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INACTIVATION AND SURVIVAL OF BACTERIOPHAGE $\Phi 6$ ON TYVEK SUITS

by

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Bachelor of Engineering, Beijing Institute of Technology, Zhuhai

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

MASTER OF PUBLIC HEALTH

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Abstract

Inactivation and survival of Bacteriophage $\Phi 6$ on Tyvek suits

by

Weiyu Chen

Thesis Chair: Dr. Lisa Casanova

Healthcare providers encounter a wide range of hazards on the job, including exposure to infectious diseases. Protecting them from occupational infectious disease is very important. Healthcare workers use personal protective equipment (PPE) as a measure to decrease the risk of getting infected during patient care. For high-risk diseases like Ebola, Tyvek suits are coverall suits that protect the body and reduce the risk of body fluid exposure. However, a person removing a contaminated suit may also be exposed to virus. Previous studies have shown that enveloped viruses can survive on different types of surfaces, so the objective of this study is to determine the inactivation of bacteriophage $\Phi 6$, a surrogate for enveloped human virus, on the surface of Tyvek suits at two different relative humidity levels, 40% and 60% at 22 °C. The results showed the inactivation rate of virus was higher at 60% RH than 40% RH. There was $\sim 3\log_{10}$ (99.9%) reduction of virus inactivation after 6 hours at 40% but $\sim 3\log_{10}$ (99.9%) inactivation took 9 hours at 60%. This suggests that enveloped viruses can survive on the surface of Tyvek suits for more than 6 hours, and should be considered a potential risk for contamination when they are taken off after use.

INDEX WORDS: Relative humidity, Temperature, Virus survival, Enveloped virus, PPE

APPROVAL PAGE

INACTIVATION AND SURVIVAL OF BACTERIOPHAGE $\Phi 6$ ON TYVEK SUITS

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1 Introduction

Healthcare workers are a group of workers who encounter a wide range of hazards on the job including infectious diseases. Many severe diseases can be transmitted to them through close contact with patients including Ebola, severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) (17). Pandemic influenza virus (H1N1) was first identified in 2009. During the outbreak of this virus, half of the infected health care workers were probably infected from other ill patients or coworkers, suggesting that healthcare workers who are exposed to H1N1 may be at risk (Wise et al., 2011). During the pandemic, Occupational Safety and Health Administration (OSHA) considered healthcare workers to be at high risk of exposure during this outbreak. Additionally, research was done by Sepkowitz et al. (2005) showing that more than 20% (1,707 of 8,098) of healthcare workers were infected with SARS in Hong Kong. Two American healthcare workers were infected with Ebola while caring for patients in the Africa and sent back to U.S. for treatment, causing extensive concern about healthcare workers protection in U.S. hospitals while caring for Ebola patients. (Isakov et al., 2014).

Personal protective equipment (PPE) is equipment that minimizes exposure to illnesses and injuries in the workplace. PPE not only includes masks, gloves, respirators, but can also include full body suits and coveralls (OSHA). Tyvek suits are full body suits that cover the whole body from toes to the head to provide barrier protection

against body fluid exposures. They are widely used for preventing exposures to virus from patients' body fluids that healthcare workers commonly encounter when caring for patients with Ebola (Fischer et al., 2015). The Centers for Disease Control and Prevention (CDC) recommends healthcare workers use coverall suits as a protector when they are facing infectious diseases such as Ebola.

However, contaminated coveralls could place healthcare workers at risk. Research done by Otake et al. (2002) showed that contaminated coveralls and boots might transmit porcine reproductive and respiratory syndrome virus (PRRSV) from infected pigs to susceptible pigs. Research done by Lai et al. (2005) mentioned that contaminated PPE could become a potential fomite to transmit SARS coronavirus (SARS-CoV) to health care workers because the virus could survive on the surface of nonabsorbent disposable gown. Similar research on the survival of viruses on PPE suggested viruses might survive on the surface of PPE for hours, and may indicated potential for infection for people who are using the PPE (Casanova et al., 2010). To further understand the risk of handling contaminated PPE, examining virus survival on PPE surfaces is very important.

The objective of this project is to determine how relative humidity; time and temperature influence virus survival on the surface of PPE, specifically Tyvek suits. The experiment used a bacteriophage $\Phi 6$, which is a surrogate virus that has a similar structure to influenza virus and coronavirus. A surrogate virus is less expensive to use compared to a pathogenic virus. Moreover, it is less difficult and risky for who work with the virus. Some former studies use inactivation experiments of coronavirus

showed the surrogate virus can stable under different humidity and temperature which indicated that it as a credible for the pathogenic virus (Adcock et al., 2009).

It is important to examine the survival rate of the virus on the surface of Tyvek suits because these suits are widely used as PPE. Furthermore, some types of Tyvek suits are reusable, and some are one-time use. This experiment can help understand if reuse of Tyvek suits during an outbreak will be a risk to healthcare workers or not, and understand how long viruses that contaminate a Tyvek suit can pose a risk. Therefore, the purpose of the study was to measure the inactivation rate of bacteriophage $\Phi 6$ on the surface of Tyvek suits at 40% and 60% relative humidity level at room temperature (~20 °C).

2 Literature review

2.1 Background information

In 2014, there was a disease outbreak worldwide named Ebola. The outbreak drew the world's attention to the importance of personal protective equipment (PPE) because two American healthcare workers were infected with Ebola virus. PPE is a very important prevention method that prevents disease spread from patient to healthcare workers (Isakov et al., 2014). Moreover, contaminated PPE can be a potential fomite that some research showed the PPE could be an agent that virus can survive on the surface of PPE, and the virus could be transmitted to healthcare workers (Casanova et al., 2009a; Lai et al., 2005).

SARS, a similar pandemic outbreak as Ebola, which was took place in 2003 showed there is a high risk that healthcare workers who are caring for patients can be affected by the infectious diseases (Casanova et al., 2009; Lai et al., 2005). A fairly large proportion of occupied healthcare workers that nearly 37%-63% of them have doubted infected with SARS in highly affect areas (Park et al., 2004). Since SARS is a type of virus that belongs to coronaviruses; a common cause of respiratory disease, people began to realize that coronavirus infection can be acute and even fatal infection (Casanova et al., 2010b).

2.2 Bacteriophage Φ 6

Bacteriophage Φ 6 is an enveloped virus used for as a surrogate for human respiratory viruses. Using surrogate virus instead of infectious virus is because surrogate virus cost less expense and labor intensive than the coronavirus. Meanwhile, the researchers will need special training under biosafety level 3 (BSL-3) laboratory facilities, and it is risky for people who handle coronavirus. Moreover, doing researches with the infectious virus such as SARS will be a significant challenge and it is very difficult to acquire the virus isolates. Therefore, using the surrogate virus have many advantages especially because it is safer to work with (Casanova et al., 2009a; Casanova et al., 2010; Adcock et al., 2009). According to the research by Adcock et al. (2009), these enveloped bacteriophages would not have the limitation, and it is cost-efficiency while the results prove that the surrogate virus can use for inactivation experiments of coronavirus. Besides, this virus is as stable as the pathogenic virus under different environmental elements like humidity and temperature.

2.3 Tyvek Protective Apparels

Tyvek suits are personal protective clothing that protects the body from contact hazardous substance and reduce the risk of dermal exposure (Gao, 2012). It is a very common protective appeal for healthcare workers to build a barrier to reduce the exposure. It was widely used in West Africa for healthcare workers caring with

patients with Ebola to protect against with the pathogen from entering their body by the broken skin (CDC, 2015). According to a study by Fischer et al. (2015), the temperature, humidity and the time of wearing protective apparel will affect the risk of getting infectious. Although there were not many types of research based on Tyvek suits, another study related to PPE by Casanova et al. (2013) did the experiment about the inactivation and survival of coronavirus on an N95 respirator by using another surrogate virus for SARS. The result showed the virus can deposit on the surface of N95 mask and detectable for up to 24 hours. Based on the study, the virus can survive on the surface for a long period to infect healthcare workers. Although the study was on an N95 respirator, since Tyvek garments are widely used around the world as are N95 respirators, it is very significant to do research on how long the virus can survive on the surface.

2.4 Environmental Factors

A study done by Chan et al. (2011) showed SARS-CoV was inactive at high temperature and high humidity and could survive under low temperature, and low humidity. That may explain explained why Hong Kong has more SARS patients than Malaysia because of the virus can easy spread in high intensive use of the air-conditioning environment. Likewise, a study done by Lowen et al. (2007) provided the same opinion that the temperature and humidity will affect influenza virus by using guinea pigs for an experiment under controlled conditions. The

transmission was highly efficient that nearly 100% transmission occurred in relative humidity (RH) 20%-35%. However, only 25% transmission at 50% RH and 75% transmission at 65% RH, but there was 0% transmission at 80% RH. The experiment indicated that the virus survives better in low RH than in high RH. Besides, another research done by Casanova et al. (2010b) that focus on the relationship between environmental surface and environmental variable showed that the virus survived more in low RH. However, the author also indicated the relation between RH and virus inactivation was not fully clear and it might be different depends on the type of virus. Nevertheless, another research showed virus survival will depend on different temperature, humidity and material surface. And some virus persisted better in high RH such as hepatitis A virus enhanced on the nonporous surface and dried human rotavirus persisted at high RH on porous materials (Abad et al., 1994).

Based on the researches above, not only humidity can affect the results of virus survival, the type of surface and influenced it. A nonenveloped virus such as hepatitis A virus and human rotavirus can survive on PPE that remain infectious may transmit to healthcare workers (Casanova et al., 2008). Hands could be another infectious source because a virus can survive on it and spread to others or self-inoculated which indicated human hand could be a vehicle to transmit the virus (Mbithi et al., 1992). A study done by Sizun et al. (2000) showed human coronaviruses could survive for hours after drying such as respiratory syncytial virus were recoverable for up to 1.5 hours on the surface of rubber gloves. Although parainfluenza virus reduced rapidly on a skin, it was detectable for up to 1hour. It showed the possible that virus could

spread person to person through hand contamination.

Not only the environmental surface and relative humidity will affect virus survival; the temperature could be another factor that influenced it. A study by Casanova et al. (2009) found out the temperature is an important factor that would influence virus survival. Another research done by Lowen et al. (2007) showed virus increased transmission at 5 °C than 20 °C that turned out the transition of the virus will increase under cold condition.

Although there were some researches related to PPE such as N95 respirator masks, studies focused on virus survival on Tyvek suits are still limited. Accordingly, doing research for virus survival on Tyvek suits is very important. This research will help analyze the risk when healthcare workers are expose to hazardous circumstances and the possibility of virus transmission through the PPE surface.

3 Materials and Methods

3.1 Virus Stock Preparation

The bacteriophage $\Phi 6$ in the host bacterium *Pseudomonas Syringae* was propagated using the soft agar preparation method. Briefly, 30mL of host bacterium culture was grown on the flask shaker at 100 rpm at room temperature (22 °C) for 24 hours. Added 2mL of virus stock into the culture after 24 hours, and incubated with shaking for an additional 24 hours. Prepared soft agar by adding agar to tryptic soy broth to 0.7% concentration and dispensed into the tryptic soy agar plates. Added 0.5mL of the host culture and 0.5mL of virus culture to 30mL soft agar and incubated for 24 hours at room temperature (22 °C). Pooled and centrifuged the harvested top layer agar (5900g, 30minutes, 4 °C), and then stored as stock in tryptic soy broth with 20% glycerol at -80 °C.

3.2 Recovery Experiments

To prepare the host, 0.9mL volume of the host was added to 100mL of tryptic soy agar (TSB) and incubated on the flask shaker for 18-24 hours. Diluted 100 μ L virus stock from the 200 μ L tubes into 900 μ L phosphate buffered saline (PBS) to reach a concentration of 10^6 PFU. Placed 10 μ L of virus stock dilution on the six 1cm² autoclaved Tyvek suits pieces in two Petri dishes (three pieces in one dish), and three

pieces for the time 0 points was transferred to three tubes immediately by using sterilized forceps. Five mL of beef extract (1.5%, PH 7.5) were added into the tubes and then placed on the shaker (220 rpm, 22 °C) for 20 minutes. The remaining set of three samples were dried for 60 minutes under the hood at room temperature (22 °C) as time 60 point. After 60 minutes, each of the pieces was placed into the tubes which with 5mL of 1.5 % beef extract on the shaker (220 rpm) at 22 °C for 20 minutes. Diluted time 0 and time 60 min samples in TSB and using the double agar layer (DAL) method to assay. The rest of 100µL virus stock was using plaque assayed method. All plates were incubated at 25 °C for 24 hours and counted and recorded results after incubation.

3.3 Survival Experiments

Diluted 100 µL of virus stock into 900 µL PBS and 10 µL of the virus was added to 6 pieces of Tyvek suit samples. Using sterilized forceps transferred three pieces immediately to tubes that contained 5mL of 1.5% beef extract and placed on the shaker for 20 minutes that represent virus concentration at time 0 points. Diluted samples serially in TSB and using double agar layer method to assay and incubated plates at room temperature (~22 °C) for 24 hours. The rest of the pieces were placed into the humidity chamber with either 40% ($\pm 2\%$) or 60% ($\pm 2\%$) humidity at 22 °C. The humidity and temperature environments were controlled by saturated salt solutions (40% - Magnesium Chloride, 60% - Magnesium Nitrate) and sealed glass tanks. Every two hours, three pieces were removed from the tank and do the same procedures as above.

Since it is very difficult for healthcare workers to wear Tyvek suits for a long time, these experiments were completed at 6-hour intervals (t=2hours, t=4hours, t=6hours) up to 6 hours under both humidity levels. After 24 hours, incubation counted and recorded the number of plaques on each plate.

3.4 Statistical Analysis

The analysis was using GraphPad Prism 5 (GraphPad, San Diego, CA) and Excel 2010 (Microsoft Corp.).

4. Results

Table 1. Percent of recovery at time 0 and time 60.

	Time 0	Time 60
Recovery Percent	99.95%	99.93%

Table 1 shows this method was able to recover enough virus necessary to perform the study which means this study is able to see survival of bacteriophage $\Phi 6$ over time.

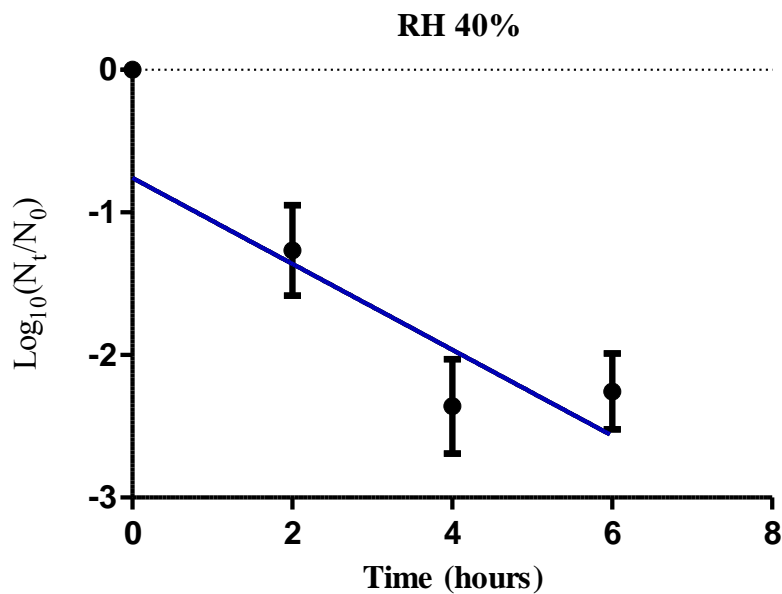


Figure 1. Bacteriophage $\Phi 6$ survival over 24 hours at 22 °C and 40% RH. 7-9 replicates per points; linear regression analysis=line.

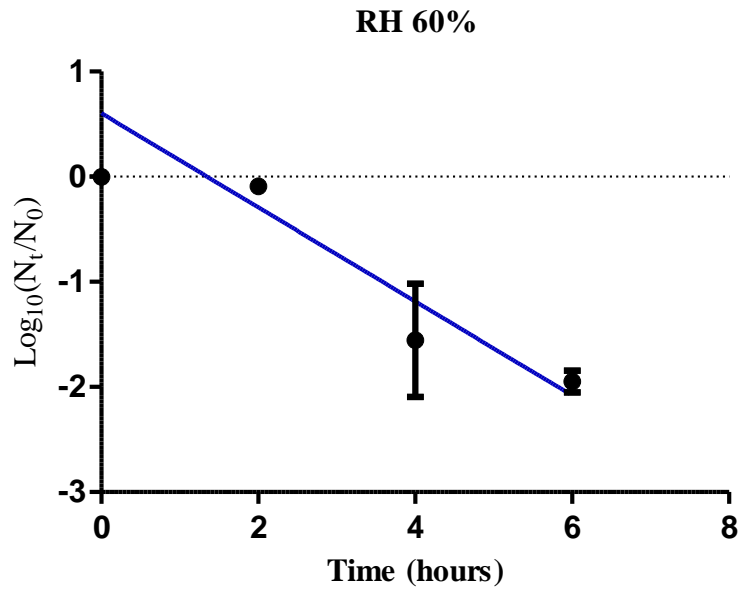


Figure 2. Bacteriophage Φ 6 survival over 24 hours at 22 °C and 60% RH. 7-12 replicates per points; linear regression analysis=line.

The survival of bacteriophage Φ 6 at 40% and 60% relative humidity and 22 °C is shown in figure 1 and 2. According to table 2, at 40% RH the slope was -0.3014 ± 0.1218 which means the 95% confidence interval at 60% RH was not overlap. Meanwhile, the slope at 60% RH was -0.4473 ± 0.0899 means the 95% confidence interval at 40% RH was not overlap. Based on this, the results suggest the virus inactivation rates at 40% RH and 60% RH were significantly different.

Table 2. Slopes of regression lines at 22 °C and 40% and 60% RH.

RH	Slope	95% CI	P value
40%	-0.3014	± 0.1218	< 0.0001
60%	-0.4473	± 0.0899	< 0.0001

Table 2 shows the 95% CI of slope of regression lines at 22 °C under both 40% and 60% RH. Both slopes show were significantly non zero, $P < 0.0001$ for both 40%

and 60% RH.

Table 3. Predicted time for virus inactivation based on log₁₀ reduction at 22 °C under both 40% and 60% RH.

RH	Reduction [log₁₀(N_t/N₀)]	Time (hours)
40%	-1(90%)	2
	-2(99%)	4
	-3(99.9%)	6
60%	-1(90%)	2
	-2(99%)	5
	-3(99.9%)	9

**Time rounded up.*

To calculate the log₁₀ reduction, I used the formula which is $(X (\log \text{reduction which is } 3) = 0.5 - 0.3014 * t)$ (t is TIME, and the formula is an example when RH is 40%)). After the calculation, use absolute value. Table 3 shows that 90% log₁₀ reduction of virus at 22 °C for both 40% and 60% RH took ~2 hours. However, there was a -3 log₁₀ reduction at 40% RH and 22 °C after ~6 hours, but it took ~9 hours for 60% RH to achieve the same log₁₀ reduction. Therefore, the results show the rate of viral inactivation is lower at higher RH.

5. Discussion

PPE is a very important measure that prevents disease spread from patients to healthcare workers. Healthcare workers must always wear it when in contact with patients who have severe diseases such as Ebola that spread via body fluids, and they may have the chance to come in contact with viruses on the surface of the suits when they take off PPE. Moreover, the Tyvek suits used in the study were disposable and one-time use, but during the outbreak of infectious disease, especially in poor settings, there may be a lack of PPE so the healthcare workers may reuse it. Based on the former research from Waka (2013), the bacteriophage was able to persist on the surface of N95 respiratory mask for 24 hours, but there is a lack of research on virus survival on Tyvek suits. In the studies done by Waka and Casanova, there was 1 log reduction in virus within 24 hours at 20 °C and 40% RH and 4 log₁₀ reduction within 24 hours at 20 °C and 60% RH. Compare with my studies, the time of virus survived on samples from Tyvek suits were much shorter than Waka's studies. The samples reached 3 log₁₀ reduction within 12 hours at both RH. Nevertheless, the Tyvek suits are tight sealing and cause heat stress after wearing for a long time, so healthcare workers do not wear it for a long period. Therefore, survival was studied for 6 hours, to reflect the amount of time a suit might be worn to care for a patient before it is taken off or changed.

The study shows that bacteriophage $\Phi 6$, a virus has a similar structure as

influenza virus, can survive at 22 °C and 40% and 60% relative humidity for 6 hours, and the inactivation rate was higher at 40% RH than 60% RH. After ~6 hours, the virus at 40% RH and 22 °C achieved 4 log₁₀ reduction but 60% RH achieved it after ~9 hours.

There are many important factors that can influence virus survival on surfaces that included temperature, humidity and the species of virus. The temperature in this experiment was controlled at around 22 °C, and the variable is the relative humidity. The results indicate that virus was inactivated more slowly at 60% than 40% RH after 6 hours. The result is different than a study done by Waka (2012). Based on Waka's research, the inactivation rate was lower at 40% than 60% RH on the N95 mask. However, the only major difference between this experiment and the Waka et al. study was the surface that the virus was applied on. Comparing with these two materials, N95 mask (model 1860, 3M) is a semi-porous material made from small fibers that allow air to pass through, and Tyvek suits are made of a completely nonporous material made of high-density polypropylene.

Research done by Tiwari et al. (2006) indicated both the avian influenza virus (AIV), avian metapneumovirus (AMPV) survived longer (for up to 48 hours) on nonporous surface (steel, tire) than on porous surface (cotton, polyester fabrics) (for less than 8 to 12 hours). Other study shows parainfluenza virus can survive longer on a nonporous surface such as laminated plastic (for 10 hours) but only can survive on a porous surface such as tissue for up to 4hours (Brady et al., 1990). Similar research showed that influenza A and B virus can survive on a stainless surface which is

nonporous material for 24 hours and could transfer to other people but only survived on tissue for few hours (Bean et al., 1982). Compare with this research and the research done by Waka, virus survival rate can be very different on nonporous surface versus a porous surface. Furthermore, there is some previous research that indicates some viruses could survive longer in high RH than low RH. A study done by Shek et al. (2003) observed the peak of respiratory syncytial virus infection was related to rain season that was a high RH environment. However, the RH has different influence on different type of virus. According to the research done by Koopmans et al. (2004), enteroviruses survived more in high humidity and hepatitis A virus survived more in low humidity. A similar research done by Bearden (2015) shows that bacteriophage $\Phi 6$ inactivated more at 40% RH than 60% RH on the surface of toy coupons which shows the approximately same results as this research. In conclusion, same type of viruses' inactive rate can be different due to different type of humidity and the type of surfaces.

Recommendation:

Under high humidity virus may survive longer, especially in places where Ebola was found that was nearly 80% RH. The study shows result that after 6 hours contact with patients, the healthcare workers still can getting infect by the virus residue which on the surface of Tyvek suits.. Because of the virus can survive on the surface of PPE, it can increase the risk for healthcare workers get infect by highly contagious diseases. The current guideline for doffing PPE is to remove it step by step while being observed by an assistant who make sure the steps are followed (CDC, 2015). This

research reinforces how important it is to make sure healthcare workers do not touch PPE during removal. Also, we recommend a thorough disinfect cleaning after each time after contact with patients because non-enveloped virus is more difficult to kill such as Ebola. The results of this study provide information that will help decrease the risk of virus infectious in the future and should be consider as an assessment for using Tyvek suits in tropical areas.

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