-minute contact time, respectively. The 95% confidence intervals of the two disinfectants do not overlap, suggesting a statistical difference in the effectiveness of the disinfectants.

Table 4.4.1 Mean Log₁₀ Reduction in Φ6 Infectivity

Disinfectant	Mean Log ₁₀ Reduction (n=18)	95% CI
Clorox® bleach	3.21	(2.59, 3.82)
Micro-Chem Plus®	4.33	(4.22, 4.44)

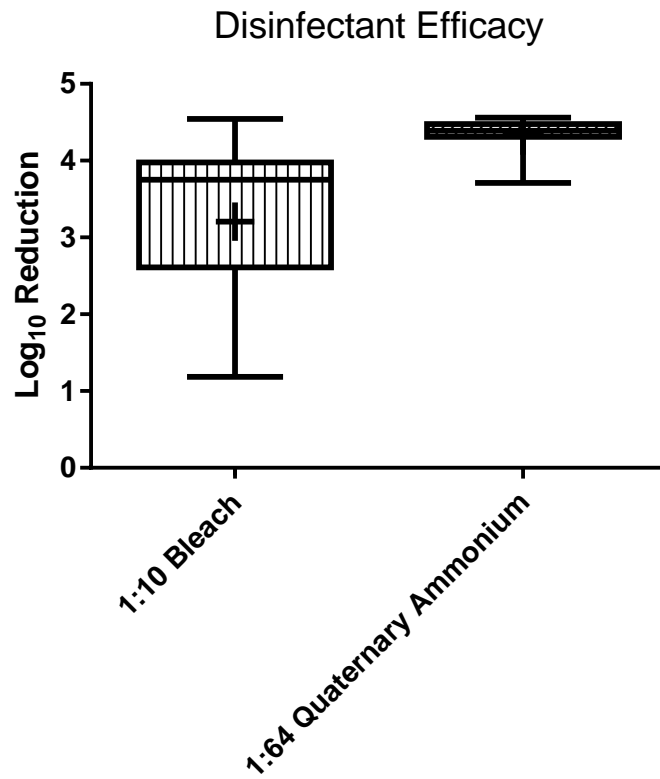


Figure 4.4.1. Disinfectant log₁₀ reduction of Φ6 on Tyvek Surface

5. DISCUSSION

PPE may serve as a vector in the transmission of pathogenic, enveloped viruses to HCWs, patients, and other environmental surfaces in healthcare settings. The use of chemical disinfectants on PPE surfaces may serve as an effective infection control method, but the efficacy of chemical disinfectants against pathogenic, enveloped human viruses is unknown. The goal of this study was to determine the effectiveness of inactivating enveloped viruses on PPE surfaces by chemical disinfection. The inactivation of bacteriophage $\Phi 6$, a surrogate for pathogenic, enveloped human viruses, on Tyvek suit surfaces using common disinfectants used in healthcare environments was evaluated. The two chemical disinfectants evaluated were sodium hypochlorite (Clorox® bleach) and quaternary ammonium (Micro-Chem Plus®).

Potential cytotoxic effects of disinfectants and their corresponding neutralizers were tested to determine if the disinfectant/neutralizer residues damaged the cell monolayers that are required for the quantification of virus in the DAL assay. A previous study by Sattar et al. (2003) suggests that disinfectants be at use-dilution for the testing of cytotoxic effects to host cell monolayers. The Clorox® bleach and the Micro-Chem Plus® were subjected to a 1:10 and 1:64 dilution, respectively, and inactivated with 0.1% sodium thiosulfate for the bleach and 3% glycine for the quaternary ammonium. No cytotoxic effects were observed upon visual inspection of host cell monolayers, so the disinfectants were then further examined by testing for interference of virus infectivity.

The exposure of host cells to disinfectant residues can interfere with the ability of the virus to infect the cells. In virus disinfection experiments, the reduction of virus can be wrongly attributed to inactivation by the disinfectant, when the disinfectant is actually interfering with the ability of the virus to infect the host cell. The loss of infectivity could possibly be from interference with viral attachment to the host cells or interference with virus replication inside

the host cell (Sattar et al. 2003). These outcomes result in overestimation of disinfectant effectiveness and underestimation of viral titer.

The viral titer of control samples (unexposed to disinfectant) and experimental samples (exposed to disinfectant) were calculated, and the subsequent 95% confidence intervals were compared to determine significance. If confidence intervals overlap, this suggests that there is no interference occurring from exposure to the disinfectant. Both the chlorine bleach and quaternary ammonium compound produced viral titers and 95% confidence intervals that did not overlap with the control. Using the 95% confidence intervals to compare, the chlorine bleach viral titer was significantly higher than the control by 0.11 log₁₀, while the quaternary ammonium viral titer was significantly lower than the control by 0.56 log₁₀. The quaternary ammonium compound produced a larger difference in viral titer, implying there was slight interference from exposure to the disinfectant. However, the interference was minimal, so the quaternary ammonium efficacy was still evaluated despite some interference. Due to this interference, the log₁₀ reduction in Micro-Chem Plus® titer is overestimated by about 0.5 log₁₀.

Before the efficacy of a disinfectant against a virus can be assessed, the efficiency of recovering the virus from a carrier using an eluting solution needs to be determined. The recovery of Φ6 from Tyvek suit surfaces using 1.5% beef extract as the eluent was determined. The virus stock (~10¹⁰ PFU/mL) was diluted in PBS to 10⁹ PFU/mL and 10μL was placed on each carrier (~10⁷ PFU). For the 0 minute trial, the percent recovery was 20.2% or a 0.69 log₁₀ reduction viral titer, and after drying, the 60 minute trial produced a 14.9% recovery or a 0.83 log₁₀ reduction in viral titer. Even though this is a low percent recovery of Φ6 from the Tyvek surface, the viral titer is still high enough to observe an efficient log reduction in disinfection efficacy experiments. For the disinfectant efficacy experiments in this study, approximately 10⁵

PFU are placed on each carrier. The \log_{10} reduction of $\Phi 6$ after the virus dried on the surface still can produce an observable reduction of $>4 \log_{10}$ after exposed to disinfectant.

Disinfectant efficacy was evaluated against bacteriophage $\Phi 6$, a surrogate for pathogenic, enveloped viruses. The disinfectants evaluate were chlorine bleach (Clorox®) and quaternary ammonium (Micro-Chem Plus®). The disinfectants were considered effective if they achieved a $3 \log_{10}$ reduction in $\Phi 6$ infectivity. This threshold value was based on previous studies that evaluate disinfectant efficacy against a variety of enveloped viruses (Rutala, 2000; Sattar, 2004).

The 1:10 (0.825% hypochlorite) Clorox® bleach produced a >3.21 (95% CI [2.59, 3.82]) \log_{10} reduction in $\Phi 6$ infectivity at a 1 minute contact time. A contact time of 1-minute was chosen to represent a more realistic scenario in the disinfection of Tyvek suits. This finding is consistent with Sattar et al. (1989), which reported a 99.9% reduction, or a $3 \log_{10}$ reduction, in human coronavirus 229E after one-minute contact time with 0.10% and 0.50% sodium hypochlorite. Another study found that 1:100 hypochlorite demonstrated a $<1 \log_{10}$ reduction in surrogate SARS-CoV, Mouse Hepatitis Virus and Transmissible Gastroenteritis Virus, but the low reduction could possibly be attributed to low concentration of hypochlorite and high chlorine demand of medium used to suspend virus (Hulkower, Casanova, Rutala, Weber, & Sobsey, 2011). A recent study evaluated hypochlorite's efficacy against Ebola virus on PPE, which found that dilute concentrations of hypochlorite at 0.5% and 1% produced 2.9 and 2.2 \log_{10} reduction, respectively, at 1 minute contact-time, and there was no recoverable virus after 5 minute contact time. (Cook et al., 2015). The Ebola virus disinfection study did not report disinfection efficacy results in \log_{10} reductions, so they were calculated from the results section, but once accounted for, the results are very similar to the present study, which indicates bacteriophage $\Phi 6$ may be an ideal surrogate for Ebola virus in disinfection studies. . Current recommendations are to use a

1:10 bleach solution for the disinfection of environmental surfaces in healthcare settings (Rutala & Weber, 2008). The present study finds that 1:10 bleach at 1-minute contact time could possibly be an effective disinfectant for the inactivation of enveloped viruses on PPE surfaces; however, the lower limit of the 95% confidence interval was slightly less than $3\log_{10}$

The Micro-Chem Plus® at 1:64 use-dilution produced a >4.33 (95% CI [4.22, 4.44]) \log_{10} reduction in $\Phi 6$ infectivity at one-minute contact time, suggesting that quaternary ammonium compounds are effective in the decontamination of enveloped viruses from Tyvek suit surfaces. However, there is an overestimation of the reduction of viral titer by approximately $0.5\log_{10}$ due to interference, but even accounting for the interference, the Micro-Chem® produces a reduction $>3\log_{10}$. Considering the interference, it is likely there is no difference in efficacy between the chlorine bleach and Micro-Chem Plus®. Quaternary ammonium compounds are membrane active agents that interact with the lipid envelope of enveloped viruses (Gerba, 2015), so this may explain the efficacy of Micro-Chem Plus against $\Phi 6$.

There is little literature on the effects of quaternary ammonium compounds on enveloped viruses, but this finding is consistent with the available literature. Also, it is important to note that a wide variety of quaternary ammonium compounds are available with different active compounds, so it is difficult to compare disinfectant efficacy in some cases. Numerous studies have evaluated the effects of quaternary ammonium compounds against norovirus and a variety of surrogates. Although non-enveloped virus was used, Jimenez & Chiang (2008) found that quaternary ammonium had a $6.4 \log_{10}$ reduction against feline calicivirus, a surrogate for norovirus, at 10 minute contact time. The present study used a 1 minute contact time to represent a worst case scenario, where a HCW does not wait around for 10 minutes for disinfection of Tyvek suit. Also, a 10 minute contact time may not be possible in an emergency situation. The

present study found a $>3\log_{10}$ reduction in viral titer after a contact time of 1 minute, suggesting that quaternary ammonium compounds achieve disinfection standards even when contact time is short.

The 2014 Ebola outbreak in West Africa saw a large number of nosocomial infections among HCWs, despite the use of PPE. Chemical disinfection of PPE before removal may serve as an infection control practice. This study shows that common disinfectants used in healthcare settings may be effective in the reduction of enveloped virus on PPE surfaces. The chlorine bleach and quaternary ammonium compounds effectively produced a $>3 \log_{10}$ reduction in $\Phi 6$ viral titer. The bleach underwent a 1:10 dilution (0.825% hypochlorite), which is the current recommendation for disinfection of hospital surfaces, and the quaternary ammonium compound was at use-dilution, as recommended by the manufacturer. Disinfectant toxicity to the wearer must first be examined to identify any negative health outcomes before disinfectants are used on PPE.

The present study finds that the disinfectants are effective in reducing viral titer on Tyvek surface, but future research needs to focus on this application in practice. Human studies that evaluate the transfer of $\Phi 6$ on PPE to underlying clothes and skin of HCWs need to be evaluated before recommendations can be made. This study also finds that bacteriophage $\Phi 6$ is a viable surrogate virus candidate in disinfectant efficacy experiments. The findings are mostly consistent with other disinfectant efficacy experiments using a variety of other enveloped viruses. The use of bacteriophage $\Phi 6$ as a surrogate virus for enveloped viruses may have significant implications for future studies, due to the virus's ease of propagation and assay and lack of pathogenicity.

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