Spring 4-23-2019

Survival of Potential Ebola Virus Surrogates (Bacteriophages Φ6 and MS2) on PAPR Hood Material

Cody Clemmons

Survival of Potential Ebola Virus Surrogates (Bacteriophages Φ6 and MS2) on PAPR Hood Material

by

Cody J. Clemmons

B.A., Biology
Coker College

A Thesis Submitted to the Graduate Faculty
of Georgia State University in Partial Fulfillment
of the Requirements for the Degree

MASTER OF PUBLIC HEALTH

ATLANTA, GEORGIA
30303

ABSTRACT

Survival of Potential Ebola Virus Surrogates (Bacteriophages Φ6 and MS2) on PAPR Hood Material

Cody J. Clemmons

04/23/2019

INTRODUCTION: One of the main lines of defense protecting healthcare workers from serious pathogens, like Ebola, during patient care is personal protective equipment (PPE). Personal protective equipment is wearable protection items worn by HCW to prevent the spread of disease, both from patient to HCW to patient and from patient to HCW. As PPE is barrier protection; it can become contaminated on its surface during patient care. This poses a risk when it is time to doff PPE at the end of use. Doffing is the detailed process of removing the PPE after patient care. Even with comprehensive training, self-contamination continues to occur. As self-contamination with PPE during the doffing process has been documented through several studies, the question of survivability of infectious diseases on PAPR hoods needs to be investigated.

AIM: The aim of this study is to determine the recovery and survival of bacteriophages phi6 and MS2 on shroud Powered Air Purifying Respirator Material (PAPR) at 45% relative humidity.

RESULTS: The linear regression line for MS2 at 45%RH showed a slope of -0.0099 ±0.0054, while the linear regression line for phi6 at 45%RH showed a slope of -0.028 ± 0.027. The projected log reduction calculated from the regression lines for phi6 is estimated to have a ~1 log10 reduction at 24 hours at 45% RH, MS2 is estimated to have a ~1 log10 reduction by 96 hours. MS2 is estimated to only have a .25 log10 reduction at 24 hours at 45% RH.

DISCUSSION: Both bacteriophage MS2 and phi6 survive on shroud PAPR hood material for over 24 hours at 45% relative humidity levels. The results provide evidence that both types of virus survive on PAPR hood material after the longest patient encounter, which is projected to be 8 hours. If a HCW's PPE were to be contaminated during their patient contact time, the virus is still active once the HCW doffs their PPE and the potential for self-contamination could occur.

APPROVAL PAGE

Survival of Potential Ebola Virus Surrogates (Bacteriophages Φ6 and MS2) on PAPR Hood Material

by

Cody J. Clemmons

Approved:

___Lisa Casanova, Ph.D._____
Committee Chair

__Christine Stauber, Ph.D. _____
Committee Member

__04/23/19_____
Date

ACKNOWLEDGEMENTS

I would like to express my appreciation to many people who guided, listened, and encouraged me throughout my thesis. First, a huge thank you to my chair, Dr. Lisa Casanova, for granting me the opportunity to work in her laboratory and explore a field of research that I find is very important in the overall aspect of public health. Secondly, thank you to Dr. Christine Stauber, who was a bright light on the early mornings of working in the laboratory. Guidance from both of these professors has been very valuable in my knowledge of the public health and environmental health fields. Another expression of gratitude goes out to Michael Kshatri who provided feedback, conversation and many laughs while in the lab together. Lastly, a special thanks goes to my supervisors, work friends and family. Without their support, laughs, and encouragement I would not have made it to the end of this program.

Author's Statement Page

In presenting this thesis as a partial fulfillment of the requirements for an advanced degree from Georgia State University, I agree that the Library of the University shall make it available for inspection and circulation in accordance with its regulations governing materials of this type. I agree that permission to quote from, to copy from, or to publish this thesis may be granted by the author or, in his/her absence, by the professor under whose direction it was written, or in his/her absence, by the Associate Dean, School of Public Health. Such quoting, copying, or publishing must be solely for scholarly purposes and will not involve potential financial gain. It is understood that any copying from or publication of this dissertation which involves potential financial gain will not be allowed without written permission of the author.

_____ Cody Clemmons_
Signature of Author

TABLE OF CONTENTS

List of Tables

List of Figures

INTRODUCTION

Background

The Ebola virus has been the cause of approximately 26 outbreaks in Africa since 1976, including three outbreaks since 2014 (WHO, 2019). Currently, the Democratic Republic of the Congo is battling an ongoing struggle with the spread of Ebola Virus Disease (EVD) across the country with around 848 cases (WHO, 2019). As hundreds of patients require healthcare to combat this highly infectious disease, the question of how healthcare workers (HCW) can be protected from transmission during patient care is particularly urgent. During the EVD 2013-2015 outbreak across Africa, the amount of HCW diagnosed with EVD was the highest reported in recorded history of EVD outbreaks, totaling 875 confirmed HCW cases out of 28,616 confirmed cases (Weber, Fischer, Wohl, and Rutala, 2015). While EVD is of great concern internationally for HCW, there is concern in the United States; patients with EVD were evacuated to the U.S for clinical care in the 2014 outbreak. Internationally and in the United States, serious pathogens of concern that may pose risks to healthcare providers also include Marburg, Lassa, pandemic influenza, and other emerging viral diseases. These pathogens can be both enveloped and non-enveloped viruses, and infection of HCW who provide care for these patients remains of great concern.

One of the main lines of defense protecting HCWs from serious pathogens like Ebola during patient care is personal protective equipment (PPE). According to the Centers for Disease Control and Prevention (2018), personal protective equipment is wearable protection items worn by HCW to prevent the spread of disease, both from patient to HCW to patient and from patient to HCW. PPE is a barrier approach, meant to prevent contact with substances that pose risks, including chemicals, body fluids, droplets, aerosols, and pathogens. PPE will vary for each disease,

depending on the route of transmission. As EVD is transmitted through direct contact with an EVD positive patient, their fluids, blood or contaminated surfaces, PPE is required for any patient interaction (CDC, 2018). EVD positive patients require the highest level of PPE, as this is a highly infectious virus and considered a risk group 4 viral agent. Risk group 4 agents most commonly cause very serious or deadly human disease for which treatment protocols are usually not obtainable (U.S. Department of Health and Human Services, 2015). In a laboratory setting, Ebola virus would only be manipulated within a biosafety level 4 (BSL4) containment laboratory, which requires a ventilated protective suit with an attached cord for breathing air. A BSL4 laboratory and the utilized PPE is the highest level of protection there is for highly infectious pathogens. The CDC (2018) currently recommends the following PPE when working with patients with confirmed EVD or unknown infectious diseases that display similar symptoms to EVD; single use impermeable coverall or gown, respiratory protection, eye protection, two pairs of disposable gloves, disposable boot covers, and a disposable single use apron. The goal of this PPE is for the provider to have no exposed skin or mucous membranes during patient care (CDC, 2018). Unlike PPE routinely worn in other types of laboratories or healthcare facilities, this type of PPE also includes some type of respiratory protection.

Respiratory protection can include either a N95 mask respirator (Figure 2.1) or a powered air purifying respirator (PAPR) (Figure 1.2). The National Institute for Occupational Safety and Health (NIOSH) (2018) describes a N95 respirator as a respirator that only covers the nose and mouth area; if eye splashes or exposure are a concern it must be combined with separate eye protection. The N95 respirator filters out approximately 95% of airborne particles. N95 respirators also must fit very closely to the face and requires fit testing by trained professionals. Additionally, N95 respirators can be more difficult to breathe in as they sit directly on your

mouth and nose, a face mask and eye protection are also needed with this type of respirator. It also cannot be worn by every individual. NIOSH (2018) describes a PAPR unit as a battery operated type of respirator. The unit utilizes a blower that delivers positive airflow (one direction) through a HEPA filter prior to the HCW breathing in the air; the HEPA filter also provides more air exchanges than a N95 mask. Another feature of the PAPR is that the hood attachment completely covers the face (without sitting directly on the mouth and nose) and a shroud PAPR hood covers the entire face, head, and neck. A PAPR is often preferred when working with EVD due to its protection against droplet and airborne particles, as well as protection against splashes. The units are bigger and more cumbersome when compared to a N95 respirator. Fit testing is not required for the use of a PAPR; therefore, it can be used more readily by those with facial hair or in an environment where fit testing is not available (NIOSH, 2018).

Figure 1.1: *Medical Products LTD: N95 respirator*

Figure 1.2: Enviro Safety Products: Shroud PAPR hood and blower

PPE is barrier protection; as such, it can become contaminated on its surface during patient care or laboratory work. This poses a risk when it is time to doff PPE at the end of use. Doffing is the detailed process of removing the PPE after patient care. For a high containment hospital room, a HCW would wear several layers of PPE, including scrubs, booties, Tyvek, apron, and double gloves. For shroud PAPR hoods, the physical challenge of doffing can be especially difficult due to the large size of the hood, multiple layers of material, and the heavy blower unit. Removing all of the pieces of PPE can be a cumbersome task and open up the opportunity for self-contamination. Specific training for the doffing process and continued proficiency testing are key in maintaining proper doffing techniques. However, even with comprehensive training, self-contamination continues to occur. As self-contamination with PPE during the doffing process has been documented through studies such as Casanova, Alfano-Sobsey, Rutala, Weber, and Sobsey (2008), Casanova et al. (2016) and Lim, Cha, Chae, and Jo (2015) the question of survivability of infectious diseases on PAPR hoods needs to be investigated.

For this study, two bacteriophages were tested on small sections of shroud PAPR hood material. A bacteriophage is a virus that infects bacteria. To reflect the behavior of a range of human viruses, an enveloped (phi6) and a non-enveloped (MS2) bacteriophage were used in this study. Bacteriophages are often utilized as surrogate viruses as while they mimic other infectious viruses; however, they do not require the high containment laboratories, the cost, and extensive biosafety training that the infectious virus requires. Previous studies such as Casanova and Waka (2013) analyzed the survivability of phi6 on a N95 respirator and Tung-Thompson et al. (2015) studied MS2 bacteriophage through aerosolization mechanisms to simulate infectious diseases. Additionally, Casanova et al. (2016) researched both phi6 and MS2 bacteriophages for

a study that analyzed how HCW can self-contaminate themselves during the doffing process of select PPE. Furthermore, a study focusing on the survivability of both bacteriophages on shroud PAPR hood material has not yet been published.

An environmental component that factors into the survivability of both bacteriophages on shroud PAPR hood material is relative humidity (RH). Several studies have explored bacteriophage survivability at multiple RH ranges from 20% RH through 98% RH and have demonstrated that infectivity and survival can be affected by RH. A study by Casanova, Rutala, Weber, and Sobsey (2010) demonstrates that RH is an important factor for survivability of virus on surfaces. Prussin et al. (2018) conducted a study that states that RH is the most important component when looking at virus infectivity in droplets and those that land on surfaces. Further studies for both enveloped and non-enveloped viruses should include RH as a factor as this environmental component plays an important role for virus survivability on PPE.

Purpose of the Study

As outbreaks of EVD and other highly infectious disease outbreaks continue to occur, HCW need to be protected against a variety of types of transmission. Shroud PAPR units are being used already in laboratory and healthcare settings in order to protect workers from infectious substances, including EVD. In order to design ways to prevent self-contamination by doffing of a shroud PAPR unit, evaluating the survivability of both phi6 and MS2 bacteriophages is important. Therefore, the objective of this research is to determine the survivability of both phi6 and MS2 bacteriophages on shroud PAPR hood material at 45%RH over selected time points within a 24 hour window.

LITERATURE REVIEW

Background

An article in the *MMWR-CDC* reported that in 2014, eleven cases of the Ebola virus disease (EVD) were diagnosed in the United States. While nine of the cases were patients that had contracted EVD prior to entering the US, two of the patients were healthcare workers that contracted the disease while performing patient care on the individuals already diagnosed with EVD. While both HCWs wore personal protective equipment (PPE), per the Centers for Disease Control and Prevention (CDC) guidelines, they still contracted EVD (Bell BP, Damon IK, Jernigan DB, et al. 2016). The World Health Organization (WHO) (2019) has reported that HCWs are 21-32 times more likely to contract EVD, versus a non HCW. During the 2014-2015 outbreak, over 8,000 health care workers, worldwide, responded (WHO, 2019). Overall, approximately 875 confirmed HCW cases of EVD were reported during the outbreak (Weber, Fischer, Wohl, and Rutala; 2015). As PPE is an important line of defense against the transmission of highly infectious diseases between the patient and healthcare worker, the PPE can be easily contaminated by a patient infected with EVD. Common symptoms of EVD include coughing, vomiting, and diarrhea. Any contact with these body fluids could contaminate the healthcare worker's PPE.

The investigation into how EVD was transmitted to the HCWs explored the possibility of self-contamination when assisting with or doffing their PPE (Petti et al, 2016). Furthermore, the 2014 outbreak made hospitals and governing officials reevaluate the plans in place for further outbreaks of highly infectious diseases, updating current CDC guidelines, reassess the current PPE requirements and training protocols for proper PPE donning and doffing when providing patient care to those infected with a highly infectious disease.

Respirators

Current CDC guidelines recommend the use of a N95 respirator or a powered, air-purifying respirator (PAPR) when working with patients specifically diagnosed ore suspected EVD (CDC, 2018). A N95 respirator can protect at 95% efficiency against certain particles (0.1 to 0.3 micron), aerosols and droplets sizes; however, is not equivalent to higher end filters, such as a HEPA (high efficiency particulate air) filter. A HEPA filter can remove up to 99.97% of particles that are greater than 0.3um in diameter (CDC, 2018). Additionally, no power source is required, they are easier to use with a stethoscope, and lastly, there is not a noise barrier. While there are many advantages to using a N95, there are disadvantages such as the required fit testing prior to use, they are harder to breathe in, and potential user error when donning (Roberts, 2014). Furthermore, the N95 must be worn with additional eye protection and a face shield. More of the HCWs skin is exposed and there is the potential for inward leakage if the respirator is exposed to body fluids, which is highly likely with an EVD patient (Coia et al., 2013).

A study by Roberts (2014) analyzes PAPRs and N95s to determine which type of respirator should be used with highly infectious agents. Per NIOSH (2018), a PAPR unit is a battery operated type of respirator. The unit utilizes a blower that delivers positive airflow (one direction) through a HEPA filter prior to the HCW breathing in the air; the HEPA filter also provides more air exchanges than a N95 mask. Another feature of the PAPR is that the hood attachment completely covers the face and a shroud PAPR hood covers the entire face, head, and neck. These features provided enhanced protection over the use of a N95 respirator. A few downfalls to the use of the PAPR include the noise associated with the blower unit. This can create difficulty when a HCW is using a stethoscope and creates a barrier for the stethoscope ear piece. Additionally, the PAPR unit is much larger and more cumbersome than the smaller N95

respirator. While the N95 respirator can be easily doffed from the face, due to the size of the PAPR hood, doffing can be more difficult and hence have the potential for a greater chance of self-contamination (Roberts, 2014). Coia et al. (2013) and Bunyan et al. (2013) have both provided research that establishes that respiratory protection when dealing with a patient with a highly infectious disease is vital in the prevention of transmission to the HCW. Additionally, both studies agree that a PAPR unit provides the greatest protection, but does require in depth training on doffing procedures in order to avoid self-contamination after patient care.

Bacteriophage Φ6

The use of bacteriophage Φ6 has been utilized in several studies to act as a surrogate to an enveloped virus, such as EVD. To study EVD directly, a high containment laboratory (HCL) would be required. High containment laboratories are extremely costly and required highly trained scientists. Most importantly, there is a greater risk when handling the EVD virus directly; therefore, gaining the information needed about the virus through a surrogate virus is ideal. Using a surrogate is less costly, can be conducted in lower level biosafety laboratories, and can be conducted more safely by removing the human pathogen aspect. One area of research that bacteriophage Ø6 is essential in studying is the infectivity and survivability of the virus on PPE that protects HCWs, specifically those that treat patients with highly infectious diseases. Several studies (Casanova et al. 2018; Brown et al., 2016; and Casanova et al., 2009) have heavily researched the bacteriophage Ø6 surrogate virus to determine the survival of enveloped viruses on personal protective equipment. All three studies conclude that bacteriophage Φ6 survives for longer than a standard session of patient care and can last up to 24 hours on PPE. Additionally, a study by Prussin et al. (2018) analyzed the infectivity of bacteriophage Φ6 with regards to relative humidity, absolute humidity, and temperature. The research demonstrates the complex

relationship between these factors and the infectivity of bacteriophage Φ6 (Prussin et al., 2018). These studies not only provide information on enveloped viruses such as EVD, but additional viruses such as influenza, coronavirus, SARS, and MERS which are all highly infectious pathogens.

Bacteriophage MS2

While enveloped viruses are of high concern in the healthcare field, non-enveloped viruses can also cause outbreaks and require HCWs to render patient care. For studies analyzing non-enveloped viruses, bacteriophage MS2 is often utilized. Brady et al. (2017) utilized bacteriophage MS2 to analyze the potential for self-contamination when HCWs doff a N95 filtering respirator. This information provides evidence that this surrogate virus has longevity (past 24 hours) on PPE and can indeed be the cause of self-contamination if the PPE is doffed improperly. Highlighting the requirement for proper and frequent training were also noted in the study in order to prevent self-contamination. An additional study, conducted by Tung-Thompson et al. (2015), performed their research with bacteriophage MS2 in order to mimic the human norovirus, a primary cause of gastrointestinal disease. Through their study, they simulated vomiting, which is a common symptom with many highly infectious diseases. The results conclude that droplets, from body fluid, are important in the route of transmission of disease. If these droplets were to land on a HCWs PPE, there is potential for self-contamination if the PPE is not doffed properly (Tung-Thompson et al., 2015). Lastly, Casanova et al. (2018) specifically analyzed the PPE that is worn by HCWs treating Ebola patients utilizing bacteriophage Φ6 and MS2. This study analyzed doffing procedures by highly experienced HCWs and also added the use of alcohol based hand rub (ABHR) during the doffing process. The use of bacteriophage MS2, as a surrogate non enveloped virus, provides evidence of the

longevity of the virus on PPE and how important proper doffing technique is for HCW to prevent self-contamination (Casanova et al., 2018).

Self-contamination from Personal Protective Equipment

There is a significant threat to the health and safety of healthcare workers that provide patient care to individuals with highly infectious pathogens. As PPE is vital in their protection against transmission of the disease, analyzing the survivability of the virus on their PPE, along with the potential for self-contamination has gained interest in the research field. Studies such as Brown et al. (2016), Casanova et al. (2010a), and Casanova et al. (2013) have all document the survivability of virus on PPE. Casanova et al. (2010a) confirmed in this study that enveloped virus can survive for up to 24 hours on PPE, which is much longer than a standard patient care session (approximately 30 minutes for non- containment). Casanova et al. (2013) discovered that an enveloped surrogate virus can persist on a N95 respirator for many hours (87 at 40% RH) if contaminated during patient care. Lastly, Brown et al. (2016) studied survivability and disinfection of surrogate virus on Tyvek suits, which is a common type of PPE used in high containment situations. This study again confirmed that enveloped virus survives on PPE longer than the standard patient encounter (Brown et al., 2016). From these studies, the importance of preventing self-contamination is demonstrated.

Several different perspectives have been addressed on how to determine how and if self-contamination occurs during the doffing process of PPE after patient care. A study by Bell et al. (2015) utilized the use of fluorescent markers to show how and where a HCW self-contaminates themselves during the doffing process. This study analyzed two sets of PPE, one commercially provided set for EVD patient care, and the other set of PPE that would be available at a hospital. Both sets of PPE were simulated with a splash to show how body fluids from a sick patient can

contaminate PPE. While all HCWs in the study were highly training on doffing techniques, the results of the study showed that self-contamination still occurred at least once with each set of PPE (Bell et al., 2015).

Another study by Chughtai et al. (2018) utilized the fluorescent markers and analyzed 10 different methods for doffing contaminated PPE to determine if one method would lessen the chances of self-contamination. PPE doffing methods were simulated from the World Health Organization, Centers for Disease Control and Prevention, Health Canada and European standard operating procedures. With 30 trained HCW volunteers, over 13% had self-contamination between the different PPE doffing methods. Overall, the least amount of self-contamination was discovered to be with the use of protocols that utilized a PAPR unit and also those with assisted doffing techniques. This study provides evidence that proper doffing technique is essential in protecting the HCW from self-contamination (Chughtai et al., 2018). A study by Zellmer et al. (2015) analyzed 30 HCWs and the doffing technique they were trained on prior to patient care. The study found that only 17% of the HCWs doffed their PPE as they were previously trained. Furthermore, the HCWs did not remove their PPE in the trained location in the patient room (Zellmer et al., 2015). Both of these findings are likely to lead to self-contamination when doffing contaminated PPE. A study by Tomas et al. (2015) also used fluorescents and bacteriophage MS2 to analyze how HCW self-contaminate during the doffing process. This study analyzed over 435 glove and gown doffing sessions within a 4 hospital system. Out of the 435 sessions, self-contamination occurred 46% of the time. While more self-contamination occurred with glove removal, than the gowns, they discovered that when being observed by a trainer or other HCW, less self-contamination was discovered (Tomas et al., 2015).

Furthermore, two studies by Casanova et al. (2016) and Casanova et al. (2018) specifically researched the doffing procedure of PPE while wearing EVD specific PPE. The study by Casanova et al. (2016) utilized bacteriophage MS2 (non-enveloped virus) and bacteriophage Φ6 (enveloped virus) to study self-contamination due to PPE doffing. The HCWs in this study were also professionally trained on how to doff their PPE. The results confirmed self-contamination (specifically with bacteriophage MS2) on their hands for 8 out of 15 HCWs (Casanova et al., 2016). Lastly, the study by Casanova et al. (2018) was conducted in a medical biocontainment unit using their standard operating procedures for doffing PPE. Again, this study analyzed bacteriophage MS2 (non-enveloped virus) and bacteriophage Φ6 (enveloped virus) for self-contamination on PPE and utilized ABHR in their doffing procedures. Through thorough and observed doffing training and techniques, along with very highly specialized HCWs, this study provided evidence that in conjunction with ABHR on hands, self- contamination did not occur for enveloped virus (Casanova et al., 2018).

Humidity

Previous studies have linked together the connection between survival and transmission of virus with humidity levels (Yang et al., 2012; Casanova et al., 2010b; and Lowen et al., 2007). Specifically, Casanova et al. (2010b) assessed how air temperature and relative humidity effect how coronavirus survives on environmental surfaces, such as stainless steel. Overall, this study confirmed that virus survival is greater at lower RH levels such as 20% or higher RH such as 80% RH when compared to a RH around 50% (Casanova et al., 2010b). The link between air temperature and relative humidity was also confirmed in this study, as previously noted in research conducted by Lowen et al. (2007). Lowen et al. (2007) demonstrated that viral transmission occurred more often at 5 degrees Celsius versus 20 or 30 degrees Celsius.

To continue the research of the relationship between humidity and survivability of virus, Prussin et al. (2018) conducted a study specifically looking at a surrogate virus (bacteriophage Φ6) and the survival of the surrogate with regards to relative humidity, absolute humidity, and temperature. This study analyzed droplets as that is a common form of contamination on HCWs, environmental surfaces and PPE. Their results showed that bacteriophage Φ6 "survived best at high (>85%) and low (<60%) RH". Overall, the study results demonstrated that "RH is the most important factor in controlling virus infectivity in droplets" (Prussin et al., 2018).

MATERIALS AND METHODS

Virus

For this experiment, two bacteriophages and corresponding hosts were utilized. Bacteriophage (Ø6) and host (*Pseudomonas syringae* HB10Y) were obtained from Dr. Leonard Mindich, University of Medicine and Dentistry New Jersey. The second bacteriophage (MS2) and host (Escherichia coli Famp) utilized in this experiment were obtained from Dr. Mark Sobsey, University of North Carolina – Chapel Hill. Host bacteria were stored in tryptic soy broth containing 20% (v/v) glycerol at -80C until use. For preparation of host culture for virus assay, *Pseudomonas syringae* bacteria was prepared by adding 1 mL of frozen host to 100 mL of tryptic soy broth. This mixture was placed on a rotating shaker (100 rpm, 21^0 C) for 18-24 hours. The second host, E. coli Famp, was prepared by scraping a small amount of frozen bacteria using a sterile wooden stick and adding to 25 mL of tryptic soy broth. Famp was placed on a rotating shaker (100rpm, 37^0 C) for 18-24 hours. As the E. coli Famp must be utilized during a log phase of growth, at 24 hours, 1 mL of the overnight host culture was placed in 100 mL fresh TSB, and placed on a rotating shaker (100 rpm, 37^0 C) for 2-4 hours until growth was clearly visible in the media. Top agar was prepared by the addition of agar to tryptic soy broth to a final concentration of 0.75% agar). For the MS2 portion of the assay, the top agar had 1 mL streptomycin/ampicillin added for every 100 mL of agar (USEPA Method 1602 is the reference here). Bottom agar was tryptic soy agar.

Titer Assay

Prior to the start of the time point experiments, each bacteriophage had a titer assay completed to determine the concentration of the virus stock (~10^{10} PFU/mL). The Ø 6 and MS2 virus stock solutions (~10^{10} PFU/mL) were both thawed and a dilution series was made with a target of ~10^{8} PFU/mL. 100uL of each ~10^{8} PFU/mL virus solution (each) was then mixed 1:1 for a final target concentration of ~10^{7} PFU/mL of each virus in the mixture. Virus mixture was used immediately after preparation.

Survival Experiment

Carriers were prepared by taking the shroud of a Powered Air Purifying Respirator (PAPR) (3M) and cutting the shroud material into 1 cm^2 squares. For each experiment, 10uL of the mixed virus solution mixture was placed onto each separate carrier. For the zero time point, three carriers were immediately placed into separate 50mL conical tubes with 1mL of 1.5% beef extract, pH 7.5, as the eluent. The three conical tubes were then placed on a rotating shaker (60 rpm, 21^0 C) for 20 minutes.

For the remaining time points (1.5, 2, 4, 6, 8 and 24 hours), 10uL of the virus mixture was placed onto three additional PAPR material carriers and positioned on a petri dish within a controlled humidity chamber at 45% (±2%) relative humidity (created by placing a saturated solution of magnesium chloride in a sealed chamber. Once each time point was reached, each carrier was immediately placed into an individual 50mL conical tubes with 1mL of 1.5% beef extract, pH 7.5, as the eluent. The three conical tubes were then placed on a rotating shaker (60 rpm, 21^0 C) for 20 minutes. Additionally, the determined drying time for the virus mixture was

determined to be 1.5 hours at 45% RH. Relative humidity of the environmental chamber was monitored during each experiment.

Each of the six conical tube's mixture was diluted out to 5 dilutions. Additionally, the virus mixture stock solution was diluted out to 5 dilutions. For each dilution, the double agar layer (DAL) plaque assay method was utilized with tryptic soy agar (TSA) plates. Each virus dilution, and corresponding top agar and host culture were mixed and distributed to individual TSA bottom agar plates. The Ø6 plates were incubated at 21^0 for 20-24 hours and the MS2 plates were incubated at 37^0 C for 20-24 hours. After each overnight incubation, the plates were read on a light box, plaques counted and the results were logged.

Statistical Analysis

Each time point was analyzed for virus survival and stated as $\log_{10} (N_t / N_0)$ for analysis. N_t denotes the virus concentration measured in PFU/mL at each time point, t. N_0 represents the original virus concentration in PFU/mL at time point zero for the (3) control samples for each time point.

Two programs were used for the statistical analysis of the data. Excel 2016 (Microsoft Corp.) and GraphPad Prism version 5 (GraphPad, San Diego, CA). For each virus, the mean of $\log_{10} (N_t / N_0)$ for each time point was utilized to complete a linear regression analysis.

RESULTS

Figures 4.1 and 4.2, below, display the survival of bacteriophage MS2 and Φ6 over a 24 hour time period at 45% RH. The p value for both bacteriophages is greater than 0.05; therefore, not statistically significant.

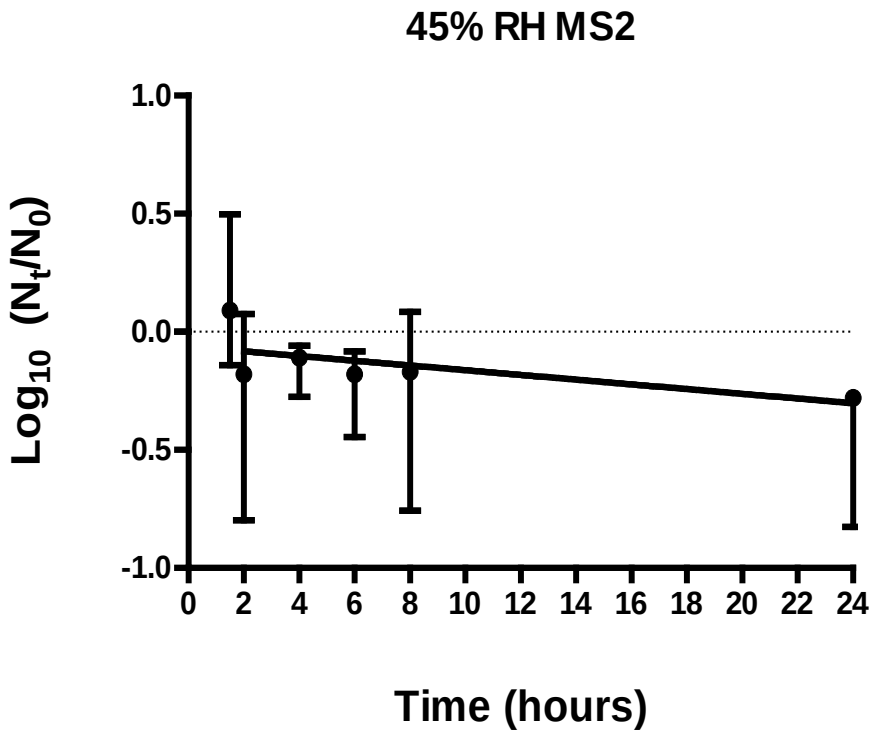

Figure 4.1 Survival of bacteriophage MS2 over 24 hours at 45% RH. 6 trials per point; 6 time points of observed data=points; bars=95% CI: linear regression analysis=line

Table 4.1: Slope of regression lines for virus inactivation of MS2 at 45% RH

RH	Slope	95% CI	P value
45%	-0.0099	+/- .1383	>0.05

Table 4.1 displays the slope of the regression line with 95% CI at 45% RH for MS2. At 45% RH, the slope was -0.0099 ± 0.0054. The p value was >0.05; therefore, not statistically significant.

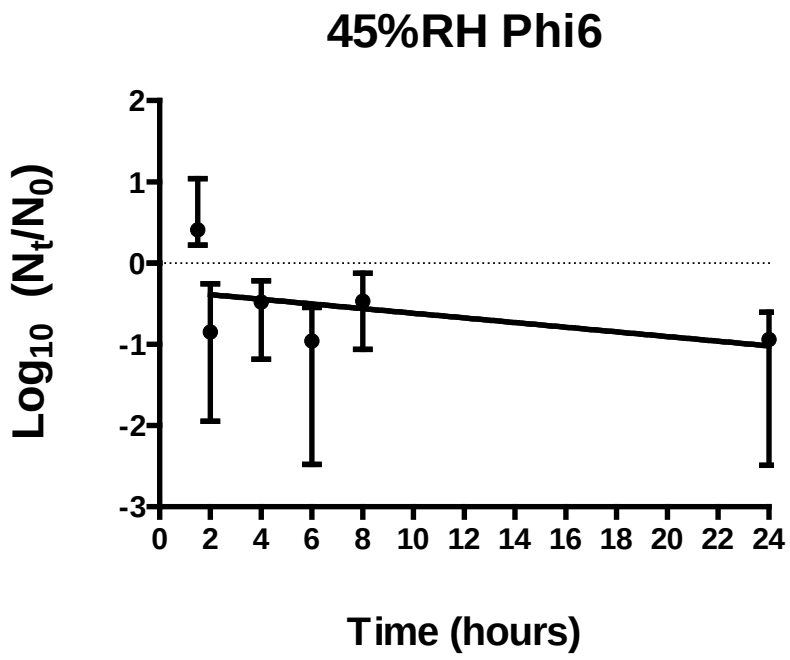

Figure 4.2 Survival of bacteriophage Φ6 over 24 hours at 45% RH. 6 trials per point; 6 time points of observed data=points; bars=95% CI: linear regression analysis=line

Table 4.2: Slope of regression lines for virus inactivation of Φ6 at 45% RH

RH	Slope	95% CI	P value
45%	-0.028	+/- 0.548	>0.05

Table 4.2 displays the slope of the regression line with 95% CI at 45% RH for Φ6. At 45% RH, the slope was -0.028 ± 0.027. The p value was >0.05; therefore, not statistically significant.

Table 4.3: Projected time (estimated in hours) for log reductions for phi6 and MS2 inactivation at 45% RH

45% RH	Reduction { $\log_{10}(N_t/N_0)$}	Inactivation Time (hour projection)
Phi6	-1 (90%)	24
	-2 (99%)	72
	-3 (99.9%)	96
	-4 (99.99%)	128
MS2	-1 (90%)	96
	-2 (99%)	192
	-3 (99.9%)	288
	-4 (99.99%)	384

Table 4.3 displays the projected log reduction was calculated from the regression lines for both phi6 and MS2. Reduction of both viruses is estimated for 90%, 99%, 99.9%, and 99.99%. While phi6 is estimated to have a ~1 $\log_{10}$ reduction at 24 hours at 45% RH, MS2 is estimated to have a ~1 $\log_{10}$ reduction by 96 hours. MS2 is estimated to only have a .25 $\log_{10}$ reduction at 24 hours at 45% RH. The longest patient encounter, while wearing a PAPR hood, is projected to be 8 hours. If a HCW were to be contaminated during their patient contact time, the virus is still active once the HCW doffs their PPE.

DISCUSSION

Discussion

The focus of this study was to analyze the survival of bacteriophage MS2 and Φ6 at 45% relative humidity over a 24 hour period on shroud hood material from PAPRs. . While the projected time for a HCW to wear a shroud PAPR hood is up to 8 hours in a high containment hospital setting, survival up to 24 hours was examined. Through the results of the current study, analyzing both enveloped and non-enveloped virus on shroud PAPR hood material, similar conclusions could be drawn when compared with previous studies such as Casanova et al. (2010) and Brown et al. (2016). These studies demonstrated that enveloped and non-enveloped virus can survive on PPE items for up to 24 hours or more. The current research established that both bacteriophages, Φ6 and MS2, underwent only X log reduction over 24 hours. While phi6 demonstrated a decrease in titer over the 24-hour window, (approximately a 1 $\log_{10}$ decrease after 24 hours,) MS2 bacteriophage showed a decrease of only ~ 0.25 $\log_{10}$ at 24 hours. To reach a ~4 $\log_{10}$ reduction for Φ6, extrapolation of results showed, would take approximately 96 hours. In the same time period of approximately 96 hours, there would be a reduction of only ~1 log10 for MS2; the results show a much longer survivability for non-enveloped virus.

Studies by Casanova et al. (2013) and Prussin et al. (2018) demonstrated survival on environmental surfaces at RH levels ranging from 20% to 98% RH. These studies provide evidence that RH is an essential variable influencing the survival of both types of viruses. Prussin et al. (2018) states that “relative humidity is the most important factor in controlling virus infectivity in droplets”. Relative humidity, in conjunction with temperature, affects the virus through what Prussin et al. (2018) believe is an “interaction between droplet physiochemical characteristics and virus physiology”. As relative humidity has played a role in

survivability of virus, the 45% RH in this study confirms the results from studies such as Prussin et al. (2018). Their study researched several relative humidity levels ranging from 23% to 98% RH, along with additional factors such as absolute humidity and temperature. This study done at 45% RH, falls in line with conclusions from other studies about how RH affects virus on surfaces, and in this case, shroud PAPR hood material (Prussin et al., 2018).

Furthermore, the results of this study are similar to previous research on survival of Ebola virus (enveloped) specifically (Schuit et al. (2016), Cook et al. (2015), and Sagripanti and Lytle (2011)). Schuit et al.'s (2016) research studied different surfaces such as stainless steel and polypropylene, which are common hospital materials, and contaminated those surfaces with Ebola virus. Their conclusions were similar in that the results showed survival much longer than 24 hours, specifically, Sagripanti et al. demonstrated only a 0.68 log reduction over a 24 hour period. Additionally, Sagripanti and Lytle (2011) provided evidence that even with ultraviolet radiation, which is a common method for decontamination for infectious substances, Ebola virus did not fully inactivate on a non-porous surface even with 254-nm radiation over six selected time periods, not exceeding 30 seconds. Cook et al. (2015) showed that Ebola virus can persist up to 192 hours on steel and plastic surfaces, again, both types of surfaces commonly found in a hospital setting. Furthermore, there is only a 4 log reduction at 365 hours for stainless steel. The Cook et al. (2015) study continued to provide evidence that proper decontamination of surfaces requires strict contact times and a disinfectant proven to inactivate the virus in question.

Additional information from the current study confirms the potential for self-contamination during the doffing process is a possibility as both Φ6 and MS2, have longevity on PPE longer than a typical 8 hour patient care time period. This means that virus may still be present at high concentrations on PAPR material when it is time for the HCW to remove the

PAPR. Studies such as Mumma et al. (2018), Casanova et al. (2018), Suen et al. (2018), Verbeek et al. (2016), and Zellmer et al. (2015) have demonstrated that even after extensive training on proper doffing techniques, self –contamination during doffing is still of great concern for HCW. With the potential for highly infectious substances remaining on shroud PAPR hoods after patient care, the doffing process is critical in prevention of transmission of the virus to not only the HCW, but also to other environmental surfaces and potentially other individuals. Mumma et al. (2018) analyzed 11 trained HCWs through the doffing process of PPE, including a PAPR unit. The study utilized human factors, fault tree analysis and behavioral coding to determine the steps of contamination. Overall, the results demonstrated 51 types of errors that could lead to self-contamination or the spread of contamination to other surfaces by the HCW doffing techniques. Specifically, PAPR hood doffing was one of the riskiest processes and the study showed that many errors occurred in that step of doffing. By analyzing each detailed step of the doffing process, the steps of self-contamination can be identified and solutions to prevent these occurrences can be put into place (Mumma et al., 2018). A study by Casanova et al. (2018) analyzed the specific doffing procedures for 10 HCWs that wear specifically, Ebola-level PPE. This study utilized both enveloped and non-enveloped bacteriophages, trained observers, and also utilized the alcohol based hand rub within the doffing procedures. The outcome from Casanova et al. (2018) confirmed the results of the current study in that if either virus is on Ebola type PPE surfaces, self-contamination is possible even with full training and observed doffing. Lastly, Suen et al. (2018) studied the doffing process of three types of PPE with 59 participants by utilizing a florescent stain to follow the route of transmission. This study showed the viral transmission does vary with the PPE that is worn, the ergonomics associated with donning/doffing of the PPE, and lastly if the standard protocol is followed or if any deviations

from the protocol occur (Suen et al., 2018). All of these studies confirm the findings from the current study in that if virus is on PPE, careful attention must be paid to the doffing procedure in order to prevent self-contamination to the HCW or their surroundings. As PPE is essential in the safety of the HCW, one specific piece of PPE that must be discussed is respiratory protection.

When analyzing PPE for HCW protection, there are two main types of respiratory protection, N95 and PAPRs, utilized by HCW when working directly with patients that have a highly infectious disease. There have been several studies on which type of respiratory protection provides the best protection against transmission and the possibility of self-contamination during the doffing process. A review of respiratory protection by Roberts, V. (2014), analyzed both N95 and PAPR usage amongst HCWs. The review concludes that there are advantages and disadvantages of both types of respiratory protection. While the PAPR units provide additional protection against droplets, they are more cumbersome, louder (due to the blower unit), and are difficult to use with a stethoscope for patient care. N95 respirators can be less expensive and more accessible; however, require fit testing prior to usage. Additionally, as N95 respirators fit closely to the face, they can cause discomfort due to heat, breathing resistance and can be tight or itchy on the facial skin. Furthermore, both types of respirators require training for proper doffing or self-contamination could occur (Roberts, V., 2014). Additional studies analyzing respiratory protection include studies by Hanoa et al. (2016) and MacIntyre et al. (2014). Both of these studies break down the variety of respiratory protection and the lack of consensus amongst policy makers (internationally and in the United States) on what is the best protection for HCW when providing patient care to patients with highly infectious diseases, such as EVD. Infection control programs at hospitals must conduct thorough risk assessments to determine the best type of respiratory protection for their staff. As both enveloped and non-enveloped viruses have

longevity on PPE, as shown in Casanova et al. (2010) and Brown et al. (2016) studies, providing evidence of survivability on shroud PAPR hoods is vital to reinforce the potential danger even when utilizing a respirator choice that is more protective than alternatives choices.

Recommendations

On a daily basis, the health and safety of healthcare workers is a top priority. As highly infectious diseases will continue to be a burden worldwide, protecting the people that provide patient care to individuals that contract the disease is essential for their health and safety, and preventing further spread of the disease. Studies have demonstrated that respiratory protection is a vital piece of PPE that can directly protect HCWs from highly infectious diseases; therefore, having properly trained HCWs is an essential part of infection control program for hospitals. Training these individuals on proper donning and doffing techniques will limit the amount of cases of self-contamination from the PPE doffing process. Continued training and competency training should play a role in the HCWs in order to maintain their knowledge and proficiency in the doffing of their PPE.

In addition to strict and practiced doffing procedures, one step of the doffing process should include a decontamination step of the PPE, including the PAPR hood. Several studies, such as Casanova et al. (2018) and Brown et al. (2016) have proven that decontaminating the PPE with either hypochlorite, a quaternary ammonium compound, or an alcohol based hand rub can greatly reduce the potential for viral transmission to other pieces of PPE, the surrounding environment, and most importantly, the healthcare worker. This important step in the doffing process should be considered, evaluated, and practiced by hospitals through their infection control procedures. Determining which disinfectant is best for their hospital for the doffing process will limit the opportunities for self-contamination of the HCW to themselves and surrounding environment.

Furthermore, hospitals should conduct risk assessments to determine the best type of respiratory protection for their HCWs when handling the variety of highly infectious agents they may come in contact with at their hospital. Analyzing trends for all the respirator types used, such as N95, PAPR or a shroud PAPR hood should be reviewed to determine cases of self-contamination and if standard operating procedures need to be updated or changed. From the research of the shroud PAPR hood study with two different bacteriophages, the results only strengthen the argument that proper doffing is essential in preventing self-contamination with highly infectious pathogens.

References

Bell BP, Damon IK, Jernigan DB, et al. Overview, Control Strategies, and Lessons Learned in the CDC Response to the 2014–2016 Ebola Epidemic. MMWR Suppl 2016;65 (Suppl-3):4–11. DOI: http://dx.doi.org/10.15585/mmwr.su6503a2

Bell, T., Smoot, J., Patterson, J., Smalligan, R., & Jordan, R. (2015). Ebola virus disease: The use of fluorescents as markers of contamination for personal protective equipment. *IDCases, 2*(1), 27-30. doi:10.1016/j.idcr.2014.12.003

Brady, T. M., Strauch, A. L., Almaguer, C. M., Niezgoda, G., Shaffer, R. E., Yorio, P. L., & Fisher, E. M. (2017). Transfer of bacteriophage MS2 and fluorescein from N95 filtering facepiece respirators to hands: Measuring fomite potential. *Journal of Occupational and Environmental Hygiene, 14*(11), 898-906. doi:10.1080/15459624.2017.1346799

Brown, T. W., Chen, W., & Casanova, L. M. (2016). Survival and disinfection of an enveloped surrogate virus on Tyvek suits used for health care personal protective equipment. *American Journal of Infection Control, 44*(12), 1734-1735. doi:10.1016/j.ajic.2016.04.247

Bunyan, D., Ritchie, L., Jenkins, D., & Coia, J. E. (2013). Respiratory and facial protection: a critical review of recent literature. *Journal of Hospital Infection, 85*(3), 165-169. doi:10.1016/j.jhin.2013.07.011

Casanova, L., Alfano-Sobsey, E., Rutala, W. A., Weber, D. J., & Sobsey, M. (2008). Virus transfer from personal protective equipment to healthcare employees' skin and clothing. *Emerging Infectious Diseases, 14*(8), 1291-1293. doi:10.3201/eid1408.080085

Casanova, L., Rutala, W. A., Weber, D. J., & Sobsey, M. D. (2009). Methods for the recovery of a model virus from healthcare personal protective equipment. *Journal of Applied Microbiology, 106*(4), 1244-1251. doi:10.1111/j.1365-2672.2008.04093.x

Casanova, L., Rutala, W. A., Weber, D. J., & Sobsey, M. D. (2010a). Coronavirus survival on healthcare personal protective equipment. *Infection Control Hospital Epidemiology, 31*(5), 560-561. doi:10.1086/652452

Casanova, L. M., Jeon, S., Rutala, W. A., Weber, D. J., & Sobsey, M. D. (2010). Effects of air temperature and relative humidity on coronavirus survival on surfaces. *Applied Environmental Microbiology, 76*(9), 2712-2717. doi:10.1128/AEM.02291-09

Casanova, L. M., & Waka, B. (2013). Survival of a surrogate virus on N95 respirator material. *Infection Control Hospital Epidemiology, 34*(12), 1334-1335. doi:10.1086/673994

Casanova, L. M., Teal, L. J., Sickbert-Bennett, E. E., Anderson, D. J., Sexton, D. J., Rutala, W. A., Program, C. D. C. P. E. (2016). Assessment of Self-Contamination During Removal of Personal Protective Equipment for Ebola Patient Care. *Infection Control Hospital Epidemiology, 37*(10), 1156-1161. doi:10.1017/ice.2016.169

Casanova, L. M., Erukunuakpor, K., Kraft, C. S., Mumma, J. M., Durso, F. T., Ferguson, A. N., . Prevention Epicenters Program, D. o. H. Q. P. (2018). Assessing Viral Transfer During Doffing of Ebola-Level Personal Protective Equipment in a Biocontainment Unit. *Clinical Infectious Diseases, 66*(6), 945-949. doi:10.1093/cid/cix956

Centers for Disease Control and Prevention (CDC). (2018) Guidance on Personal Protective Equipment (PPE) To Be Used By Healthcare Workers during Management of Patients with Confirmed Ebola or Persons under Investigation (PUIs) for Ebola who are Clinically Unstable or Have Bleeding, Vomiting, or Diarrhea in U.S. Hospitals, Including Procedures for Donning and Doffing PPE. Retrieved from https://www.cdc.gov/vhf/ebola/healthcare-us/ppe/guidance.html. Accessed March 2019.

Chughtai, A. A., Chen, X., & Macintyre, C. R. (2018). Risk of self-contamination during doffing of personal protective equipment. *American Journal of Infection Control*. doi:10.1016/j.ajic.2018.06.003

Coia, J. E., Ritchie, L., Adisesh, A., Makison Booth, C., Bradley, C., Bunyan, D., . . . Facial, P. (2013). Guidance on the use of respiratory and facial protection equipment. *Journal of Hospital Infections, 85*(3), 170-182. doi:10.1016/j.jhin.2013.06.020

Cook, B. W., Cutts, T. A., Nikiforuk, A. M., Poliquin, P. G., Court, D. A., Strong, J. E., & Theriault, S. S. (2015). Evaluating environmental persistence and disinfection of the Ebola virus Makona variant. *Viruses, 7*(4), 1975-1986. doi:10.3390/v7041975

Hanoa, R. O., & Moen, B. E. (2016). Ebola Care and Lack of Consensus on Personal Protective Respiratory Equipment. *Workplace Health Safey, 64*(2), 48-50. doi:10.1177/2165079915608405

Lim, S. M., Cha, W. C., Chae, M. K., & Jo, I. J. (2015). Contamination during doffing of personal protective equipment by healthcare providers. *Clinical and Experimental Emergency Medicine, 2*(3), 162-167. doi:10.15441/ceem.15.019

Lowen, A. C., Mubareka, S., Steel, J., & Palese, P. (2007). Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathogen, 3*(10), 1470-1476. doi:10.1371/journal.ppat.0030151

MacIntyre, C. R., Chughtai, A. A., Seale, H., Richards, G. A., & Davidson, P. M. (2014). Respiratory protection for healthcare workers treating Ebola virus disease (EVD): are facemasks sufficient to meet occupational health and safety obligations? *International Journal of Nursing Studies, 51*(11), 1421-1426. doi:10.1016/j.ijnurstu.2014.09.002

Mumma, J. M., Durso, F. T., Ferguson, A. N., Gipson, C. L., Casanova, L., Erukunuakpor, K., . . . Prevention Epicenters Program, D. o. H. Q. P. (2018). Human Factors Risk Analyses of a Doffing Protocol for Ebola-Level Personal Protective Equipment: Mapping Errors to Contamination. *Clinical Infectious Diseases, 66*(6), 950-958. doi:10.1093/cid/cix957

National Institute for Occupational Safety and Health (NIOSH). (2018). The National Personal Protective Technology Laboratory. Retrieved from https://www.cdc.gov/niosh/npptl/default.html. Accessed April 25, 2019.

Petti, S., Protano, C., Messano, G. A., & Scully, C. (2016). Ebola Virus Infection among Western Healthcare Workers Unable to Recall the Transmission Route. *Biomed Research International, 2016*, 8054709. doi:10.1155/2016/8054709

Prussin, A. J., 2nd, Schwake, D. O., Lin, K., Gallagher, D. L., Buttling, L., & Marr, L. C. (2018). Survival of the Enveloped Virus Phi6 in Droplets as a Function of Relative Humidity, Absolute Humidity, and Temperature. *Applied Environmental Microbiology, 84*(12). doi:10.1128/AEM.00551-18

Roberts, V. (2014). To PAPR or not to PAPR? *Can Journal of Respiratory Therapy, 50*(3), 87-90.

Sagripanti, J.-L., & Lytle, C. D. J. A. o. V. (2011). Sensitivity to ultraviolet radiation of Lassa, vaccinia, and Ebola viruses dried on surfaces. *Archives of Virology, 156*(3), 489-494. doi:10.1007/s00705-010-0847-1

Schuit, M., Miller, D. M., Reddick-Elick, M. S., Wlazlowski, C. B., Filone, C. M., Herzog, A., . . . Noah, J. W. (2016). Differences in the Comparative Stability of Ebola Virus Makona-C05 and Yambuku-Mayinga in Blood. *PLoS One, 11*(2), e0148476. doi:10.1371/journal.pone.0148476

Suen, L. K. P., Guo, Y. P., Tong, D. W. K., Leung, P. H. M., Lung, D., Ng, M. S. P., . . . Yu, W. (2018). Self-contamination during doffing of personal protective equipment by healthcare workers to prevent Ebola transmission. *Antimicrobial Resistant Infection Control, 7*, 157. doi:10.1186/s13756-018-0433-y

Tomas, M. E., Kundrapu, S., Thota, P., Sunkesula, V. C., Cadnum, J. L., Mana, T. S., . . . Donskey, C. J. (2015). Contamination of Health Care Personnel During Removal of Personal Protective Equipment. *JAMA Internal Medicine, 175*(12), 1904-1910. doi:10.1001/jamainternmed.2015.4535

Tung-Thompson, G., Libera, D. A., Koch, K. L., de Los Reyes, F. L., 3rd, & Jaykus, L. A. (2015). Aerosolization of a Human Norovirus Surrogate, Bacteriophage MS2, during Simulated Vomiting. *PLoS One, 10*(8), e0134277. doi:10.1371/journal.pone.0134277

U.S. Department of Health and Human Services. (2015). Risk Groups. Retrieved from https://www.phe.gov/s3/BioriskManagement/biosafety/Pages/Risk-Groups.aspx. Accessed March 15, 2019.

Verbeek, J. H., Ijaz, S., Mischke, C., Ruotsalainen, J. H., Makela, E., Neuvonen, K., . . . Mihalache, R. C. (2016). Personal protective equipment for preventing highly infectious diseases due to exposure to contaminated body fluids in healthcare staff. *Cochrane Database System Review, 4*, CD011621. doi:10.1002/14651858.CD011621.pub2

Weber, D. J., Fischer, W. A., Wohl, D. A., & Rutala, W. A. (2015). Protecting healthcare personnel from acquiring Ebola virus disease. *Infection Control Hospital Epidemiology, 36*(10), 1229-1232. doi:10.1017/ice.2015.205

World Health Organization. (2019). Ebola Virus Disease. Retrieved from https://www.who.int/ebola/en/. Accessed on March 1, 2019.

Yang, W., Elankumaran, S., & Marr, L. C. (2012). Relationship between humidity and influenza A viability in droplets and implications for influenza's seasonality. *PLoS One, 7*(10), e46789. doi:10.1371/journal.pone.0046789

Zellmer, C., Van Hoof, S., & Safdar, N. (2015). Variation in health care worker removal of personal protective equipment. *American Journal of Infection Control, 43*(7), 750-751. doi:10.1016/j.ajic.2015.02.005