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Laboratory study of a scavenging mask system to evaluate and control airborne pathogens for healthcare workers in the Post Anesthesia Care Unit (PACU) and Intensive Care Unit (ICU)

Josh Lee Horton
Purdue University

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For the degree of Master of Science

Is approved by the final examining committee:

Dr. James D. McGlothlin

Dr. Bruce M. Applegate

Dr. James F. Schweitzer

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Dr. James D. McGlothlin

Approved by Major Professor(s): _____

Approved by: Dr. Keith Stantz 08/22/2014

Head of the Department Graduate Program Date

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A Thesis

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Joshua Lee Horton

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This Master's thesis is dedicated to my friends and family that have helped me through these past few years. I want to especially thank my family: Dad, Ashton, Joey, Tanya, Jamie, and Allie for all the love and support to help me through this challenging time. They have made me the person I am today.

I would also like to dedicate this thesis to the loving memory of a dear friend and brother in the Sigma Chi Fraternity, Chace Wimberley. Rest in Peace.

"If you ain't wild, you're mild"

I may have gotten my degree at Purdue, but I also got an education.

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ABSTRACT

Horton, Joshua L. M.S., Purdue University, December, 2014. Laboratory Study of a Scavenging System to Evaluate and Control Airborne Pathogens for Health Care Workers in the Post Anesthesia Care Unit (PACU) and Intensive Care Unit (ICU). Major Professor: Dr. James McGlothlin.

This laboratory study evaluated the usefulness of a new market available scavenging system (patient mask and filter) in controlling the spread of airborne pathogens by: 1. Develop a laboratory simulation of an infectious patient exhaling a range of respirable bacteria and viruses into a laboratory hood. 2. Compare and contrast capabilities of the market-available scavenging system to reduce and control pathogens in a laboratory setting versus not using a scavenging system. 3. Evaluate the effectiveness of the scavenging system's filter and alternative HEPA filters in capturing these pathogens. A life-like manikin head equipped with a bioaerosol collision nebulizer was set up to simulate a person exhausting pathogenic droplets. The study's hypothesis was tested by using two different scenarios: 1. The scavenging system was used the entire duration of the trial (Case) 2. The scavenging system was not used at all during the trial (Control). The nebulizer used multiple types of respirable pathogens (bacteria and viruses) to represent different size pathogens to evaluate the scavenging system's ability to capture a range of pathogens likely to be found in infectious patients. Pathogens that may escape the scavenging system were captured using liquid impingers, and pathogens inside the scavenging system were captured by the filter that came with the market available mask. A filter flask was used to capture pathogens that broke through the scavenging system's supplied filter. The captured pathogens were analyzed and quantified by spread plate analyses for both bacteria and viruses. The filter equipped with the market available scavenging system did not consistently prevent all pathogens from breaking through the

filter ($p \geq .05$). The HEPA filters in the scavenging mask followed a general trend showing a higher percentage of the smaller viruses passed through the HEPA filter compared to the larger viruses. However, overall the market available scavenging system proved to reduce the exposure to pathogens by 93.2% when exposed to the smallest viruses used (27nm) and as much as 99.9998% for the largest bacteria used (3 μm). Based on this laboratory research, it appears the market available scavenging system may help protect healthcare workers working in the PACU and ICU against airborne pathogen exposure. Further research in clinical trials will help validate these laboratory results.

CHAPTER 1. INTRODUCTION

1.1 Thesis Statement

The overarching goal of this research is to reduce, prevent and control pathogen exposure among healthcare workers in the post anesthesia care unit and intensive care unit by controlling the exposure at the source (the patient). To help achieve this goal a laboratory study was conducted to simulate pathogen exposures to healthcare workers including those that work in the PACU and ICU. The objective was to simulate an infectious patient wearing a market available waste anesthetic gas scavenging system versus a patient not wearing a scavenging system. We hypothesize that the scavenging system may be as useful in pathogen control as it has proved to be useful in the reduction of waste anesthetic gas exposure.

To test this hypothesis, a patient off-gassing airborne pathogens was simulated using a manikin equipped with an atomizer nebulizing a bioaerosol while the scavenging system mask and filter is being used and the mask and filter not being used. Each simulation was analyzed comparing the amount of pathogens (bioaerosols) captured while using the scavenging mask and when it is not being used. The amount of bioaerosols captured on the filter was compared to how much was captured passed the filter to understand the amount of breakthrough that occurs.

1.2 What are Bioaerosols?

The human conception of danger is what the mind perceives as harmful to the body. It is well known that human beings are more careless if they cannot perceive their environment as dangerous. Coming into contact with unseen particles in the air is

inevitable and for the most part are never thought of by most people. Aerosols can be defined as microscopic particles composed of liquids and/or solids that are suspended in a gas. The most essential focus in aerosols is understanding its physical, chemical, and biological properties. The aerosol's size (usually measured in micrometers (μm) or nanometers (nm)), shape and density are parameters that express its abilities to move in the atmosphere, along with the duration it can travel and its capability to penetrate across barriers. The chemical and biological properties of aerosols are also very important in the sense that they tell us potentially of their origins, the quality of the air we breathe, and the health effects to humans. These properties are used to comprehend how a particular aerosol acts while suspended in the air, reacts to the human body, and interacts with other aerosols and aspects of the environment. (Hinds, 1999)

An important category of aerosols which has been a concern in public health for years and has begun to get attention in occupational health is bioaerosols. As the name implies, bioaerosols are aerosols of biological origins. These can include viruses, viable organisms, endospores, and products of organisms. Bioaerosols have a large range of sizes from less than 20nm to 100 μm which makes "blanket" generalizations about them, collectively, near impossible to draw accurate conclusions. Exposure to bioaerosols come with potential health effects such as infectious disease and allergic reactions. (Hinds, 1999). Potentially being pathogenic, viruses and viable organisms (mainly bacteria) are being exhibited as a health hazard that needs to be controlled. In order to understand how to control them, the understanding of how they become aerosolized and transmitted is essential.

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1.3 Historical Epidemics

The spread of pathogenic bioaerosols have historically shown to be lethal. One pathogen that has been an issue for centuries is Tuberculosis (TB). TB is known to spread in areas of condensed populations living in unsanitary conditions. In the late 1800s and early 1900s, people were leaving the farms and hillsides to come work in factories inside the city limits creating an era called the industrial revolution. This city living was a great reservoir for TB causing what is known as The Great White Plague. (Sucre, 1995). It was estimated that 70% - 90% of urban areas in Europe and North America were infected with TB, and 80% of this population died either directly or indirectly by this disease. (Library, 2013). Just in America, it was estimated that roughly 110,000 people died every year from TB in the early 1900s. (Sucre, 1995)

TB was also responsible for 40% of all middle class deaths at this time. In order to control the spread of this disease, the sanatoria movement occurred, which called for sick people to be quarantined and removed from the healthy population. (Library, 2013) This proved to be very helpful and the mortality rate for TB steadily decreased to a controllable level.

Every year, influenza impacts the world's population. Every so often, a strain becomes so powerful or resistant to vaccines that it causes a world-wide emergency. The Spanish Flu of 1918-1919 is one of the worst cases of influenza in recorded history. Severe illness escalated very quickly after contracting the disease. It was documented that people would feel fine when they woke up and would have died by the end of the day. People who did not die from contracting this strain weren't safe, as a significant portion of the population would later die from complications. Approximately 20% to 40% of the world's population became ill with the Spanish Flu, and about 50 million people died with 675,000 of them living in the United States. The lack of scientific knowledge and resources allowed the Spanish Flu to be placed in history as one of the worst influenza season in history (Pandemic Flu History, 2012).

Most historical epidemics occurred long ago, which would make the average person feel comfortable that it could not happen in this day and age. In 2003, a new pathogen emerged that no one had ever seen before, Severe Acute Respiratory Syndrome (SARs). SARs is part of the coronavirus and has been described as a severe form of pneumonia. This pathogen is believed to have its origins in Asia. It became an international issue when a doctor from the Guandong Province, that had been treating patients with SARs, stayed at the Metropole Hotel in Hong Kong. Staying on the same floor of this hotel was a tourist couple from Canada. The couple transmitted the disease over from Hong Kong introducing it to North America. (Sciences, 2014,) The World Health Organization states that there were a total of 8,098 people infected with SARs in this outbreak which 774 people died. The majority of the people who died were healthcare workers, patients, and visitors. (SARS Basics Fact Sheet, 2004) The SARs outbreak of 2003 has required

government and public health officials to take note of illness happening in other countries, knowing the possibility that one person can spread a disease globally.

The Ebola outbreak of 2014 has been one of the largest in recorded history and is considered the first in West Africa. Ebola is predominant in small villages in Africa and usually confined to these areas because the lack of traveling. Ebola is mostly transmitted by human to human interaction through bodily secretions such as blood or saliva. As of August 19, 2014 there were 2612 suspected and laboratory confirmed deaths. Major international health organizations such as the WHO, the Center for Disease Control (CDC) and other international organizations are helping treat these ill patients and reduce this outbreak from spreading. The Ebola outbreak in West Africa has the potential of becoming a worldwide pandemic if the proper techniques are not taken by these organizations to eliminate the chance of spreading the disease to their home country. (CDC, 2014)

1.4 Healthcare Workers (HCWs) and Their Environment

The Bureau of Labor Statistic recently shown that there are about 2.7 million nurses working in the United States with an increase need of an additional 1.2 million jobs by the year 2020. (Statistics, 2012) Along with this increase in jobs, studies have shown that 55% of nurses surveyed plan to retire between the years of 2011 and 2020. (Hader, 2006) This large growth and retirement may insure a shortage of nurses in the near future, potentially forcing nurses to care for more patients and work for longer hours. Some hospitals have combatted these shortages by recruiting foreign nurses. Even though these nurses are trained as well as any other nurse, there are still barriers that may cause difficulty in fitting into the hospital setting. Some of difficulties that can come with recruiting foreign nurses are: language barriers, unfamiliar with the healthcare system in federal, local, and organizational level, and personal/societal value gaps (Wong, 2012).

With the added workload on these nurses, it is well understood why nursing is considered a very stressful occupation. As of 2005, it was estimated that the Registered Nurse (RN) turnover rate was 13.9% and a vacancy rate of 16.1%. With each nurse that quits, another must take its place, which can cost on average $2,821

per new hire. (Group, 2005) (Hunt, 2009) As one would expect, the majority of these nurses that quit are newer to the field. It is estimated that about 13% of newly RNs had changed their principal jobs after one year and 37% were ready to quit. The demanding jobs that nurses have can be very stressful and may affect their performance. These high stress work conditions can cause nurses to forget training, not take the proper measurements to protect themselves, or make other mental errors that would not normally occur under less severe conditions. With newly licensed RNs starting repeatedly, many may not have the proper training or experience to take all necessary precautions and can increase the chances for medical/performance errors. Two departments that are both physically and mentally stressful are the Intensive Care Units (ICU) and the Post Anesthesia Care Units (PACU).

There are over a half million intensive care nurses practicing in the United States today. (About Critical Care Nursing, 2013). These nurses work in close proximity to critically ill patients that have a wide variety of issues such as: shock, acute/chronic respiratory failure, infections, renal failure, neurological condition, and bleeding/clotting. (Health) With these different types of health conditions the nurses have to use different treatments that can cause harmful exposures. Depending on the individual patient, intensive care nurses may be exposed to many different exposures like: radiation from x-rays and radioisotope sources, waste anesthetic gases (WAG), long term exposure to medications, airborne pathogens, and blood/bodily fluids. (IIOSH, 2000) The diversity of patient issues demand that intensive care nurses must be well trained and very focused on the patient, the environment, and their personal safety.

Another group of nurses that may be smaller in population but have just as stressful of a job are perianesthesia nurses. In the United States, there are over 55,000 perianesthsia nurses employed. (ASPAN, 2013) These nurses mainly work in close proximity to patients before and after surgery and are potentially exposed to WAG and biological pathogens, where the patient is the reservoir. In the PACU, patients coughing is very common because of mechanical and pharmacological irritation caused from the anesthetic gases and surgery. Not only can the coughing expel WAGs, but it can also expel saliva and blood due to the disruption in the mucosa

membrane from tubes inserted during surgery. Coughing is also common in certain surgeries like ear, nose, and throat (ENT) and dental surgery. (Csete, 1996) Coughing up blood and saliva generates bio-aerosols within the area of the perianesthesia nurse causing potential transmission of pathogens. When nurses work in close proximity to sick/healing patients, they are being exposed to treatments given to the patients such as WAG, and they are potentially exposed to pathogens brought in by the patient.

1.5 Human Transmission

The majority of bioaerosols transmitted by means other than direct contact often exits a sick person through their respiratory system. Activities like coughing, sneezing, talking and even breathing can cause formations of respiratory droplets. There are three different types of bioaerosol-respiratory transmission: droplet, droplet nuclei, and airborne transmission. Droplet transmission can be defined as respiratory droplets that carry pathogens and traveling directly from the respiratory tract of an ill person to the mucosal surfaces of a susceptible host; physically, they are greater than 5 μm in size and can only travel up to 3 feet from the patient's head. Droplet nuclei are a lot smaller in size going from less than or equal to 5 μm in size and can travel up to 6 feet from the patient's head. The final type that is discussed is airborne transmissions. Contrary to droplet and droplet nuclei, these do not have to be caused by sneezing or coughing. Airborne particles can travel by dust or any other particulate in the air. (Siegel, Rhinehart, Jackson, & Chiarello, 2007) All three of these transmissions are important, but this research focuses on droplet and droplet nuclei transmission spread by breathing, coughing, sneezing and talking.

Out of all of the mechanisms for the dispersal of droplets, normal breathing can possibly be the most critical. Coughing and sneezing may produce more aerosolization at once, but breathing occurs more than the other mechanisms. (Tang, et al., 2013) Human breathing has a tidal volume of about 0.5 liters per breath taken. Since the average person (77kg) takes 20 breathes per minute, we can calculate the average flow rate to be about 10 liters per minute. (Green, et al., 2012) At this flow rate, an average of about 38 particles of droplet and droplet nuclei are expelled from breathing with every liter of breath exhaled. (Wurie, et al., 2013) These particles can

travel an average of 2 or 2.6 feet depending whether it exits through the nose or mouth, respectively. (Tang, et al., 2013)

Coughing is a reaction that occurs when there is irritation in the throat. The dimensions of a cough, along with other methods of aerosolization, depends on gender, height, and age. The exhaled volume varies from 400 – 1600 ml for males and 250 – 1250 ml for females. These are also fast with a peak velocity time between 57 and 110 ms. (Tang, et al., 2013) This violent displacement of air disperses about 710 particles per cough that can expose people up to 3 feet. (Fernstrom & Goldblatt, 2013) (Guptaa, Linb, & Chena, 2009) Coughs not only expel droplet/droplet nuclei into the environment, but also bits of the mucosal lining of the throat that can also harbor pathogens.

A sneeze is a sudden, forceful, uncontrolled expulsion of air from the lungs that travels through the nose and mouth cause by irritation of the mucosal membrane of the nose and throat. (Sneezing, 2012) Even though this definition seems similar to a cough, sneezing is more forceful and is produced by a lot more energy. The average sneeze exits the mouth traveling at about 4.5 meters per second. (Tang, et al., 2013) Different from a cough, sneezes are estimated to release on average 40,000 particles per sneeze covering a range of over 2 feet (Fernstrom & Goldblatt, 2013; Tang, et al., 2013).

The final mechanism for droplet transmission is the most essential part of communication and obtaining of knowledge. Talking stands second to breathing as far as actions that are done on a frequent basis. Studies have shown that talking has an exhaled volume of about 25 liters (for reciting a 2 minute passage) and a peak flow rate of 1.6 liter per second. This exhaled breath causes the release of about 36 particulates per 100 words with an exposure range of over 2 feet. (Fernstrom & Goldblatt, 2013) (Gupta, Lin, & Chen, 2010)

1.6 Healthcare Exposure Controls

Occupational Safety and Health Administration (OSHA) defines industrial hygiene as, “science and art devoted to the anticipation, recognition, evaluation, and control of those environmental factors or stresses arising in or from the workplace,

which may cause sickness, impaired health and well-being, or significant discomfort among workers or among the citizens of the community." As each one of these actions builds on the next, the anticipation, recognition, and evaluation are used to develop controls. Controls that are used to harness hazardous exposures should be done by the following hierarchy: elimination/substitution, engineering, administration, and personal protective equipment (PPE).

Elimination and substitution is the top of the hierarchy because it completely removes the hazard from the area instead of controlling it. Disposable products such as needles, are a form of elimination because once it is contaminated, they are removed and never to be used again. Disposable products have played a major role in reducing the spread of infectious pathogen in healthcare settings.

Engineering controls are very important because they control the hazard at the source by designing out any hazardous condition in the system that can affect the user. In infection control, the idea is to engineer hospital rooms to contain any pathogens that may be in the room, or keep out any pathogens from entering the room. HEPA (High Efficiency Particulate Air) filters are used to competently clean the air coming into the rooms by capturing most particles (pathogens included), providing quality air. For rooms that house infectious patients, these rooms need to be under negative pressure with about 12 air changes per hour of non-recirculated air. The windows, floors and ceiling should also be sealed to keep air from leaking out (Siegel, Rhinehart, Jackson, & Chiarello, 2007).

Administrative controls are used to control hazardous exposures by way of policies and standard operating procedures. Common administrative controls are: surveillance, education, patient placement, and hand hygiene. Surveillance in healthcare gathers and analyze data with an overall goal of reducing mortality and morbidity for the faculty, patients, and visitors. Data collected through surveillance can predict the spread of infections by monitoring high-risk populations, device use, and facility location. Educating healthcare personnel by giving them the scientific rationale for policies is essential for them to fully understand why policies are set. By truly understanding the scientific rationale, the healthcare personnel will be able to modify precautions as the environment or the situation changes. Another

administrative control is patient isolation. If a patient has a known infection, they must get top priority in having their own room. By isolating infected patients from the noninfected, this can reduce the spread of disease while containing the infection into a single area. The final and arguably the most important administrative control is hand hygiene. Traditionally hand washing has been done with antiseptic soap and water; however, alcohol based hand sanitizers are being used more often. They have become a popular choice when hands aren't visibly soiled because of their superior microbicidal capabilities and convenience. These administrative controls along with the other controls above allow for cleaner and safer healthcare facilities (Siegel, Rhinehart, Jackson, & Chiarello, 2007).

All of the control methods so far has attempted to keep everyone in the healthcare facility safe. Personal protection equipment (PPE) is solely used to keep the user safe. The reason it is the lowest is because it doesn't actually remove or contain the hazard, it just makes the user capable to work in the hazardous conditions. Common forms of PPE used in healthcare settings are gloves, gowns/aprons, facesheilds/goggles and respirators. As one would guess, the types and amount of PPE used depends on the environment and expected potential exposures that correspond to the task. There are some guidelines that are recommended by the Center for Disease Control and Prevention. Gloves should no longer be latex due to allergic reactions. Gowns and aprons should be made of a fluid resistant material so to not have bodily fluids soak through the material. Respirators should be rated as a N95, N99, or N100 to ensure sufficient filtering of air the user is breathing. For all PPE, the most important thing is to ensure it fits properly. If it does not fit, then the PPE is not fully protecting the worker.

The hierarchy of controls model has been used and proven worthy time and time again. Even though it is a very important tool, it is not perfect because the people who use it are not perfect. The more human interaction the controls have, the more chances for error to occur. The administrative controls mentioned are very helpful in infection control but the chaotic environment of healthcare may cause healthcare workers to forget their training, education, overlook policies, or rush through procedures and not properly complete them. PPE is very similar. The user must don

the PPE on correctly and take all precautions that are required. Also since the hazardous exposure is not actually removed or contained, if the PPE failed due to a tear or malfunction, then the person is being exposed to the hazardous agent. With some PPE, such as respirators, the slightest thing such as an unshaven face can cause failure. As long as human beings are controlling their fate, there will always be human error. The priority is to use the top tiers of the hierarchy (elimination and engineering) as much as possible without abandoning the lower tiers for extra safety (Siegel, Rhinehart, Jackson, & Chiarello, 2007).

1.7 Epidemiologic Studies

As mentioned earlier, the SARs outbreak that occurred in 2003 was one of the most devastating outbreaks in recent history. This retrospective study described one of the most impacted hospitals in Toronto where 144 persons contracted SARS. One of the most devastating circumstances that left the Toronto area vulnerable (not just this hospital) was that the SARS was spreading to other patients and healthcare personnel before awareness of the presence of this disease by the Canadian medical community; therefore, they did not have proper respiratory precautions implemented. Out of the 144 people who came ill, 111 (77%) contracted the disease in a hospital. The majority of these people contracted it in the same hospital that the son of the index case checked in after he fell ill. Out of all of the ill, 23% of the patients had to stay in the hospital for extended stays due to complications and suppressed immune systems. Less than 10% of the patients had to return to the hospital due to reoccurring symptoms. Unfortunately 8 people died (6.5% mortality rate). The major modes of transmission were believed to be during interaction between patients and healthcare personnel and also during the transportation of sick patients. (Booth, et al., 2003)

In 2008, an outbreak of measles occurred in a hospital in Tucson, AZ. A Swiss traveler was traveling back from Mexico when he entered the Tucson hospital after feeling ill. The healthcare personnel came to the conclusion that he had the measles and other patients and healthcare personnel became ill. Because of this exposure to measles, there were 363 suspected cases of measles that had to be screened, 8 probable case, and 14 confirmed. When tracking down the origins of the transmission,

it was concluded that the second person to contract measles was sitting beside the Swiss traveler in the emergency waiting room. The third was found to be the HCW that assisted the second patient. From there the disease spread through the hospital. The overall cost of this outbreak was almost $800,000 dollar in which 56% was accredited to the lost of time from the HCWs (15,120 hours of sick leave) (Chen, et al., 2011).

In 2005, a study showed a nosocomial transmission of Group A Streptococcus (GAS) from a patient to HCWs. The index case was a homeless Native American woman who entered into the hospital with respiratory issues, weakness, arthrulgas, and vomiting. She was also coughing up greenish sputum with a sore throat. Even after more than 48 hours of antimicrobial therapy, her respiratory therapist still contracted a genetically identical strain of GAS. Because of this woman, 705 healthcare workers had to be screened. Only 14 HCW were tested positive, but 10 of the 14 showed no symptoms even though they had the disease. Seven of the respiratory therapist family members also tested positive for GAS (Lacy & Horn, 2009).

Adenovirus is a febrile respiratory illness that has been a noteworthy problem with military trainees. In March 2007, military officials reported a 3 fold increase in febrile respiratory illness which were later identified as the Ad14 adenovirus among military trainees. Fifteen military trainees were admitted into the hospital, three of which had to be put in intensive care with one death. An investigation was done from August 1, 2007 to June 14, 2007to assess the impact of exposure on the HCWs in this hospital. Outcomes happened as followed: Out of 483 HCWs that were identified, 218 agreed to participate in the study. Forty-two tested positive for Ad 14 with 28 being confirmed. Eighty nine percent of the confirmed cases were symptomatic by definition with 16 of these patients additionally reporting fever. Of the 16 cases, fourteen continued working while they were sick. Nine of these cases were nurses, three were medical technicians, one was a respiratory therapist and one was a medical resident (Lessa, et al., 2009).

1.8 Review Summary of the Literature

As mentioned earlier, nurses and other healthcare personnel have very stressful occupations. The more stressed a worker is, the more likely he/she is to make mental errors. These mental errors can affect the patients, workers, families or a combination of all. Not only can stress cause mental errors that can lead to hazardous conditions, but it also decreases the workers immune system. With a weakened immune system, healthcare workers are more apt to be absent due to illness or continue working while sick which is a rich source of spreading infections. These mistakes made by the healthcare personnel can be costly and become a vicious circle. Figure 1.1 indicates a possible route of how infections spread. Generally an infectious person transmit their illness to surrounding areas and it survives to pass to the next victim. Most methods used currently try to control the exposure after the infection has spread through good hygiene and sterile techniques, but very few actually try to control it at the source of exposure, which is the patient. To reduce infections from spreading, healthcare facilities implement controls at the source of exposure to protect the workers from illness along with the patients, family, and other visitors. A new market available scavenging system has been developed a scavenging system to reduce healthcare worker's exposure to waste anesthetic gas. If it has the ability to control pathogens exposure as well as it controls waste anesthetic gas, this can be a very powerful tool in reducing the spread of infectious disease in hospital settings, especially in the PACU and ICU.

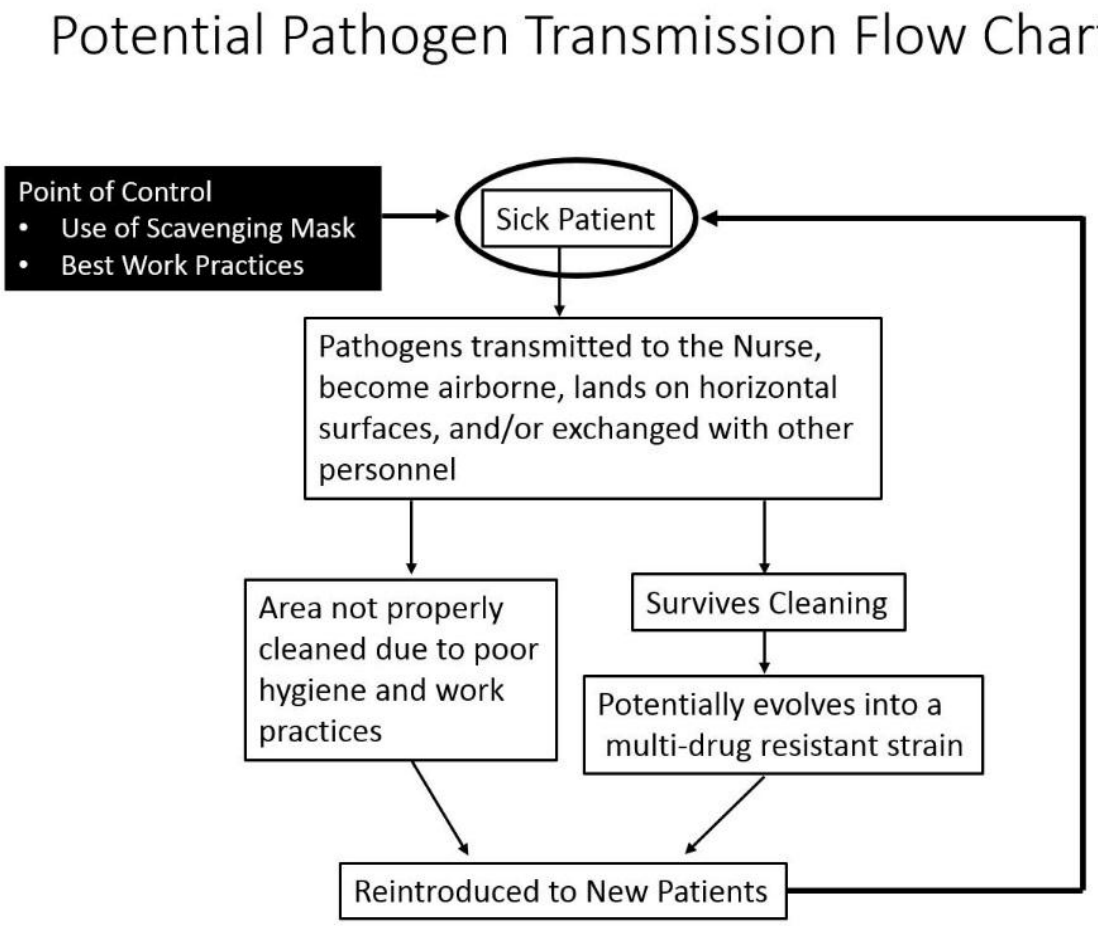


Figure 1.1 Possible Routes for Pathogen Transmission

CHAPTER 2. MATERIALS AND METHODS

2.1 Procedure

This study was divided into two separate parts: bacterial trials and virus trials. The trials were separated in order to ensure that the results are accurate and issues with unwanted interactions between the viruses and bacteria will not occur during analysis. The first part of this project had 3 steps to each trial: standard curve, control, and case trials. The standard curve was important in understanding the boundaries of the sampling equipment by observing its efficiency in capturing the bioaerosols in the air with known concentrations being nebulized. The controls were used to understand at what concentration of bioaerosols the sampling methods can expect to capture if there aren't engineering control or barriers used. Finally, the case trials are the last to be completed which include the scavenging mask used as it would be in the PACU and ICU. Figure 2.1 outlines the steps of the study.

The live bacteria and viruses were used to give a practical understanding of the issues involved when microorganisms are being released into the air. The primary characteristic was the range of sizes that could correlate with common infections such as the Methicillin Resistant *Staphycoccus aureus* (MRSA) and the common cold . The sizes in between the maximum (bacteria) and minimum (viruses) help identify trends in the data.

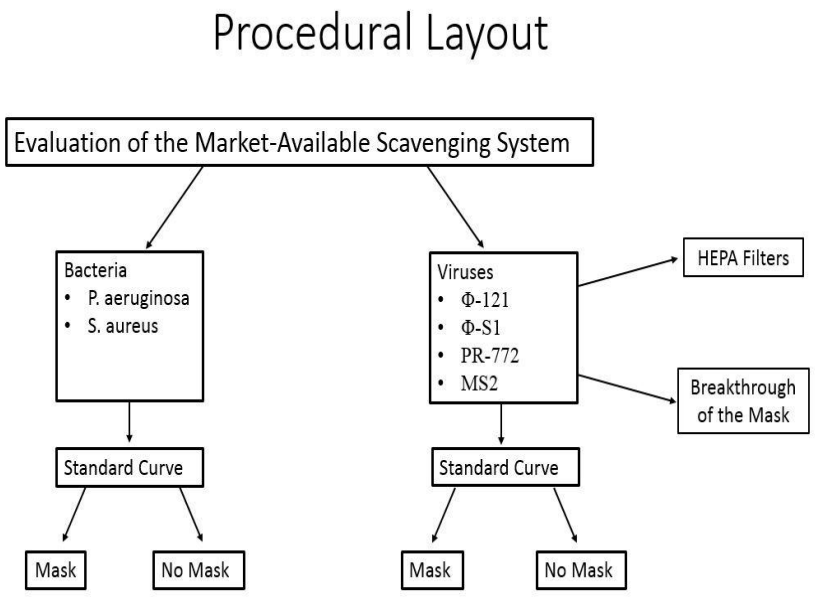


Figure 2.1 Layout of the Primary Procedures of the Study

2.2 Microorganisms Used

For the validity of this study, viable microorganisms were used as our aerosol as oppose to using an artificial representation. In choosing the microorganisms to use in this study, size was an essential characteristic. The goal of choosing microorganisms were to represent a large pathogenic bacteria such as *mycobacterium tuberculosis* (2 – 4 μm) and through the smallest of pathogenic viruses like the ones that can cause the common cold (~ 30 nm). (Bryan, 2011) (Kowalski., 2008). Microorganisms that are within this range were also used. By having a broad range of microorganism sizes, our study can potentially identify any changes that may occur due to size differentials. In choosing our bacteria, we wanted to choose one bacteria that was gram positive and one was stained gram negative in the event that the morphological differences may be a factor in the behavior of the bacteria. It was also important from an analytic standpoint that both bacteria would be able to grow on selective media in attempt to quantify each microorganism separately. In choosing the study's viruses, the surrogate viruses were picked based on size, but also picked on the type of host cell required. Much like the selective media, surrogate viruses that

use the same host cell cannot, under normal circumstances, be quantified individually. The chosen microorganisms used for this study were: *Pseudomonas aeruginosa* and *Staphylococcus aureus* (surrogate bacteria) and *Φ-121, Φ-S1, PR-772*, and *MS2* (surrogate viruses). Additional information about these microorganisms can be found in Appendix A.

2.2.1 Preparation

The *P. aeruginosa* and *S. aureus* was obtain from Food Microbiology Laboratory at Purdue University (Bruce Applegate, 2014). The *S. aureus* strain used was a safer strain that is not resistant to antibiotics and was vulnerable to sterilization. The *P. aeruginosa* strain used in this study has the *lux* genes that allowed it to luminesce at a wavelength of 490 nm to ensure a visible difference between the two bacteria. (Lin & Meighe, 2009) These bacteria were grown in 100 ml flask of Luria Broth (LB) overnight. This overnight culture was mixed with glycerol in a 1:2 ratio, placed in 1.5 ml eppendorf tubes, and stored in a -80 ˚C freezer. When a fresh culture was needed, a sample from the tube was removed and placed in a fresh flask of LB broth and grown overnight. The optical density (OD) was measured using a biophotometer to get an accurate concentration of cells for each set of runs. An OD of 1.7 for *P. aeruginosa* indicated around $4.4x10^8$ CFUS/ml (Colony Forming Units per milliliter) and an OD of 1.5 about 7.9×10^7 CFUS/ml for *S. aureus*. In most cases, the cultures in the flasks were too concentrated in which case 0.05M phosphate buffer was added to dilute to proper concentration. Twenty milliliters of each culture were used in the nebulizer to make a total volume of 40 ml.

The surrogate viruses (*Φ-121*, *Φ-S1*, *PR-772*, and *MS2*) were also obtained from stocks in Food Microbiology Laboratory. Each of these surrogate viruses are different in sizes and also use an array of cell hosts. All surrogate viruses use different strains of *Escherichia coli* except for *Φ-S1* which uses *Psuedomonas fluorecens M3A* as its host. These surrogate viruses were grown using standard protocol for growing bacteriophage as described in Appendix C. The grown surrogate viruses were suspended in phage buffer and tittered using plaque assay which is described in

Appendix B. These surrogate viruses were filter sterilized using 0.2 µm filtered syringe and stored in 1.6˚C refrigeration. Since the concentration of surrogate viruses was known from the stocks, calculating how much was needed for 50 ml by using the dilution formula $C_1V_1 = C_2V_2$ was possible. For each surrogate viruses, three values were known: C1 from the stock surrogate viruses, C2 was desired concentration (10^7), and final volume of 50 ml. This provided the equation: (Stock Conc.) x (?) = (10^7) x (50ml). The determined amount was mixed together in a 50ml conical tube and surrogate viruses buffer was added to get a final volume of 50 ml which was used in the nebulizer for testing. The step by step equation methods for each surrogate viruses are shown in Appendix D.

2.3 Scavenging System Used

The original purpose of the scavenging system is to reduce the amount of WAG that perianesthesia nurses are exposed to during the work shift. The system uses a combination of both negative pressure (suction/scavenging) and positive pressure (oxygen delivery) to keep a constant flow of oxygen to the patient as needed and removal of the waste anesthetic gases which reduces the perianesthesia nurses' exposure. The scavenging system is designed like a normal oxygen tent, but has added an opening around the oral cavity region that is connected to a flexible, extendible suction tube. The oxygen being delivered to the mask comes at a flow rate of 10 liters per minute (lpm) and the suction has a flow rate of about 45 lpm. The scavenging system is designed so that it is not required to fit tightly on the patient in order to keep them calm and comfortable. At the end of the suction tube is a filter whose purpose is to capture debris from the mouth such as blood or sputum. (ISO-Gard, 2014) The aspects of engineering controls that remove the hazardous exposure away from the workers makes this scavenging mask an attractive solution for this study.

2.4 Nebulizer

The nebulizer used for this study was the 6-jet CN-25 MRE (Microbiological Research Establishment) Collision Nebulizer from BGI USA instruments. It was chosen because of its impressive performance in similar studies and its specificity to aerobiological research. (Green, et al., 2012) (BGI Instruments, 2006). The collision nebulizer is made of 316 stainless steel and glass which allows it to be autoclaved. The amount of air pressure applied to the nebulizer determines the amount of liquid that is released. The equation $Q_{liq} = -0.84859 + 0.2336 * \ln (psig)^2$ allow users to determine how much liquid is being dispersed in the air in units of ml/hr. (BGI Instruments, 2006) For the purpose of this project, 27-28 psi of air was applied to the nebulizer which produces 0.52ml of liquid for every 15 minute run.

2.5 Laboratory Setup

The purpose of the project's design is to simulate an infectious patient breathing to the best of the ability of the resources available. A life size manikin head was donated to the project by the Purdue School of Nursing. A piece of $^5/_8$ inch inner-diameter corrugated tubing was fed through the end of the neck and pulled through the mouth stopping just inside the opening of the mouth. This gave the bioaerosols being nebulized direction and kept it contained in the mouth until it was released out of the opening. The nebulizer was connected to the neck end of the manikin where it was inserted in the corrugated tubing. After some minor testing, it was apparent that minimal aerosol coming out of the nebulizer would leak through the tube on the end of the nebulizer so this end was not sealed. The aerosol coming out of the nebulizer was to represent a human breath leaving the body. The average person has a tidal volume of 5 to 7 ml/kg (MacIntyre , 2005). A large 100 kg (220 lbs) man in average condition should have an exhaled flowrate of about 12 lpm. This is calculated under the assumption of a tidal volume of 6ml/kg and 20 breathes per minute. (Green, et al., 2012) The nebulizer in this study had an air supply of 27 – 28 psi measured using a thermo-anemometer to give an exhaled volume of about 12 lpm. Even though breathing consist of inhaling and exhaling, these calculations are for what is exhaled

out of the body; therefore, it is acceptable to have the nebulizer aerosolize at a constant rate. The sampling methods used for this study were two liquid impingers from SKC Inc. that were connected to separate Gilian high flow personal air samplers running at a flowrate of 3 lpm. The impingers were filled with buffer (0.05M phosphate buffer for the bacteria and phage buffer for the surrogate viruses. The volumes were different for the bacteria compared to the surrogate virus trials. The bacteria trials had 15 ml of phosphate buffer while the surrogate virus trials used 10 ml of surrogate viruses buffer. This is because the samplers were pulling the phage buffer into the sampler and 10 ml gave a volume that would not let this occur. The bioaerosols entered in the impingers through a piece of tygon tubing with the inlet equipped with an empty 25mm styrene cassette. Every run was done for 15 minutes. At the end of the 15 minutes, the nebulizer air supply was stopped, followed by the air sampler, and then any other devices that were being used.

2.5.1 Standard Curve

The standard curve was used to identify the limit of detection of our liquid impingers and determine its capture efficiency of bioaerosols. The goal of this part is to set up the ideal conditions to capture as much bioaerosols as possible. With this, our air sampler cassettes were placed directly above the mouth of the manikin and rested on its lips. Ideally anything that exited the mouth had the opportunity to be captured by the impingers. The undiluted mixture of microorganisms that were placed in the nebulizer were the same concentration that was going to be nebulized in the other trials. From this stock solution, serial dilutions were made to make 9 different solutions that ranged in concentration from 0 (stock) to 10^{-8}. The standard curve started with the 10^{-8} dilution with three runs of each dilution and continue to higher concentrations. The reason the mixture was nebulized from lower to higher is to reduce the cleaning and preparation times since whatever is in the nebulizer and other equipment shouldn't affect the higher concentrations. Each sample was collected in a 50 ml conical tube and refrigerated until analysis.

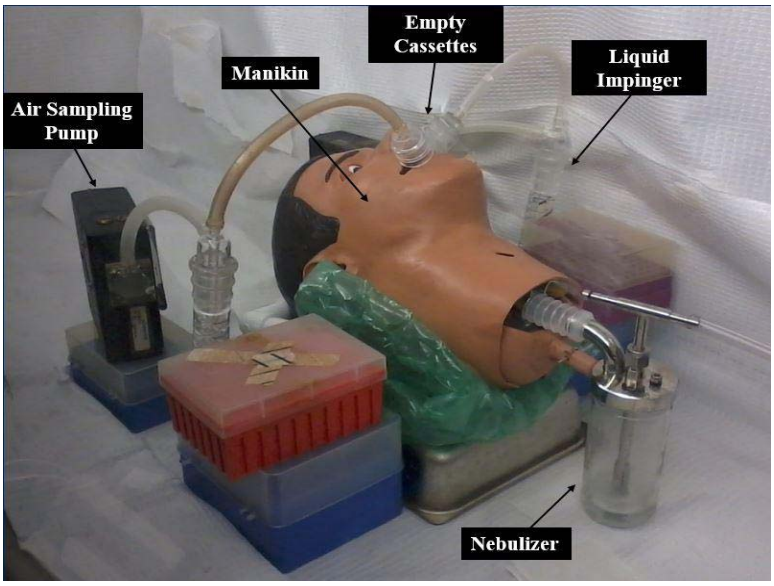


Figure 2.2 Standard Curve Setup with Components Labeled

2.5.2 Control (No Scavenging System Used)

The control and the case were essentially set up the same way only with the scavenging system present in the case trials. In the control runs, the sampling cassettes were positioned six inches above the manikin's mouth to simulate a healthcare worker in close proximity to the patient which would be a vulnerable position for the healthcare worker. (McGlothlin, Moenning,, & Cole, 2013) All samples used the same stock concentration in the nebulizer. There were 12 runs at which the impingers were cleaned and sterilized before the each use. The idea of the control is to see how much exposure to bioaerosols will be captured (represent the exposure to the healthcare worker) without using any sort of control to harness the exposure.

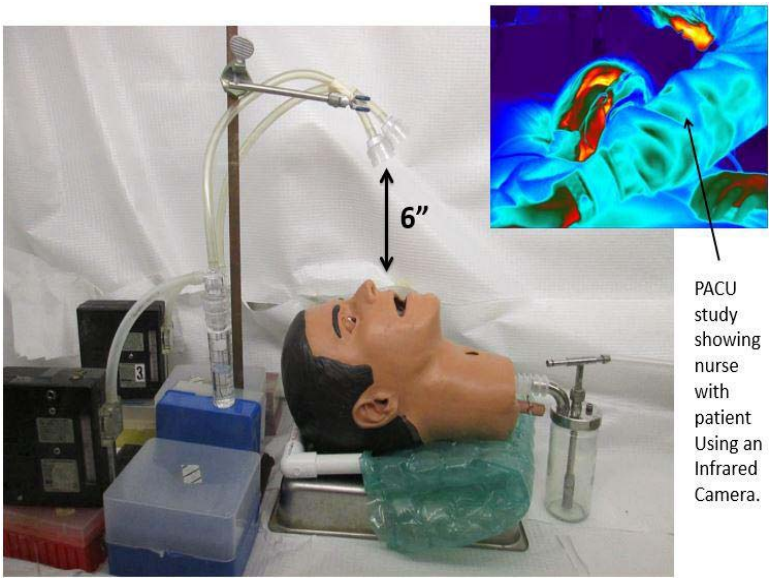


Figure 2.3 Control Setup with Sampler Inlet 6 Inches Above the Manikin

2.5.3 Case (Scavenging System Used)

Everything used in the control runs were used in the case runs and in the same fashion. The scavenging mask was placed on the manikin's face to insure a snug fit. The suction tubing of the mask was directed to a filter flask which contained 50 ml of buffer. The scavenging masked was connected to the filter flask by a piece of tubing that went inside the filter flask to make contact with the buffer. The filter flask was connected to a ¼ horsepower General Electric vacuum that pulled in air at 45 lpm which was also measured by the thermo anemometer. Once a sample was completed, all liquid from both impingers were placed in a conical tube, the filter from the scavenging mask was extracted using a Dremel® tool and placed in a conical tube with 20ml of buffer where it was then vortexed for 5 minutes to extract the microorganisms from the filter. The liquid in the filter flask was rolled around to collect as much as and placed in a conical tube until analysis.

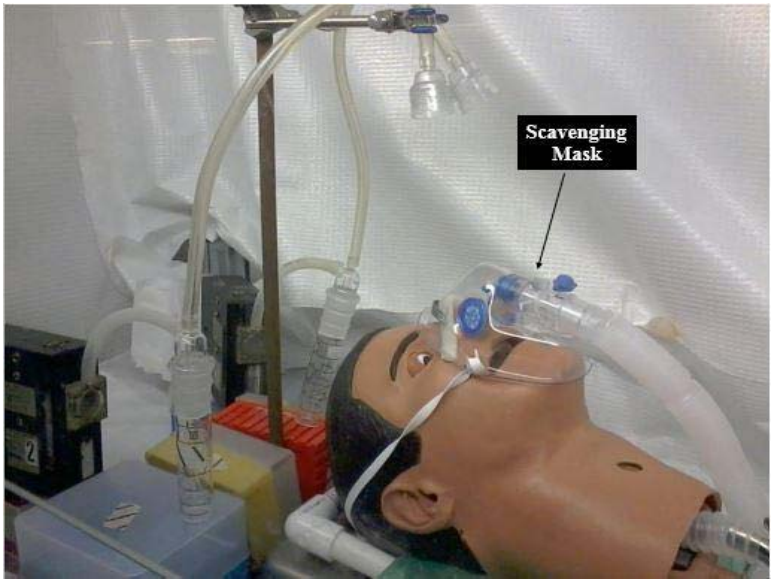


Figure 2.4 Case Setup with the Scavenging Mask

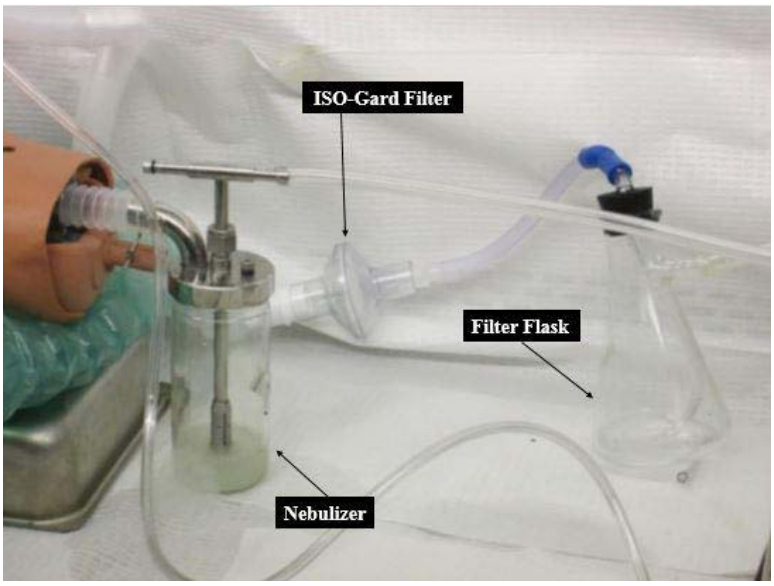


Figure 2.5 Case Setup with the Nebulizer, Filter, and Filter Flask Labeled

2.5.4 HEPA Filter and Breakthrough Protocol

While working on the virus trials, it was evident that more simulations could be done to increase the understanding of how bioaerosols interact with the mask. Surrogate viruses are much smaller than bacteria, usually being measured in

nanometers (nm) as opposed to bacteria which generally is measured in micrometers (μm). These tiny bioparticles are likely to have the ability to escape the mask and break through the filter. For one of the additional trials, the scavenging mask's filter was replaced by a High Efficiency Particulate Air (HEPA) filter. Four runs were done exactly how the case trials were conducted. The HEPA filter was placed in a centrifuge tube with 50 ml of surrogate viruses buffer and vortexed for 10 minutes. The liquid from the tube was then placed in a 50 ml conical tube. The filter flask was collected the same way as before. The surrogate viruses also allowed us to test breakthrough of the scavenging mask. Once again, everything was set up just as in the case trials. The dilutions used in the standard curve was used in the same fashion starting from 10^{-8} dilution to 0 dilution, but only with one run per dilution. The only thing that was collected in these runs were the liquid from the impingers since what was found outside the mask was all that was of concern.

2.6 Analyses

2.6.1 Captured Microorganisms

For both the bacteria and surrogate virus trials, the spread plate method was used to analyze the results. This works by taking the captured sample and diluting the sample by factors of 10. In the bacteria trials, media was used that would cause selective growth of the bacteria being analyzed. This means that the media offers the optimal growth conditions for the said bacteria but is not optimal for the other or any contamination that may occur. As mentioned before, the surrogate viruses used has individual host cells that they attacked to create plaques. Just like the cells, the samples were diluted by factors of 10, then plaque assay was performed as mentioned in Appendix B. Since 100 μL out of 1 ml of sample was taken to analyze, all data was multiplied by 10 and then by however much liquid the bioaerosols was captured in to represent the overall capture of the impinger, filter, or filter flask.

2.6.2 Statistical Approach

Initially, descriptive statistics were used (i.e., mean, standard deviation, and standard error) in this project instead of more advanced methods because of the number of groups that are being compared at once. At any given portion of the study, no more than two groups were being compared at the same time.

A one tailed 2 sample t-test, descriptive statistics, and a regression line were generated using Microsoft Excel 2013. The t-test were used to find significant differences between two points in three different scenarios: impingers from control and case (mask vs no mask), difference between what is captured outside the mask to what is captured inside, and difference in what is captured by the filter and what passed through.

CHAPTER 3. RESULTS

3.1 Surrogate Bacteria Trials

3.1.1 Standard Curve

The "0" dilution on the standard curve, shown in Figure 3.1, has an average capture of 2.36E+08 CFUs and 5.76E+07 CFUs (Colony Forming Units) for *P. aeruginosa* and *S. aureus* respectively. Looking at the figure, it does not appear to be within the saturation part of the curve. The limit of detection seems to begin at dilution -6 for *P. aeruginosa* and -5 for *S. aureus*, which means the indicated linear range for this this concentration is 0 to -6 dilution for *P. aeruginosa* and 0 to -5 for *S. aureus*. This showed the impingers essentially captured the majority of the bacteria nebulized in the air. In both cases the concentration of bacteria is higher in the 0 dilution than what was calculated being nebulized into the air. Looking at the overall trend of the standard curve, it decreased in CFUs as expected by a one log reduction with each dilution. For *P. aeruginosa*, the second and third dilution was not a one log reduction, but in the fourth dilution was reduced by 2 log. The raw data can be found in Appendix E.

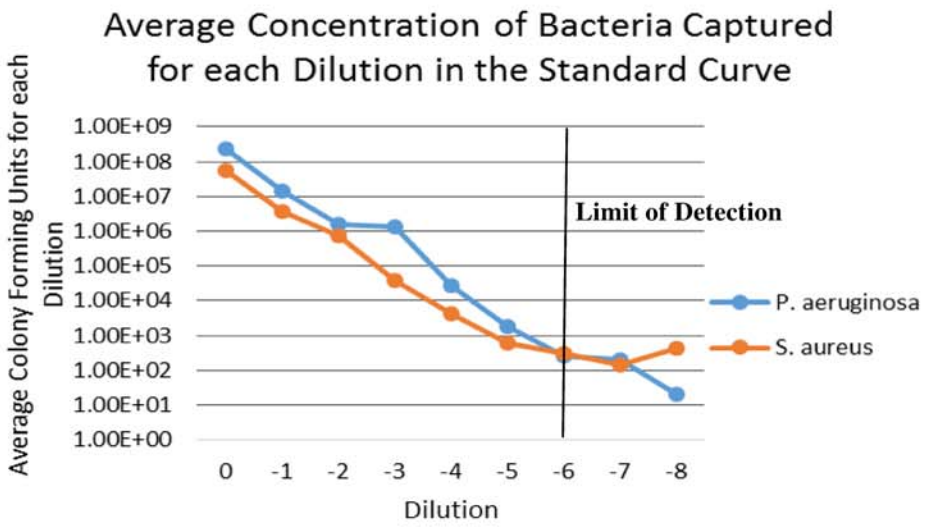


Figure 3.1 Standard Curve for the Bacteria Trials

3.1.2 Controls (No Scavenging System Used)

The average captured colonies from the control runs seen in Figure 3.2 for *P. aeruginosa* and *S. aureus* were 3.25E+06 CFUs (Standard Error = 1.54E+06) and 5.73E+06 CFUs (Standard Error = 1.14E+06), respectively. When compared to the 0 dilution of the standard curve, it is evident that this six inch difference in the sampling inlet has shown a two log reduction in *P. aeruginosa* and a one log reduction in *S. aureus*. The raw data for the control runs of the bacteria can be seen in Appendix F.

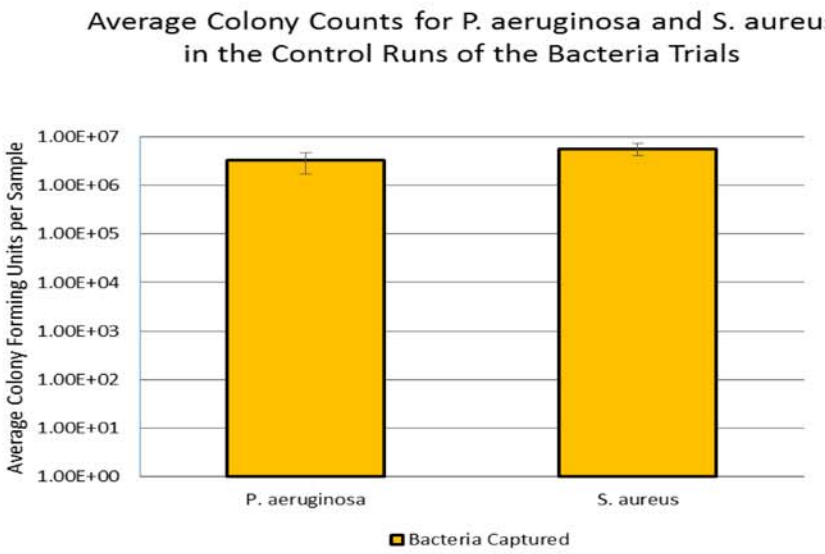


Figure 3.2 Average Capture in the Control Runs of the Bacteria Trials

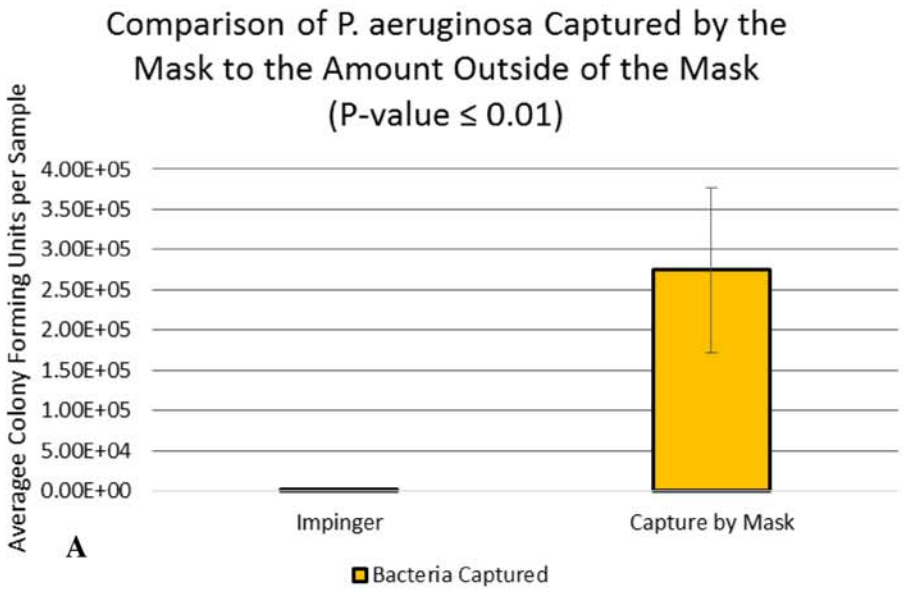
3.1.3 Case (Scavenging System Used)

The case runs had three different points to analyze: impingers, the mask's filter, and the filter flask. The average, standard deviation, and standard error for each point is summarized in Table 3.1 below. The complete raw data can be viewed in Appendix G.

Table 3.1 Descriptive Statistics for the Case Runs of the Bacteria Trials

	P. aeruginosa				S. aureus				
	Impinger	Filter	Filter Flask	Capture by Mask		Impinger	Filter	Filter Flask	Capture by Mask
Average	8.00E+02	2.19E+05	5.57E+04	2.75E+05	Average	3.50E+05	8.89E+06	5.86E+05	9.47E+06
Stan Dev	1.12E+03	3.20E+05	5.03E+04	3.56E+05	Stan. Dev	4.80E+05	1.88E+07	1.65E+06	1.87E+07
Stan. Error	3.23E+02	9.24E+04	1.45E+04	1.03E+05	Stan Error	1.38E+05	5.43E+06	4.76E+05	5.39E+06

When comparing what was captured outside the mask to what was captured inside, the *P. aeruginosa* was found to have much less captured outside as opposed to *S. aureus*. This could be caused by *P. aeruginosa*'s morphology. Figure 3.3 compares the difference in *P. aeruginosa* and *S. aureus* of what escaped the mask and what was captured.



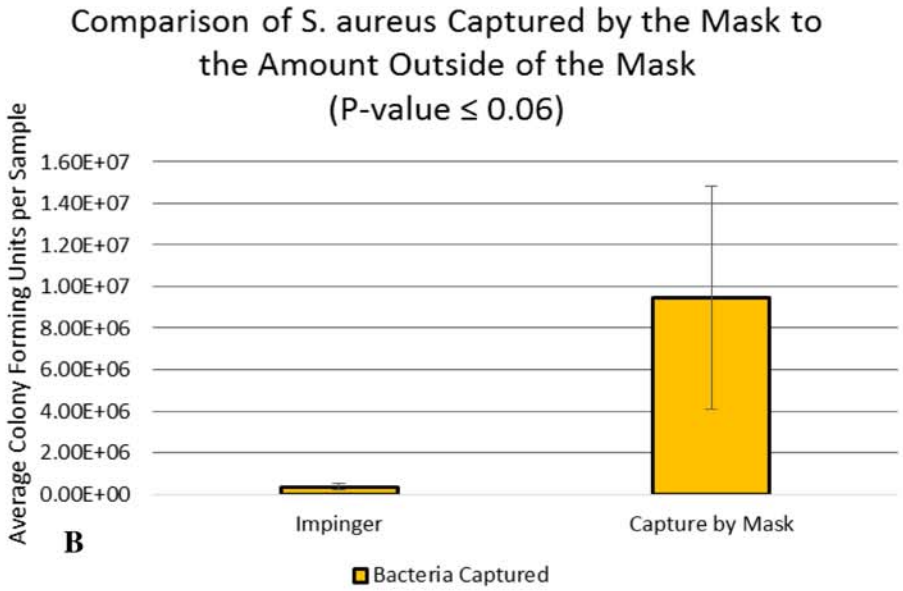


Figure 3.3 (A,B) Average Captured Bacteria Outside the Mask and Inside the Mask

From the case runs, the suitability of the scavenging mask's filter in stopping bioaerosols from passing through the filter was also analyzed. The average amount, shown in Figure 3.4, of *P. aeruginosa* captured by the filter compared to the amount that broke through to the filter flask is 2.19E+05 CFUs and 5.57E+04 CFUs (P-value ≤ 0.05) and *S. aureus* was 8.89E+06 CFUs and 5.86E+05 CFUs (P-value ≤ 0.08).

Both of these bacteria show that the filter will capture more than it will let pass through but will also reduce the amount that can potentially travel to the facilities ventilation unit.

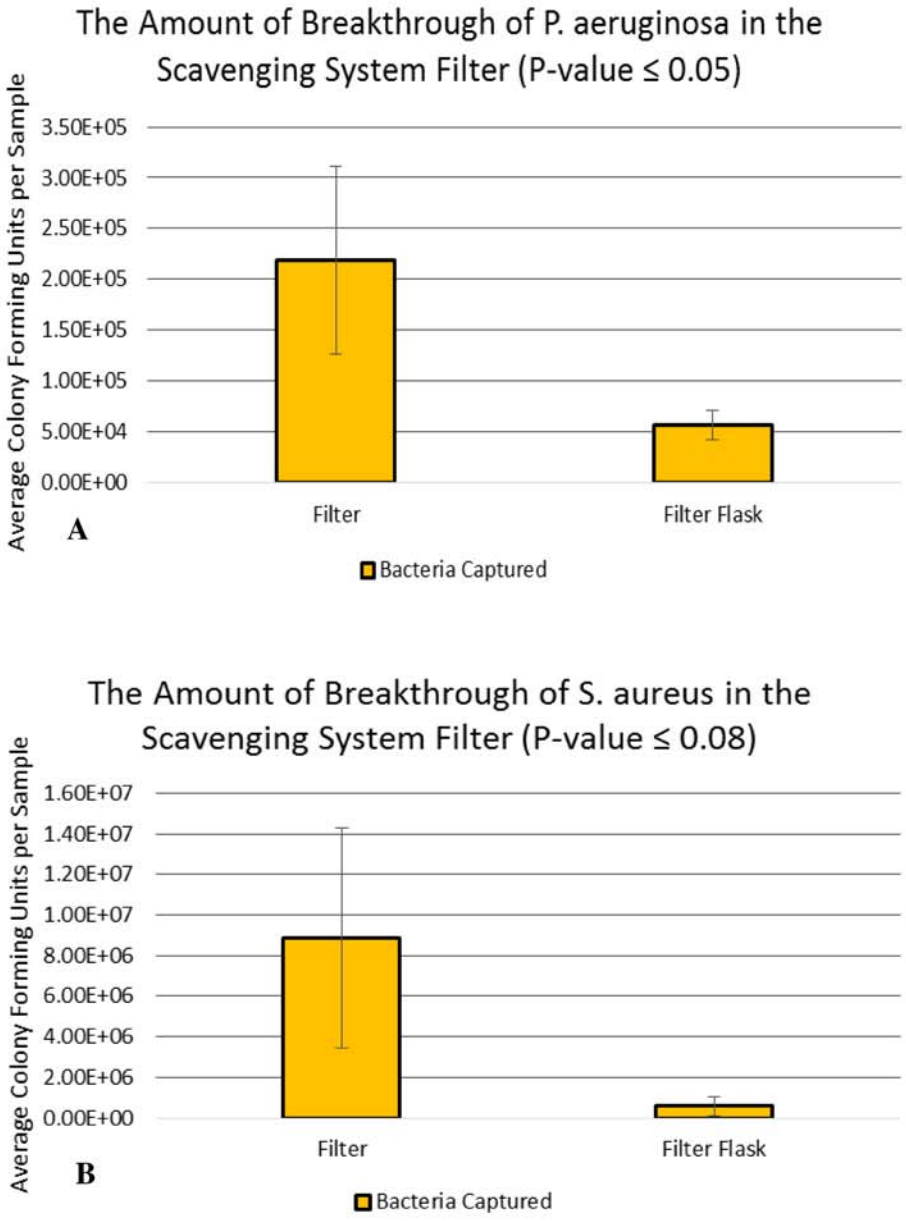


Figure 3.4 (A,B) The Average Amount of Breakthrough of the Filter for Each Bacteria

Even though most of the data for the case runs show the scavenging mask working, there is a great amount of bacteria that is unaccounted for. The amount that is nebulized out of the mouth for *P. aeruginosa* and *S. aureus* is about 1.14E+08 CFUs and 2.05E+07 CFUs respectively. Adding the average amount captured by the impinger, filter, and filter flask gives the amount of bacteria that is accounted for which equals to about 2.76E+05 CFUs for *P. aeruginosa* and 9.83E+06 CFUs for *S. aureus*. This means that over 99% of *P. aeruginosa* and 51% of the *S. aureus* are unaccounted for. Theses three points that are summed to get the overall amount of bioaerosols do not represent the vast majority of surface area of the mask that can cause the bioaerosols to impact or settle that would make it unaccounted.

3.1.4 Mask vs No Mask

With knowing how much bioaerosols was captured in the air without using the mask, and how much was captured with the mask, a comparison of the usefulness of the scavenging system can be determined. Figure 3.5 shows the amount of reduction in these bacteria when using the scavenging mask. The *P. aeruginosa* had on average 3.25E+06 CFUs when the mask was not used and 8.00E+02 when the mask was used. This gives a 99.9998% indicated reduction of this bacteria when using the mask (P-value $\leq$ 0.029). The *S. aureus* had on average 5.73E+06 CFUs when the mask was not used and 9.47E+06 CFUs when the mask was used. This gives a 94% indicated reduction in *S. aureus* when using the mask (P-value $\leq$ 0.00033).

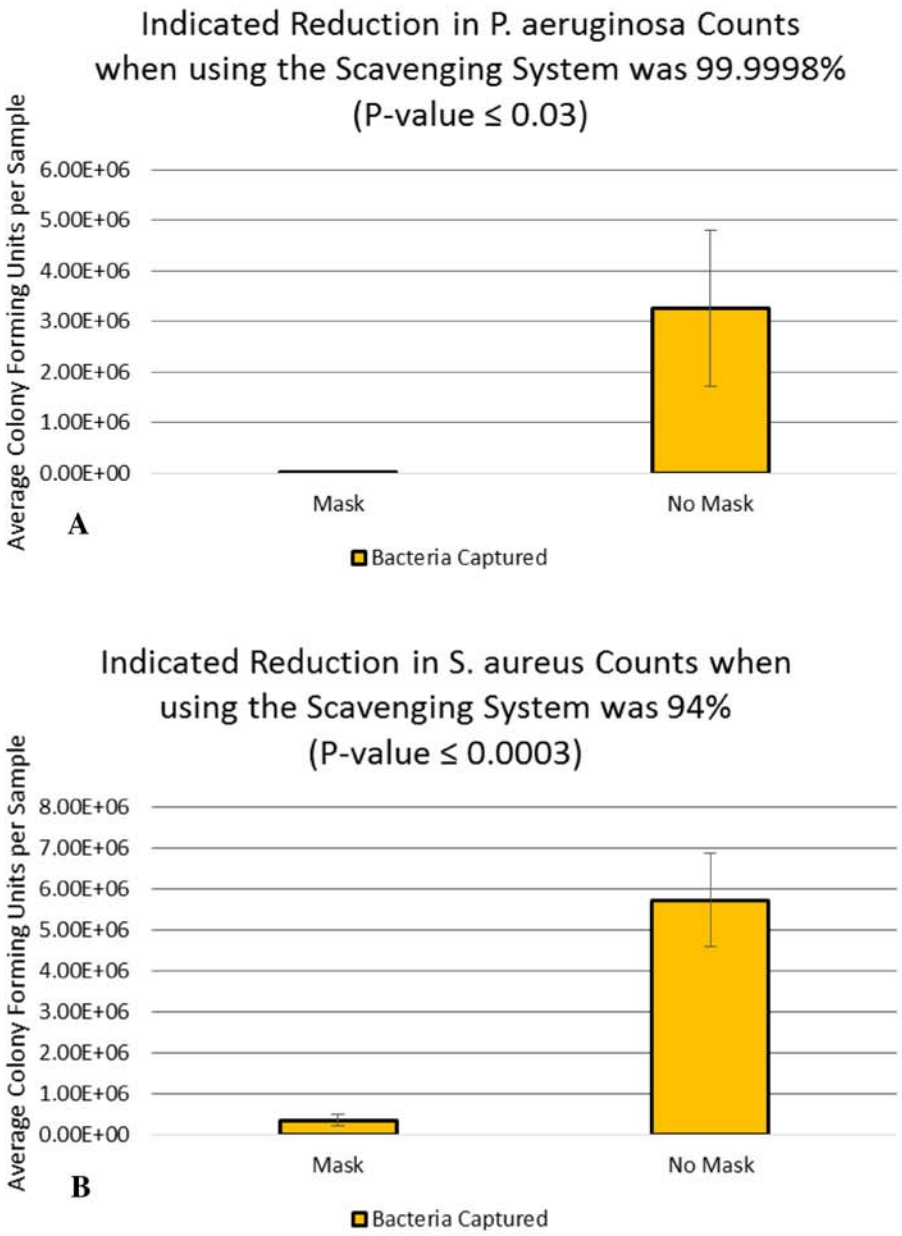


Figure 3.5 (A,B) Comparison of Exposure to Bacteria With and Without the Mask

3.2 Surrogate Virus Trials

3.2.1 Standard Curve

As mentioned before, the concentration of each surrogate virus used in the nebulizer were diluted to get 10^7 surrogate virus per ml for 50 ml. It was calculated that approximately 5.2E+07 surrogate viruses were dispersed from the manikin per 15 minute run. The average amount captured by the impingers are very close to this concentration, indicating that the sampling method is capable of effectively capturing almost everything that is being dispersed. The data plotted on Figure 3.6 shows the average captured plaque forming units (PFUs) for each dilution in the standard curve. The standard curve graph shows that the amount of captured surrogate viruses of different sizes at the "0" dilution is about 5.0E+07 PFUs per sample which relates very well to what was estimated to have been nebulized in the air. The standard curve indicates that the "0" dilution which was used in all other runs was not at the saturation point. It also shows the linear range for all surrogate viruses were from the "0" dilution to the "-3" dilution with everything below "-3" being below the limit of detection. There was a two log reduction between dilutions "-2" and "-3" for all four surrogate viruses that could have been caused by multiple reasons. Besides this 2 log reduction, all surrogate viruses decreased by one log up until the limit of detection. The raw data for the standard curve can be seen in Appendix H.

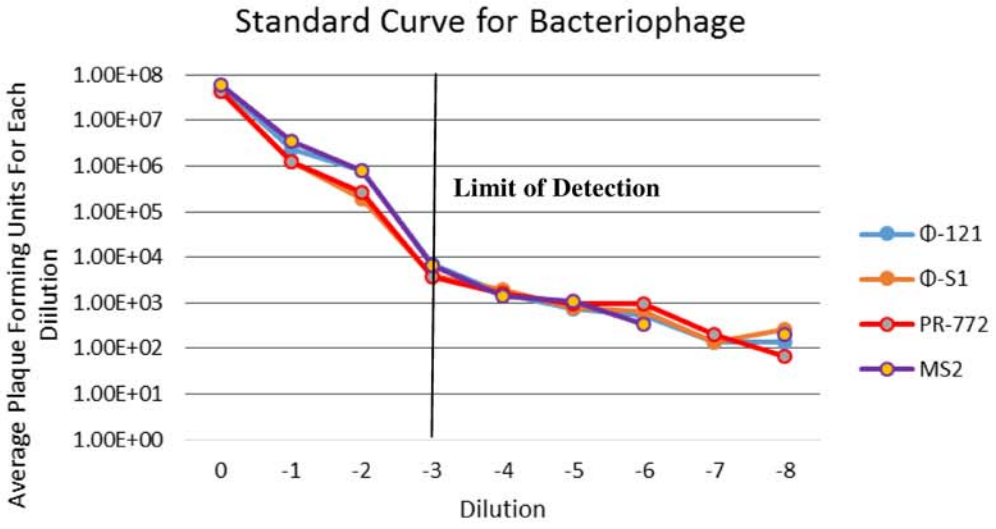


Figure 3.6 Standard Curve for the Surrogate Viruses Trials

3.2.2 Controls (No Scavenging System Used)

The collective descriptive statistics for each surrogate virus is described in Tables 3.2. and 3.3. This table presents two separate sets of data. The data in Table 3.2 is derived from the original raw data collected from the first set of plating. In this data, there were several points that were below the limit of detection or 0E+00. In order to confirm that the sample is below the limit of detection, these points were replated a using the same spread plate analysis method. The descriptive statistics that include the replated points are seen in Table 3.3.

Table 3.2 Descriptive Statistics of the Initial Plating of the Control Runs

	Φ-121	Φ-S1	PR-772	MS2
n	12	12	12	12
Average	2.23E+06	2.09E+06	1.43E+06	1.30E+06
Stan. Dev	2.72E+06	2.38E+06	1.43E+06	1.16E+06
Stan. Error	7.85E+05	6.88E+05	4.13E+05	3.34E+05

Table 3.3 Descriptive Statistics of the Replating of the Control Runs

	Φ-121	Φ-S1	PR-772	MS2
n	12	12	12	12
Average	2.50E+06	9.93E+05	7.15E+05	1.10E+06
Stan. Dev	3.53E+06	1.49E+06	1.05E+06	1.37E+06
Stan. Error	1.02E+06	4.29E+05	3.04E+05	3.96E+05

If referred to the raw data in Appendix H, it is evident that there is a one log increase between the samples 1-6 and 7-12. The raw data for the replated samples can be seen in Appendix I. As seen on the two tables the averages were very similar for *Φ-121*, *Φ-S1*, and *MS2*, while *PR-772* varied from each other. Referring to the standard curve "0" dilution, the surrogate viruses generally presented a 1 log reduction in capture efficiency when the impingers were moved six inches from the manikin. The means that the distance of 6 inches can cause dispersion of the bioaerosols that can cause it to not be captured by the impingers

3.2.3 Case (Scavenging System Used)

The descriptive statistics for the impingers, filter, and filter flask of the surrogate viruses trials are listed in Tables 3.3 below. During the initial plaque assay plating, there were several data points that were reported as 0.00E+00. These data points were replated to see if any plaques could be recorded from the sample. The descriptive statistics shown are calculated with the second platings findings. All four surrogate viruses showed that 99.8% of the surrogate viruses nebulized out of the manikin remain unaccounted when the averages for each point is summed. As with the bacteria, this can be explained because of the vast amount of surface area of the mask that isn't accounted for. The initial set of raw data can be seen in Appendix J and the replated data can be seen in Appendix K.

Table 3.4 Descriptive Statistics for the Surrogate Viruses in the Case Runs

	Φ-121					Φ-S1			
	Impinger	Filter	Filter Flask	Capture By Mask		Impinger	Filter	Filter Flask	Captured By Mask
n	12	12	12	N/A	*n*	12	12	12	N/A
Average	3.08E+04	4.10E+04	4.38E+04	8.48E+04	Average	3.18E+04	2.43E+04	2.52E+04	4.95E+04
Stan. Dev	4.42E+04	3.40E+04	4.20E+04	5.26E+04	Stan. Dev	2.84E+04	1.93E+04	2.69E+04	3.32E+04
Stan. Error	1.28E+04	9.80E+03	1.21E+04	1.52E+04	Stan. Error	8.21E+03	5.56E+03	7.76E+03	9.57E+03

	PR-772					MS2			
	Impinger	Filter	Filter Flask	Capture By Mask		Impinger	Filter	Filter Flask	Capture By Mask
n	12	12	12	N/A	*n*	12	12	12	N/A
Average	1.88E+04	1.50E+04	4.67E+04	6.17E+04	Average	1.62E+04	4.08E+04	5.00E+04	9.08E+04
Stan. Dev	1.45E+04	1.16E+04	2.74E+04	3.14E+04	Stan. Dev	2.03E+04	3.09E+04	3.36E+04	4.89E+04
Stan. Error	4.20E+03	3.33E+03	7.91E+03	9.07E+03	Stan. Error	5.87E+03	8.92E+03	9.69E+03	1.41E+04

Capture By Mask is the Sum of the Captured Bioaerosol Extracted from the Filter and by the Filter Flask.

When comparing what was captured inside the mask to what was captured outside the mask, Figure 3.7 shows the details for each surrogate virus. *Φ-121* had an average of 3.08E+04 PFUs captured outside the mask and 8.48E+04 PFUs captured inside the mask (P-value ≤ 0.0064). *Φ-S1*had an average of 3.18E+04 captured outside the mask and 4.95E+04 captured inside the mask (P-value ≤ 0.08). *PR-772* had an average captured outside and inside of 1.88E+04 and 6.17E+04. Finally, *MS2* shows an average of 1.62E+04 and 9.08E+04 PFUs for the amount of surrogate viruses captured outside the mask and inside, respectively.

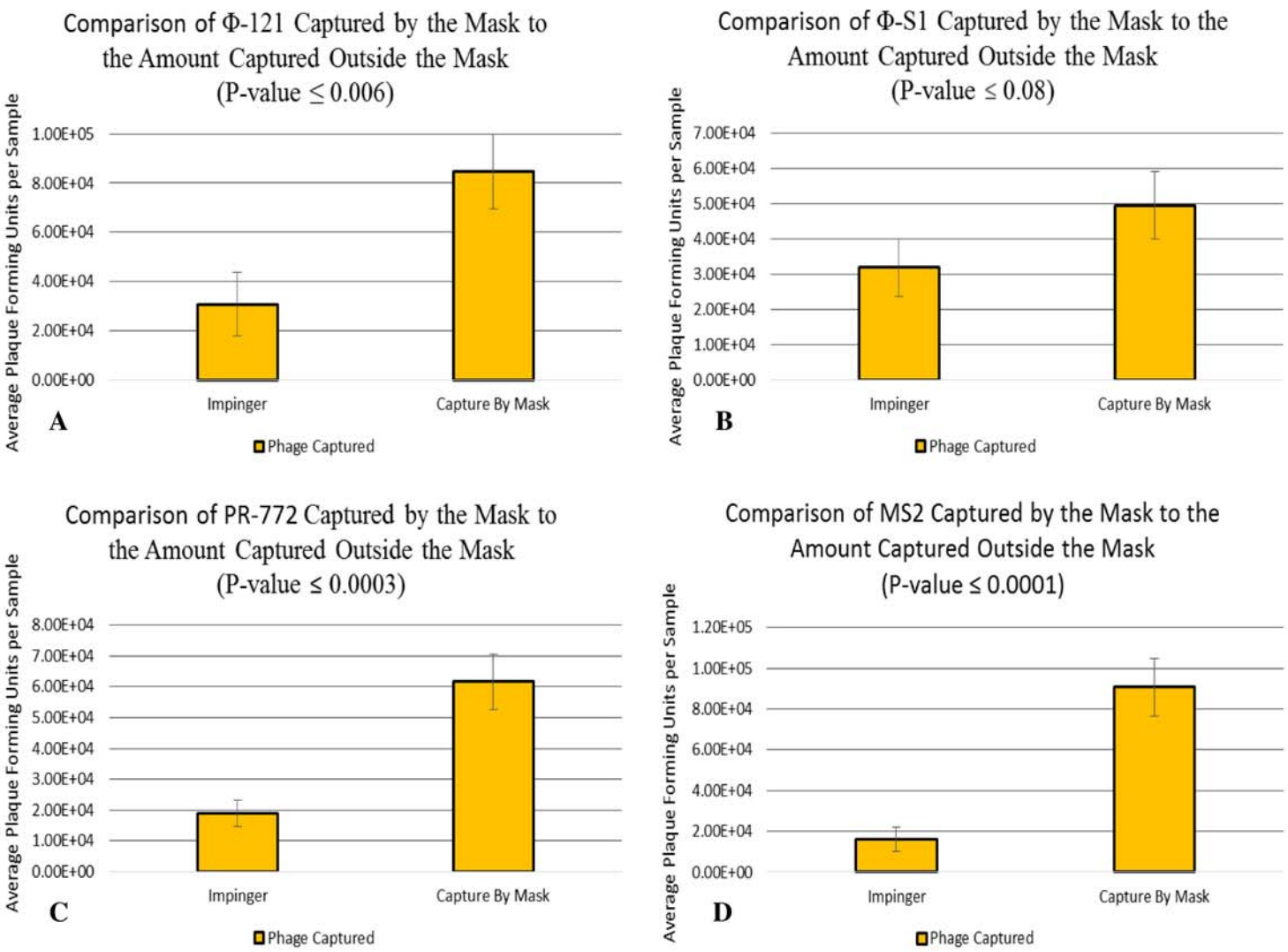


Figure 3.7 (A,B,C,D) Average Captured Surrogate Viruses Outside the Mask and Inside the Mask

The amount of surrogate viruses that broke through the mask's filter can also be analyzed as shown in Figure 3.8. The average amount *Φ-121* recovered from the filter was 4.1E+04 and 4.4E+04 PFUs broke through the filter barrier (P-value ≤ 0.43). This figure also indicates that an average amount of *Φ-S1* captured by the filter was 2.4E+04 PFUs and the amount found in the filter flask was 2.5E+04 PFUs (P-values ≤ 0.47). The amount of *PR-772* found on the filter was 1.5E+04 PFUs and 4.7E+04 PFUs (P-value ≤ 0.0011) had broken through, and *MS2* showed almost 4.1E+04 in the filter and 5.0E+04 PFUs (P-value ≤ 0.25) in the filter flask.

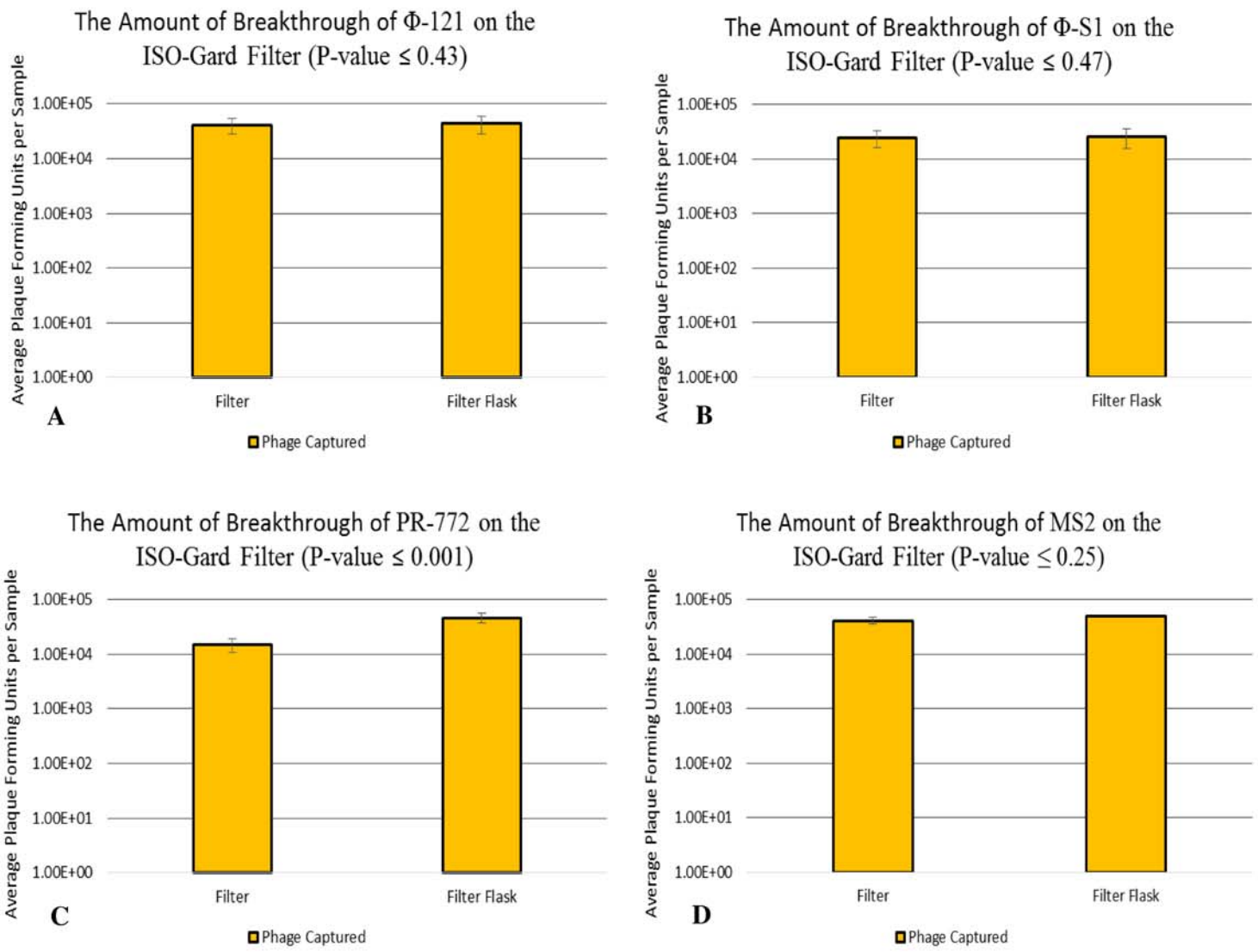


Figure 3.8 The Average Amount of Breakthrough of the Filter for Each Surrogate Viruses

When comparing the concentration of surrogate viruses captured using the mask as opposed to not using the mask, a significant reduction for all surrogate viruses were found as seen in Figures 3.9. *Φ-121* had an average of 3.08E+04 PFUs captured by the impingers when the mask was used and 2.50E+06 PFUs when the mask was not in use (P-value ≤ 0.018). *Φ-S1* had an average capture of 3.18E+04 PFUs while using the mask and 9.93E+05 PFUs when no mask was used (P-value ≤ 0.023). *PR-772* indicated an average of 1.88E+04 PFUs while using the mask and 7.15E+05 PFUs when it wasn't used (P-value ≤ 0.022). Lastly, *MS2* captured by the impinger were 1.62E+04 PFUs for the mask and 1.10E+06 without using the mask (P-value ≤ 0.0096). These large differences in captured bioaerosols and the low P-values indicate that there is significant reduction in bioaerosols going into the surround air when using the scavenging system.

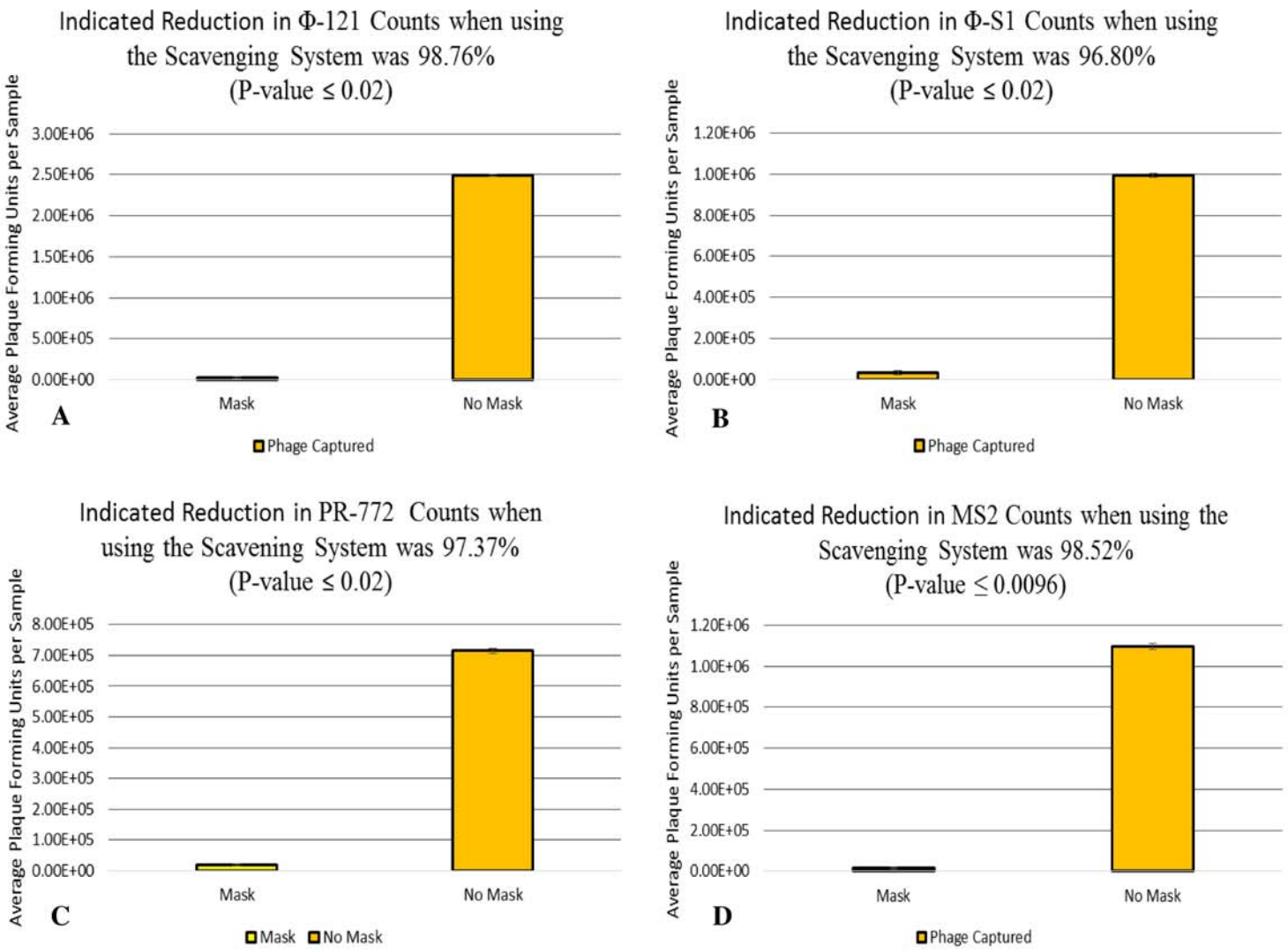


Figure 3.9 (A,B,C,D) Comparison of Exposure to Surrogate Viruses With and Without the Mask

3.2.4 Breakthrough

When looking at the amount of breakthrough seen by the mask per concentration, it did not have a 1 log reduction like the concentration in the nebulizer. It did generally decrease as the nebulized dilution decreased but not at the same rate. *MS2* had the highest amount of breakthrough out of all of the surrogate viruses, followed by *PR-772*, *Φ-121*, and *Φ-S1*. The point that the aerosolized surrogate viruses reached the limit of detection with each surrogate viruses were seen as such: *Φ-121* = -5, *Φ-S1* = -6, *PR-772* = -4, and *MS2* = -7. The general trend is that the smaller surrogate viruses (with exemption of *PR-772*) has more breakthrough than the larger ones. The raw data can be viewed in Appendix L.

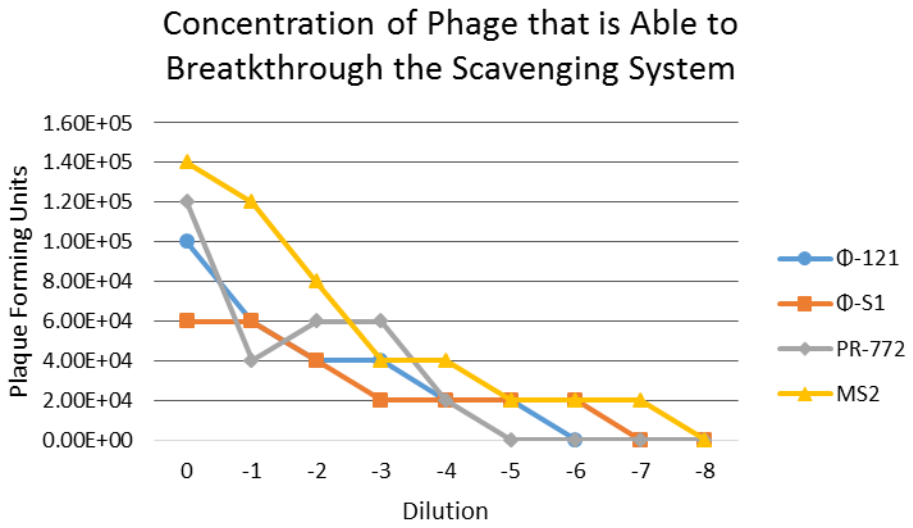


Figure 3.10 Breakthrough of Surrogate Viruses by Concentration

The data from the impingers in the case trials for bacteria and surrogate viruses were plotted in a scatter plot to see if there is a correlation between size of the microorganism and its ability to escape from the mask. Three charts were plotted: using all the microorganisms, surrogate viruses only, excluding *P. aeruginosa*. The general trend in all plots is that the larger microorganisms escaped better than the smaller ones. When all microorganisms were plotted the data had R^2 value of 0.0005. When not including the bacteria, the R^2 value raised to 0.8546. When the lowest value

(*P. aeruginosa*) was removed from the graph, the R^2 value was 0.6153. Figure 3.10 depicts these lines on a graph.

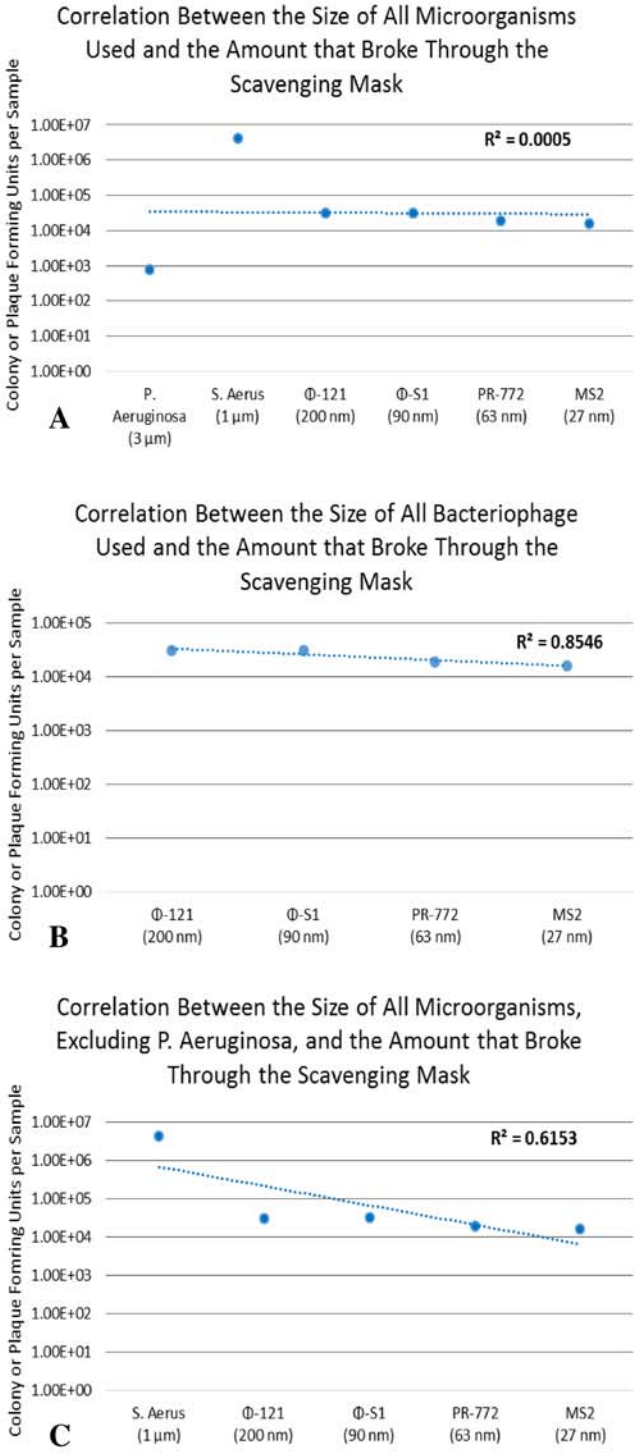


Figure 3.11 (A,B, C) Regression Lines on Scatterplots

3.2.5 HEPA Filters

Table 3.5 provides the average amount of surrogate viruses that was captured from the four runs using HEPA filters in the scavenging mask. Looking at Figure 3.11 the general trend, with the exception of *PR-772*, is that more of the smaller surrogate viruses passed through the filter compared to the larger viruses that were used. The raw data collected can be seen in Appendix M.

Table 3.5 Descriptive Statistics for the Recovered Surrogate Viruses from the HEPA Filters

Φ-121		
	Filter	Filter Flask
n	4	4
Average	2.75E+04	5.00E+03
Stan. Dev.	1.50E+04	1.00E+04
Stan. Error	4.33E+03	2.89E+03

Φ-S1		
	Filter	Filter Flask
n	4	4
Average	8.50E+04	1.50E+04
Stan. Dev.	3.79E+04	1.73E+04
Stan. Error	1.09E+04	5.00E+03

PR-772		
	Filter	Filter Flask
n	4	4
Average	6.50E+04	7.50E+03
Stan. Dev.	2.52E+04	1.50E+04
Stan. Error	7.26E+03	4.33E+03

MS2		
	Filter	Filter Flask
n	4	4
Average	8.50E+04	3.00E+04
Stan. Dev.	2.52E+04	2.45E+04
Stan. Error	7.26E+03	7.07E+03

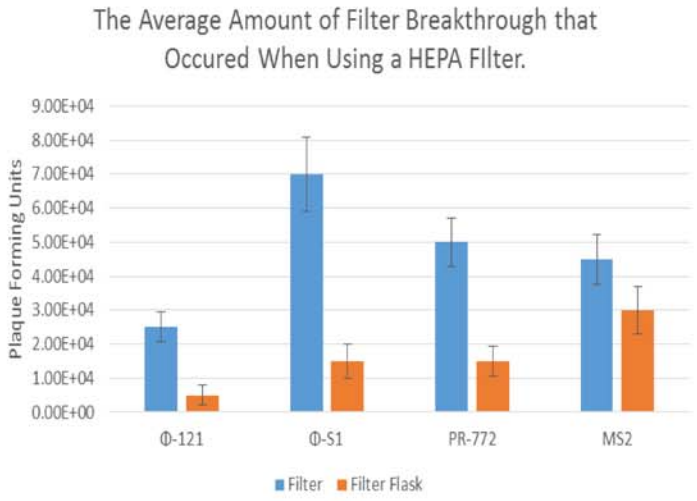


Figure 3.12 Average Recovered Surrogate viruses from the HEPA Filters and Filter Flask

CHAPTER 4. DISCUSSION

4.1 Explanation of the Results

4.1.1 Standard Curve

A standard curve can generally be separated into three parts: point of saturation, linear range, and limit of detection. It is ideal to have the bioaerosols concentration in the linear range as to not overload the impingers (saturation) or too low of concentration to collect meaningful data. When taking a sample in any circumstance, having data that comes back negative or "0" does not mean that there isn't anything in the sample, it means that the sample analyzed was below the limit of detection. On the other side of the spectrum, saturation can be a big problem. Saturation can caused inaccurate results due to the sampler and analyzing methods not being able to detect more or less of the substance being analyzed. After calculating the amount of surrogate bacteria and viruses in the nebulizer during their respective runs and how much can be expected to be released during one run, it was found that about 1.14E+08 *P. aeruginosa* cells, 2.05E+07 *S. aureus* cells, and 5.2E+07 for all viruses were nebulized with each 15 minute run.

In both in the bacteria trials and *MS2* in the virus trials had higher concentrations of in the 0 dilution than what was calculated being nebulized into the air. First, spread plate analysis is a reasonable and proven quantifying method for microorganisms, but it's not exact. This is because it takes a small sample of the culture and quantifies it. Sampling error can occur taking the sample from a less populous area of the culture. Second, these microorganisms can't be seen with the human eye; therefore, it is unknown how well they stay mixed in the nebulizer or if they cluster together during the run.

When looking at the raw data for *P. aeruginosa*, there is not much difference between the CFU counts for the -2 dilution as is for the -3. This may indicate that *P. aeruginosa* contaminated this dilution after the dilutions were done. The contamination would have had to occur afterwards, because the -4 dilution is what is expected if each dilution was a one log reduction. Looking at the raw data for all of the viruses, there is less than a 1 log reduction for each virus between the "-3" dilution and "-4" dilution. Since this occurs in all four viruses, it can mostly be explained by an error while diluting. This can be caused by not properly mixing dilution "-3" before making dilution "-4" using a vortex.

4.1.2 Control (No Scavenging System Used)

When compared to the 0 dilution of the standard curve, it is evident that this six inch difference in the sampling inlet has shown a roughly two log reduction in *P. aeruginosa* and a one log reduction in *S. aureus* and all four viruses. It's expected to see a reduction when the sampling inlet of the impinger is 6 inches away, because as the bioaerosols exits from the manikin, it disperses not only upward but also outward into the air in many different directions. The reason *P. aeruginosa* has a two log reduction as opposed to a one log reduction can possibly be explained by the fact that it's 1.5 to 3 times larger than the *S. aureus* and may begin to descend before it reaches the air sampler due to gravity and air resistance.

In the virus data, it is evident that there is a one log increase between the samples 1-6 and 7-12. To ensure the accuracy of this data, the samples were replated to see if it would yield any plaques. Even though the trend of the first half being less than the second half is still present, the numbers are less uniform and closer to what would be expected from the data. This may be explained by the viruses settling in the bottom of the nebulizer so fewer viruses are nebulized until later samples when viruses closer to the bottom of the nebulizer may be aerosolized.

4.1.3 Case (Scavenging System Used)

The "Captured by the Mask" is the sum of the concentration found on the filter and filter flask. This was useful in comparing the CFUs found in the mask to what was captured on the outside by the impingers. When comparing what was captured outside the mask to what was captured inside, the *P. aeruginosa* was found to have much less captured outside as opposed to *S. aureus*. *P. aeruginosa* has been known as an issue with hospitals because of its ability of sticking to surfaces because of its exopolysaccride secretion. (Evans & Linker, 1973). This exopolysaccride secretion means that it is likely to stick to any surfaces that it comes into contact. The reason for much higher outside counts of *S. aureus* maybe because it wasn't sticking to the mask like the *P. aeruginosa*. This can also explain why less *P. aeruginosa* was recovered from the filter and filter flask. *Φ-S1* had data that was a little out of the ordinary compared to the other three surrogate viruses. With a P-value of 0.08, there wasn't a large difference between what was captured outside the mask by the impinger and what was collected inside the mask by the filter and filter flask. Looking at the raw data of the filter flask for *Φ-S1*, there were a 4 samples that were below the limit of dectection or "0.00E+00". This decreased the average for "Captured by Mask" and created the higher P-value. This can be caused by more being attached to the filter and could not be removed or that *Φ-S1* never reached the mask but instead attached itself to the mask.

The P-values for *P. aeruginosa* and *S. aureus* are at or slightly above 0.5 which indicates that the filter helps some with the breakthrough of bacteria, but doesn't greatly reduce the breakthrough of bacteria. The surrogate virus data shows even a larger P-value for *Φ-121*, *Φ-S1*, and *MS2* ranging from 0.25 to 0.47. This means that the viruses being nebulized were passing the filter with very little resistance from the filter.

In additional runs, the traditional filter was replaced with a HEPA filter insert. The definition of a HEPA filter is: "A throwaway, extended-media, dry type filter with a rigid casing enclosing the full depth of the pleats. The filter shall exhibit a minimum efficiency of 99.97% when tested at an aerosol of 0.3 micrometers

diameter." (Department of Energy, 2005) With the largest virus used being about 200 nm, it is evident that HEPA filter do help but it will not be fully protective. The graph on Figure 3.12 showed the HEPA filters in the scavenging mask followed a general trend showing a higher percentage of the smaller viruses passed through the HEPA filter compared to the larger viruses. Since the definition of a HEPA filter states that it can capture 99.97% of particles at 300nm or larger, it was assumed the HEPA filters would capture the bacteria, so only viruses were used. The P-values for the HEPA filters were all below 0.05, but would need to have further studies done to derive a confident answer.

The scavenging mask has a large amount of surface area and the suction tube is very rigid due to it being corrugated. There are endless possibilities of where the rest of the surrogate bacteria and viruses settled. It's also possible some of it landed of the manikin's face which could pose an issue of nurses wiping the patients face after using the mask. All surrogate viruses and *P. aeruginosa* had over 99% of the particles nebulized unaccounted for and 51% unaccounted for with *S. aureus*. Even though there is a noticeable amount of surrogate bacteria and viruses that are not accounted for, it is still being captured by the mask and is reducing the exposure to healthcare workers. Since the mask is a one-time use, disposable scavenging system there is no cleaning involved and can be discarded after use.

The scavenging mask is made with soft clear vinyl plastic. (Teleflex, 2014) Most vinyl plastics used in medical devices are made with plasticizers such as DEHP (di-2-ethylhexyl phthalate) which makes the plastic flexible and versatile. It is also a major component in wire and cable insulation making the coating around the wire neutrally charged. (European Council for Plasticisers and Intermediates, 2010) Generally, both bacteria and viruses have a net negative charge on the surface. (Krueger, Ritter, & Smith, 1929) (Dickson & Koohmarie, 1989) Since the mask is neutrally charged and the bioaerosols are negatively charged a temporary charge polarization can possibly occur. When the negatively charged bioaerosols makes contact to the neutral plastic, the electrons in the plastic may realign to be as far away from the bioaerosols as possible, leaving the plastic surface near the bioaerosols with a temporary positive charge which could cause an adhesion of the two.

When comparing how well the mask reduces bioaerosols exposure to no mask, there is a notable difference. There is a 94% reduction or greater in bioaerosols in all surrogate bacteria and viruses used in this study. When the concentration of all microorganisms captured outside the mask by the impingers is plotted on a scatter plot, there is no real trend as the R^2 value equals 0.0005. This shows no trend and is unable to make predictions using this data. Since the *P. aeruginosa* has the exopolysaccride coating that is believed to cause issues with this study, it was removed in a second scatter plot to see if there is a better correlation. Without *P. aeruginosa* the R^2 value increased to 0.6153. Then only the viruses were plotted where the R^2 value increased to 0.8546. The difference between the two values could be attributed to the gap in particle sizes between *S. aureus* (1 μm) and *Φ-121* (~200 nm).

There are very few research papers that have evaluated a way to reduce exposure to bioaerosols as done in this study. As part of a larger study, Christopher Green (2012) led a team in testing how well N95 respirators work in reducing bioaerosols exposure to healthcare workers; however, there are a few differences in the two studies. Their study used a breathing apparataus that "inhaled" and "exhaled" where ours only exhaled. Since the flowrate used in this study was based off of tidal volume, the amount nebulized in the air should be similar. Their sampling equipment was designed for flowrates up to 12.5 lpm as the ones in this project were restriced to 3 lpm, but as mentioned before, there was little bioaerosols lost from the used methods. In their study, the manikin was faced vertically and the sampling system was set up one meter away. Even with these differences, the best reduction using a N95 respirator was approximately 75% using bacteria, and the worst reduction in this research, using the smallest surrogate virus (*MS2*), was 93.2 %. Even though this is only a comparison of a single study, it shows that the scavenging system is much more effective in pathogen control than a N95 respirator.

4.2 Challenges and Recommendations

Future researchers who replicate this study may want to make some changes to further better this research. As mentioned earlier, the *P. aeruginosa* and its

exopolysaccride characteristics cause unforeseen challenges with this study. The *P. aeruginosa* should be replaced by another bacteria that is similar in size but does not have characteristics that make it stick to surfaces as easy. When all data was plotted on a graph to make a regression line, the large size gap between the bacteria and surrogate viruses may have caused some misleading results with the R^2 values. Adding microorganisms that are between 200 nm and 1μm in size may, give more accurate description on how particle size and breakthrough of the mask are related.

There are also some recommendations with the procedure as well. All captured surrogate bacteria and viruses in the case, control and HEPA filter runs should have been plated in triplicate instead of only once to ensure accurate results in the data. The extraction of viruses from the HEPA filter was less than optimal. After each run, the HEPA filters should have been cut in half and placed in a smaller container. It may also be desired to use more buffer while vortexing the filters but it should be noted that too much buffer will dilute the sample and give false representation of what was captured. The two bacteria used in this study had nearly a one log difference in the concentrations even though the OD readings were similar. The bacteria should have been tittered to understand what concentration of bacteria corresponds to a particular OD reading. Finally, the impingers should have remained sampling longer after the sample was taken (possibly 5 minutes) to ensure that all bioaerosols in the air was captured.

4.3 Future Research

This pilot study opened up many opportunities for future research. First and foremost, a study needs to be done to understand where the bioaerosols are going and account for as much as possible. This is important in understanding how much bioaerosols is truly captured by the mask and how much is not being captured and not being detected. An additional part of this future study would be to identify how much is landing back on the patients face. If this is being seen, then additional protocols for cleaning the patients face may need to be implemented to help keep the surrounding environment sterile. A second important future study is to identify common places

that the bioaerosols are escaping. As mentioned before the mask isn't made to have a snug fit on the patient but identifying common areas of escape can create adjustments in the redesign to optimize the mask's capabilities. This can be done by nebulizing smoke or visible tracers to see how the particles flow through the system, Finally, more research needs to be done using the HEPA filters as a replacement and optimizing its capabilities.

CHAPTER 5. CONCLUSIONS

The Center of Disease Control and Prevention (CDC) estimates that hospital associated infections have a direct cost up $33.8 billion dollars per year. These cost effect not only patients who have a compromised immunity, but also visitors, healthcare workers, insurance companies, and with the new Affordable Care Act, taxpayers. Referring to the industrial hygiene hierarchy of controls, healthcare facilities generally exercise substitution, administration, and PPE controls, but not as much in engineering controls. These engineering controls can be used to reduce the spread of infection at the source, which would make their infection control program more proactive and less reactive. The scavenging system's original purpose is to reduce waste anestethic gases, but has all the components of an essential tool in the reduction of pathogen exposure to healthcare workers. This study proves that this mask has the potential to reduce the amount of airborne pathogens expelled by the patient as high as 99%. Even though the data reports that there is an abundant of bioaerosols that were unaccounted for, the scavenging system indicates that it can reduce the amount of pathogen exposures to healthcare workers. Since the scavenging system is disposable, no matter where it is captured in the mask, the pathogens can be safely removed from the area. Even though the scavenging system has proven reduction, the it can still be improved upon by redesigning the filter system along with other components. This pilot laboratory study showed the scavenging mask as a promising tool; however, future research needs to be conducted to further validate its use as an infection control tool and redesign small details to increase its ability to protect workers.

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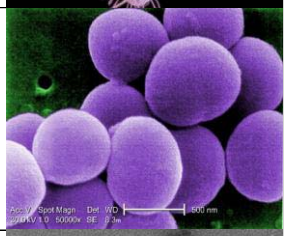
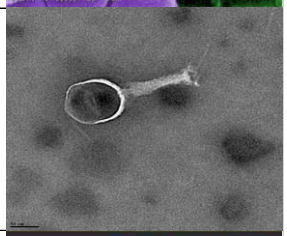
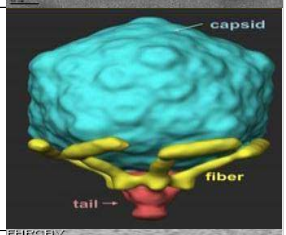
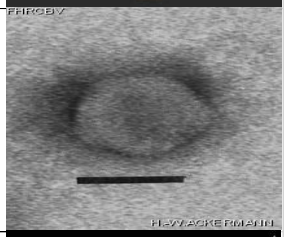
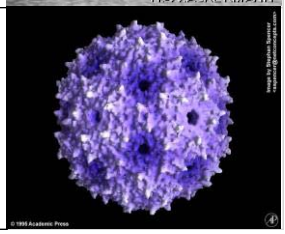
APPENDICES

Appendix A List of Microorganisms with Additional Information

Table A 1 Description of Each Microorganism Used

Name	Type of Organism	Size	Selective Media Host Cell	Picture
Pseudomonas aeruginosa	Bacteria (Gram -)	1.5 - 3µm long	LB with Kanamycin & Salicylate	
Staphylcoccus aureus	Bacteria (Gram +)	1 µm diameter	Mannitol Salt Agar	
Φ-121	Bacteriophage Specific to E. Coli	200 nm long 150 nm tail fibers	E. Coli 0121:H19	
Φ-S1	Bacteriophage Specific to Psuedomonas Fluorescens	60 nm head 30 nm tail	Psuedomonas Fluorescens M3A	
PR-772	Bacteriophage Specific to E. Coli	63 nm in diameter	E. Coli K12	
MS2	Bacteriophage Specific to E. Coli	27 nm in diameter	E. Coli F' Top 10	

Appendix B Procedure for Plaque Assay

1. Determine how many dilutions is required to obtain the optimal results. For tittering purposes, generally diluting to the negative tenth power is usually acceptable.
2. For each dilution, fill a 1.5 ml effendorf tube with .9ml of surrogate viruses buffer.
3. Place 100µL of surrogate viruses into the first tube and vortex.
4. Transfer 100µL from the first tube and place in the second tube, vortex and repeat for the next tube.
5. Have tubes with 4mls of LB top agar preheated and placed in a hot water bath.
6. Add 200µL of fresh overnight grown host culture in the top agar.
7. Add 100µL of bacteriophage in the tube and vortex.
8. Pour in a LB media plate and tilt the plate to ensure complete coverage of the plate.
9. Allow the top agar to solidify.
10. Repeat for each dilution.
11. Allow cells and bacteriophage to grow over night.
12. The following days, count the plaques on the plates in the same fashion as one would colonies in a bacteria spread plate analysis.

Appendix C Standard Procedure for Growing Bacteriophage

1. Make 1 liter of liquid LB for each bacteriophage being grown and have it prewarm over night at 37˚C.
2. Inoculate each 1 liter flask with 5 ml of overnight cell culture of the host cell that is appropriate for the bacteriophage.
3. Monitor OD_{600} for the culture until it reaches 0.2 – 0.3 (about 10^6 cells/ml) and inoculate with bacteriophage for a multiplicity of infection of about 0.1 bacteriophage per cell (about 10^8).
4. Continue to monitor the OD_{600}. The readings should increase with a sharp drop back down the OD before inoculation. Add 4 ml of chloroform per liter culture.
5. Add enough NaCl to make 1M NaCl. For LB that has 10g of NaCl, 48g/L should be added to the flask. Mix until completely dissolved.
6. Centrifuge at 10,000 rpm for 10 minutes to pellet cell debris. Decant the supernatant into sterile bottles.
7. Add 70-80g of polyethylene glycol (M.W. 6000) and dissolve thoroughly. Allow it to sit overnight in at least 4˚C
8. Centrifuge at 10,500 rpm for 15 minutes to pellet surrogate viruses. Decant the supernatant.
9. Resuspend in surrogate viruses buffer.
10. Titter your bacteriophage using plaque assay.

** This protocol was taken from the standard operating procedures (SOPs) of Dr. Applegate's Food Microbiology Laboratory.

Appendix D Equation and Calculation for Determining the Volume of Surrogate Viruses to get a 10^7 Concentration

Φ-121

$C_1V_1 = C_2V_2$

$(1.3 \times 10^{10}$ surrogate viruses/ml) x $(V_1) = (10^7$ surrogate viruses/ml) x (50ml)

$V_1 = 0.385$ ml

Φ-S1

$C_1V_1 = C_2V_2$

$(1.6 \times 10^{10}$ surrogate viruses/ml) x $(V_1) = (10^7$ surrogate viruses/ml) x (50ml)

$V_1 = 0.313$ ml

PR-772

$C_1V_1 = C_2V_2$

$(3.6 \times 10^{10}$ surrogate viruses/ml) x $(V_1) = (10^7$ surrogate viruses/ml) x (50ml)

$V_1 = 0.139$ ml

MS2

$C_1V_1 = C_2V_2$

$(1.28 \times 10^{10}$ surrogate viruses/ml) x $(V_1) = (10^8$ surrogate viruses/ml) x (50ml)

$V_1 = 0.391$ ml

Appendix E Raw Data for the Standard Curve (Surrogate Bacteria)

Table E 1 Raw Data for *P. aeruginosa* and *S. aureus* in the Standard Curve

P. aeruginosa					
Dilution	Sample 1	Sample 2	Sample 3	Average	Sample Adjustment
0	1.60E+06	9.10E+06	1.11E+06	3.94E+06	2.36E+08
-1	2.80E+05	3.81E+04	4.20E+05	2.46E+05	1.48E+07
-2	2.25E+04	2.75E+04	2.96E+04	2.65E+04	1.59E+06
-3	3.25E+04	8.90E+02	3.30E+04	2.21E+04	1.33E+06
-4	2.90E+02	3.36E+02	7.10E+02	4.45E+02	2.67E+04
-5	3.10E+01	3.40E+01	2.80E+01	3.10E+01	1.86E+03
-6	3.00E+00	1.00E+00	9.00E+00	4.33E+00	2.60E+02
-7	0.00E+00	0.00E+00	1.00E+01	3.33E+00	2.00E+02
-8	1.00E+00	0.00E+00	0.00E+00	3.33E-01	2.00E+01

S. aureus					
Dilution	Sample 1	Sample 2	Sample 3	Average	Sample Adjustment
0	1.01E+06	1.50E+06	3.68E+05	9.59E+05	5.76E+07
-1	1.06E+05	1.60E+03	8.20E+04	6.32E+04	3.79E+06
-2	7.40E+03	1.50E+04	1.35E+04	1.20E+04	7.18E+05
-3	6.30E+02	3.20E+02	9.30E+02	6.27E+02	3.76E+04
-4	3.50E+01	6.80E+01	1.08E+02	7.03E+01	4.22E+03
-5	9.00E+00	1.40E+01	8.00E+00	1.03E+01	6.20E+02
-6	5.00E+00	4.00E+00	7.00E+00	5.33E+00	3.20E+02
-7	3.00E+00	3.00E+00	1.00E+00	2.33E+00	1.40E+02
-8	4.00E+00	4.00E+00	1.30E+01	7.00E+00	4.20E+02

Appendix F Raw and Adjusted Data for the Control Runs (Surrogate Bacteria)

Table F 1 Data for the Control Runs of the Bacteria Trials

P. aeruginosa			S. Aureus		
Sample	CFU/100ml	CFU/Sample	Sample	CFU/100ml	CFU/Sample
1	1.22E+04	3.66E+06	1	3.07E+04	6.14E+06
2	5.00E+04	1.50E+07	2	3.90E+04	7.80E+06
3	1.03E+03	3.09E+05	3	1.95E+04	3.90E+06
4	5.30E+02	1.59E+05	4	2.54E+04	5.08E+06
5	1.48E+02	4.44E+04	5	1.16E+04	2.32E+06
6	2.20E+02	6.60E+04	6	1.21E+04	2.42E+06
7	2.87E+02	8.61E+04	7	1.52E+04	3.04E+06
8	5.20E+02	1.56E+05	8	3.50E+04	7.00E+06
9	1.43E+02	4.29E+04	9	1.24E+04	2.48E+06
10	4.50E+04	1.35E+07	10	7.30E+04	1.46E+07
11	7.40E+03	2.22E+06	11	5.70E+04	1.14E+07
12	1.27E+04	3.81E+06	12	1.27E+04	2.54E+06
Average	1.08E+04	3.25E+06	Average	2.86E+04	5.73E+06
Stan. Dev.	1.78E+04	5.34E+06	Stan. Dev.	1.97E+04	3.95E+06
Stan Error	5.13E+03	1.54E+06	Stan. Error	5.69E+03	1.14E+06

Appendix G Raw and Adjusted Data for the Case Runs (Bacteria)

Table G 1 Raw and Adjusted Data for the Case Runs of *P. aeruginosa*

P. aeruginosa

Sample	Impinger	Filter	Filter Flask	Capture by Mask
1	2.00E+00	1.70E+02	1.52E+02	3.22E+02
2	0.00E+00	3.80E+02	8.10E+01	4.61E+02
3	3.00E+00	1.70E+01	8.00E+01	9.70E+01
4	2.00E+00	5.60E+01	3.70E+01	9.30E+01
5	1.00E+00	6.40E+02	6.30E+01	7.03E+02
6	3.00E+00	3.50E+03	1.57E+02	3.66E+03
7	1.00E+00	2.80E+02	6.30E+01	3.43E+02
8	0.00E+00	4.70E+03	3.80E+02	5.08E+03
9	1.00E+00	1.30E+02	9.30E+01	2.23E+02
10	1.40E+01	2.70E+03	3.10E+01	2.73E+03
11	2.00E+00	4.70E+01	1.00E+01	5.70E+01
12	3.00E+00	5.20E+02	1.90E+02	7.10E+02
Average	2.67E+00	1.10E+03	1.11E+02	1.21E+03
Stan Dev	3.73E+00	1.60E+03	1.01E+02	1.67E+03
Stan Error	1.08E+00	4.62E+02	2.90E+01	4.82E+02

P. aeruginosa Adjusted*

Sample	Impinger	Filter	Filter Flask	Capture by Mask
1	6.00E+02	3.40E+04	7.60E+04	1.10E+05
2	0.00E+00	7.60E+04	4.05E+04	1.17E+05
3	9.00E+02	3.40E+03	4.00E+04	4.34E+04
4	6.00E+02	1.12E+04	1.85E+04	2.97E+04
5	3.00E+02	1.28E+05	3.15E+04	1.60E+05
6	9.00E+02	7.00E+05	7.85E+04	7.79E+05
7	3.00E+02	5.60E+04	3.15E+04	8.75E+04
8	0.00E+00	9.40E+05	1.90E+05	1.13E+06
9	3.00E+02	2.60E+04	4.65E+04	7.25E+04
10	4.20E+03	5.40E+05	1.55E+04	5.56E+05
11	6.00E+02	9.40E+03	5.00E+03	1.44E+04
12	9.00E+02	1.04E+05	9.50E+04	1.99E+05
Average	8.00E+02	2.19E+05	5.57E+04	2.75E+05
Stan Dev	1.12E+03	3.20E+05	5.03E+04	3.56E+05
Stan Error	3.23E+02	9.24E+04	1.45E+04	1.03E+05

Table G 2 Raw and Adjusted Data for the Case Runs of *S. aureus*

S. aureus

Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	1.66E+03	3.10E+03	1.24E+02	3.22E+03
2	1.66E+03	4.90E+03	1.16E+04	1.65E+04
3	3.50E+02	7.30E+03	3.20E+01	7.33E+03
4	2.93E+02	4.50E+02	1.40E+01	4.64E+02
5	4.40E+01	4.90E+03	4.50E+01	4.95E+03
6	3.60E+02	8.10E+03	4.00E+02	8.50E+03
7	0.00E+00	5.80E+03	1.12E+03	6.92E+03
8	9.70E+01	7.60E+03	7.10E+01	7.67E+03
9	1.30E+02	3.60E+04	1.90E+02	3.62E+04
10	1.92E+03	3.80E+04	7.70E+01	3.81E+04
11	5.60E+03	8.40E+04	4.40E+01	8.40E+04
12	1.87E+03	3.33E+05	3.40E+02	3.33E+05
Average	1.17E+03	4.44E+04	1.17E+03	4.56E+04
Stan. Dev	1.60E+03	9.40E+04	3.30E+03	9.37E+04
Stan Error	4.62E+02	2.71E+04	9.52E+02	2.70E+04

S. aureus Adjusted*

Sample	Impinger	Filter	Filter Flask	Capture by Mask
1	4.98E+05	6.20E+05	6.20E+04	6.82E+05
2	4.98E+05	9.80E+05	5.80E+06	6.78E+06
3	1.05E+05	1.46E+06	1.60E+04	1.48E+06
4	8.79E+04	9.00E+04	7.00E+03	9.70E+04
5	1.32E+04	9.80E+05	2.25E+04	1.00E+06
6	1.08E+05	1.62E+06	2.00E+05	1.82E+06
7	0.00E+00	1.16E+06	5.60E+05	1.72E+06
8	2.91E+04	1.52E+06	3.55E+04	1.56E+06
9	3.90E+04	7.20E+06	9.50E+04	7.30E+06
10	5.76E+05	7.60E+06	3.85E+04	7.64E+06
11	1.68E+06	1.68E+07	2.20E+04	1.68E+07
12	5.61E+05	6.66E+07	1.70E+05	6.68E+07
Average	3.50E+05	8.89E+06	5.86E+05	9.47E+06
Stan. Dev	4.80E+05	1.88E+07	1.65E+06	1.87E+07
Stan Error	1.38E+05	5.43E+06	4.76E+05	5.39E+06

Appendix H Raw Data for the Standard Curve (Surrogate Viruses)

Table H 1 Raw Data for the Standard Curve for *Φ-121 & Φ-S1*

Φ-121	Dilution	Sample 1	Sample 2	Sample 3	Average	Sample adjustment
	0	1.10E+05	2.90E+05	4.30E+05	2.77E+05	5.53E+07
	-1	2.80E+04	6.00E+03	2.00E+03	1.20E+04	2.40E+06
	-2	4.70E+01	7.10E+03	4.70E+03	3.95E+03	7.90E+05
	-3	3.40E+01	2.80E+01	4.10E+01	3.43E+01	6.87E+03
	-4	8.00E+00	7.00E+00	9.00E+00	8.00E+00	1.60E+03
	-5	4.00E+00	5.00E+00	2.00E+00	3.67E+00	7.33E+02
	-6	2.00E+00	3.00E+00	3.00E+00	2.67E+00	5.33E+02
	-7	0.00E+00	2.00E+00	0.00E+00	6.67E-01	1.33E+02
	-8	0.00E+00	0.00E+00	2.00E+00	6.67E-01	1.33E+02

Φ-S1	Dilution	Sample 1	Sample 2	Sample 3	Average	Sample adjustment
	0	1.20E+05	2.30E+05	3.00E+05	2.17E+05	4.33E+07
	-1	1.00E+04	5.00E+03	4.00E+03	6.33E+03	1.27E+06
	-2	5.50E+01	1.60E+03	1.20E+03	9.52E+02	1.90E+05
	-3	2.50E+01	1.50E+01	1.80E+01	1.93E+01	3.87E+03
	-4	9.00E+00	1.00E+01	1.10E+01	1.00E+01	2.00E+03
	-5	5.00E+00	4.00E+00	3.00E+00	4.00E+00	8.00E+02
	-6	3.00E+00	3.00E+00	4.00E+00	3.33E+00	6.67E+02
	-7	1.00E+00	0.00E+00	1.00E+00	6.67E-01	1.33E+02
	-8	2.00E+00	1.00E+00	1.00E+00	1.33E+00	2.67E+02

Table H 2 Raw Data for the Standard Curve for *PR-772* & *MS2*

PR-772	Dilution	Sample 1	Sample 2	Sample 3	Average	Sample adjustment
	0	1.00E+05	3.50E+05	2.10E+05	2.20E+05	4.40E+07
	-1	1.00E+04	5.00E+03	4.00E+03	6.33E+03	1.27E+06
	-2	5.10E+01	2.30E+03	1.60E+03	1.32E+03	2.63E+05
	-3	1.90E+01	1.60E+01	2.00E+01	1.83E+01	3.67E+03
	-4	7.00E+00	9.00E+00	8.00E+00	8.00E+00	1.60E+03
	-5	5.00E+00	6.00E+00	3.00E+00	4.67E+00	9.33E+02
	-6	3.00E+00	5.00E+00	6.00E+00	4.67E+00	9.33E+02
	-7	1.00E+00	0.00E+00	2.00E+00	1.00E+00	2.00E+02
	-8	0.00E+00	1.00E+00	0.00E+00	3.33E-01	6.67E+01

MS2	Dilution	Sample 1	Sample 2	Sample 3	Average	Sample adjustment
	0	4.00E+04	4.80E+05	4.10E+05	3.10E+05	6.20E+07
	-1	3.80E+04	8.00E+03	6.00E+03	1.73E+04	3.47E+06
	-2	3.80E+03	1.80E+03	6.00E+03	3.87E+03	7.73E+05
	-3	3.00E+01	3.10E+01	3.80E+01	3.30E+01	6.60E+03
	-4	1.00E+01	4.00E+00	7.00E+00	7.00E+00	1.40E+03
	-5	4.00E+00	5.00E+00	7.00E+00	5.33E+00	1.07E+03
	-6	4.00E+00	1.00E+00	0.00E+00	1.67E+00	3.33E+02
	-7	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	-8	2.00E+00	0.00E+00	1.00E+00	1.00E+00	2.00E+02

Appendix I Raw and Adjusted Data for the Control Runs (Surrogate Viruses)

Table I 1 Initial Raw Data of Surrogate viruses

	Φ-121			Φ-S1	
Sample	PFU/100µL	PFU/Sample	Sample	PFU/100µL	PFU/Sample
1	1.00E+03	2.00E+05	1	2.00E+03	4.00E+05
2	9.00E+02	1.80E+05	2	1.40E+03	2.80E+05
3	1.10E+03	2.20E+05	3	1.00E+03	2.00E+05
4	2.80E+03	5.60E+05	4	2.60E+03	5.20E+05
5	7.00E+02	1.40E+05	5	9.00E+02	1.80E+05
6	2.40E+03	4.80E+05	6	2.70E+03	5.40E+05
7	3.90E+04	7.80E+06	7	3.90E+04	7.80E+06
8	1.00E+04	2.00E+06	8	1.00E+04	2.00E+06
9	3.40E+04	6.80E+06	9	2.60E+04	5.20E+06
10	1.30E+04	2.60E+06	10	1.70E+04	3.40E+06
11	2.30E+04	4.60E+06	11	1.30E+04	2.60E+06
12	6.00E+03	1.20E+06	12	1.00E+04	2.00E+06
Average	1.12E+04	2.23E+06	Average	1.05E+04	2.09E+06
Stan. Dev	1.36E+04	2.72E+06	Stan. Dev	1.19E+04	2.38E+06
Stan. Error	3.93E+03	7.85E+05	Stan. Error	3.44E+03	6.88E+05

	PR-772			MS2	
Sample	PFU/100µL	PFU/Sample	Sample	PFU/100µL	PFU/Sample
1	2.30E+03	4.60E+05	1	2.70E+03	5.40E+05
2	1.10E+03	2.20E+05	2	2.50E+03	5.00E+05
3	1.60E+03	3.20E+05	3	2.00E+03	4.00E+05
4	2.40E+03	4.80E+05	4	1.70E+03	3.40E+05
5	1.20E+03	2.40E+05	5	1.10E+03	2.20E+05
6	2.90E+03	5.80E+05	6	3.90E+03	7.80E+05
7	2.50E+04	5.00E+06	7	1.60E+04	3.20E+06
8	5.00E+03	1.00E+06	8	6.00E+03	1.20E+06
9	1.00E+04	2.00E+06	9	1.90E+04	3.80E+06
10	1.40E+04	2.80E+06	10	7.00E+03	1.40E+06
11	1.10E+04	2.20E+06	11	1.00E+04	2.00E+06
12	9.00E+03	1.80E+06	12	6.00E+03	1.20E+06
Average	7.13E+03	1.43E+06	Average	6.49E+03	1.30E+06
Stan. Dev	7.16E+03	1.43E+06	Stan. Dev	5.79E+03	1.16E+06
Stan. Error	2.07E+03	4.13E+05	Stan. Error	1.67E+03	3.34E+05

Table I 2 Raw Data after Replating Selected Points

	Φ-121			Φ-S1	
Sample	PFU/100µL	PFU/Sample	Sample	PFU/100µL	PFU/Sample
1	2.00E+02	4.00E+04	1	4.00E+02	8.00E+04
2	4.00E+02	8.00E+04	2	7.00E+02	1.40E+05
3	3.00E+02	6.00E+04	3	2.00E+02	4.00E+04
4	1.00E+02	2.00E+04	4	2.00E+02	4.00E+04
5	5.00E+02	1.00E+05	5	5.00E+02	1.00E+05
6	1.00E+02	2.00E+04	6	3.00E+02	6.00E+04
7	4.90E+04	9.80E+06	7	2.63E+04	5.26E+06
8	8.10E+03	1.62E+06	8	6.10E+03	1.22E+06
9	4.10E+04	8.20E+06	9	7.00E+03	1.40E+06
10	1.49E+04	2.98E+06	10	6.30E+03	1.26E+06
11	3.00E+04	6.00E+06	11	8.70E+03	1.74E+06
12	5.10E+03	1.02E+06	12	2.90E+03	5.80E+05
Average	1.25E+04	2.50E+06	Average	4.97E+03	9.93E+05
Stan. Dev	1.77E+04	3.53E+06	Stan. Dev	7.43E+03	1.49E+06
Stan. Error	5.10E+03	1.02E+06	Stan. Error	2.14E+03	4.29E+05

	PR-772			MS2	
Sample	PFU/100µL	PFU/Sample	Sample	PFU/100µL	PFU/Sample
1	1.00E+02	2.00E+04	1	3.00E+02	6.00E+04
2	4.00E+02	8.00E+04	2	6.00E+02	1.20E+05
3	2.00E+02	4.00E+04	3	5.00E+02	1.00E+05
4	5.00E+02	1.00E+05	4	4.00E+02	8.00E+04
5	4.00E+02	8.00E+04	5	3.00E+02	6.00E+04
6	6.00E+02	1.20E+05	6	4.00E+02	8.00E+04
7	9.60E+03	1.92E+06	7	1.40E+04	2.80E+06
8	1.80E+03	3.60E+05	8	1.00E+04	2.00E+06
9	1.70E+04	3.40E+06	9	1.90E+04	3.80E+06
10	2.40E+03	4.80E+05	10	4.30E+03	8.60E+05
11	8.00E+03	1.60E+06	11	1.40E+04	2.80E+06
12	1.90E+03	3.80E+05	12	1.90E+03	3.80E+05
Average	3.58E+03	7.15E+05	Average	5.48E+03	1.10E+06
Stan. Dev	5.27E+03	1.05E+06	Stan. Dev	6.85E+03	1.37E+06
Stan. Error	1.52E+03	3.04E+05	Stan. Error	1.98E+03	3.96E+05

Appendix J Raw and Adjusted Data for the Case Runs (Surrogate Viruses)

Table J 1 Raw and Adjusted Data for *Φ-121*

Φ-121				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	2.00E+03	0.00E+00	2.00E+03	2.00E+03
2	0.00E+00	3.00E+03	0.00E+00	3.00E+03
3	1.00E+03	3.00E+03	4.00E+03	7.00E+03
4	1.00E+03	3.00E+03	5.00E+03	8.00E+03
5	0.00E+00	1.00E+03	5.00E+03	6.00E+03
6	1.00E+03	2.00E+03	0.00E+00	2.00E+03
7	0.00E+00	3.00E+03	1.00E+03	4.00E+03
8	7.00E+03	4.00E+03	0.00E+00	4.00E+03
9	5.00E+03	5.00E+03	3.00E+03	8.00E+03
10	0.00E+00	0.00E+00	0.00E+00	0.00E+00
11	0.00E+00	0.00E+00	5.00E+03	5.00E+03
12	1.00E+03	0.00E+00	1.00E+03	1.00E+03
Average	1.50E+03	2.00E+03	2.17E+03	4.17E+03
Stan. Dev	2.24E+03	1.76E+03	2.12E+03	2.69E+03
Stan. Error	6.45E+02	5.08E+02	6.13E+02	7.77E+02

Φ-121 Adjusted				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	4.00E+04	0.00E+00	1.00E+05	1.00E+05
2	0.00E+00	6.00E+04	0.00E+00	6.00E+04
3	2.00E+04	6.00E+04	2.00E+05	2.60E+05
4	2.00E+04	6.00E+04	2.50E+05	3.10E+05
5	0.00E+00	2.00E+04	2.50E+05	2.70E+05
6	2.00E+04	4.00E+04	0.00E+00	4.00E+04
7	0.00E+00	6.00E+04	5.00E+04	1.10E+05
8	1.40E+05	8.00E+04	0.00E+00	8.00E+04
9	1.00E+05	1.00E+05	1.50E+05	2.50E+05
10	0.00E+00	0.00E+00	0.00E+00	0.00E+00
11	0.00E+00	0.00E+00	2.50E+05	2.50E+05
12	2.00E+04	0.00E+00	5.00E+04	5.00E+04
Average	3.00E+04	4.00E+04	1.08E+05	1.48E+05
Stan. Dev	4.47E+04	3.52E+04	1.06E+05	1.10E+05
Stan. Error	1.29E+04	1.02E+04	3.07E+04	3.18E+04

Table J 2 Raw and Adjusted Data for *Φ-S1*

Φ-S1				
Sample	Impinger	Filter	Filter Flask	Captured By Mask
1	2.00E+03	0.00E+00	0.00E+00	0.00E+00
2	1.00E+03	0.00E+00	1.00E+03	1.00E+03
3	0.00E+00	3.00E+02	0.00E+00	3.00E+02
4	0.00E+00	2.00E+03	4.00E+03	6.00E+03
5	2.00E+03	3.00E+03	0.00E+00	3.00E+03
6	1.00E+03	1.00E+03	0.00E+00	1.00E+03
7	4.00E+03	1.00E+03	2.00E+03	3.00E+03
8	1.00E+03	0.00E+00	0.00E+00	0.00E+00
9	3.00E+03	1.00E+03	2.00E+03	3.00E+03
10	0.00E+00	1.00E+03	3.00E+03	4.00E+03
11	4.00E+03	0.00E+00	2.00E+03	2.00E+03
12	0.00E+00	3.00E+03	0.00E+00	3.00E+03
Average	1.50E+03	1.03E+03	1.17E+03	2.19E+03
Stan. Dev	1.51E+03	1.11E+03	1.40E+03	1.82E+03
Stan. Error	4.35E+02	3.20E+02	4.05E+02	5.25E+02

Φ-S1 Adjusted				
Sample	Imp Adjust	Fil Adjust	F.F Adjust	Captured By Mask
1	4.00E+04	0.00E+00	0.00E+00	0.00E+00
2	2.00E+04	0.00E+00	2.00E+04	2.00E+04
3	0.00E+00	6.00E+03	0.00E+00	6.00E+03
4	0.00E+00	4.00E+04	8.00E+04	1.20E+05
5	4.00E+04	6.00E+04	0.00E+00	6.00E+04
6	2.00E+04	2.00E+04	0.00E+00	2.00E+04
7	8.00E+04	2.00E+04	4.00E+04	6.00E+04
8	2.00E+04	0.00E+00	0.00E+00	0.00E+00
9	6.00E+04	2.00E+04	4.00E+04	6.00E+04
10	0.00E+00	2.00E+04	6.00E+04	8.00E+04
11	8.00E+04	0.00E+00	4.00E+04	4.00E+04
12	0.00E+00	6.00E+04	0.00E+00	6.00E+04
Average	3.00E+04	2.05E+04	2.33E+04	4.38E+04
Stan. Dev	3.02E+04	2.21E+04	2.81E+04	3.64E+04
Stan. Error	8.70E+03	6.39E+03	8.10E+03	1.05E+04

Table J 3 Raw and Adjusted Data for *PR-772*

PR-772				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	2.00E+03	0.00E+00	2.00E+03	2.00E+03
2	4.00E+02	0.00E+00	1.00E+03	1.00E+03
3	0.00E+00	1.00E+03	3.00E+03	4.00E+03
4	0.00E+00	2.00E+03	4.00E+03	6.00E+03
5	1.00E+03	1.00E+03	1.00E+03	2.00E+03
6	2.00E+03	1.00E+03	4.00E+03	5.00E+03
7	0.00E+00	1.00E+03	0.00E+00	1.00E+03
8	1.00E+03	0.00E+00	4.00E+03	4.00E+03
9	0.00E+00	1.00E+03	1.00E+03	2.00E+03
10	1.00E+03	0.00E+00	2.00E+03	2.00E+03
11	2.00E+03	1.00E+03	3.00E+03	4.00E+03
12	1.00E+03	0.00E+00	3.00E+03	3.00E+03
Average	8.67E+02	6.67E+02	1.37E+03	3.00E+03
Stan. Dev	8.06E+02	6.51E+02	1.37E+03	1.60E+03
Stan. Error	2.33E+02	1.88E+02	3.96E+02	4.61E+02

PR-772 Adjusted				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	4.00E+04	0.00E+00	4.00E+04	4.00E+04
2	8.00E+03	0.00E+00	2.00E+04	2.00E+04
3	0.00E+00	2.00E+04	6.00E+04	8.00E+04
4	0.00E+00	4.00E+04	8.00E+04	1.20E+05
5	2.00E+04	2.00E+04	2.00E+04	4.00E+04
6	4.00E+04	2.00E+04	8.00E+04	1.00E+05
7	0.00E+00	2.00E+04	0.00E+00	2.00E+04
8	2.00E+04	0.00E+00	8.00E+04	8.00E+04
9	0.00E+00	2.00E+04	2.00E+04	4.00E+04
10	2.00E+04	0.00E+00	4.00E+04	4.00E+04
11	4.00E+04	2.00E+04	6.00E+04	8.00E+04
12	2.00E+04	0.00E+00	6.00E+04	6.00E+04
Average	1.73E+04	1.33E+04	2.74E+04	6.00E+04
Stan. Dev	1.61E+04	1.30E+04	2.74E+04	3.19E+04
Stan. Error	4.65E+03	3.76E+03	7.91E+03	9.21E+03

Table J 4 Raw and Adjusted Data for *MS2*

MS2				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	0.00E+00	0.00E+00	6.00E+03	6.00E+03
2	2.00E+03	0.00E+00	1.00E+03	1.00E+03
3	1.00E+03	5.00E+03	3.00E+03	8.00E+03
4	0.00E+00	2.00E+03	1.00E+03	3.00E+03
5	0.00E+00	4.00E+03	5.00E+03	9.00E+03
6	2.00E+03	3.00E+03	1.00E+03	4.00E+03
7	0.00E+00	1.00E+03	2.00E+03	3.00E+03
8	0.00E+00	0.00E+00	1.00E+03	1.00E+03
9	3.00E+03	1.00E+03	1.00E+03	2.00E+03
10	0.00E+00	3.00E+03	3.00E+03	6.00E+03
11	1.00E+03	3.00E+03	3.00E+03	6.00E+03
12	0.00E+00	1.00E+03	3.00E+03	4.00E+03
Average	7.50E+02	1.92E+03	2.50E+03	4.42E+03
Stan. Dev	1.06E+03	1.68E+03	1.68E+03	2.61E+03
Stan. Error	3.05E+02	4.84E+02	4.85E+02	7.53E+02

MS2 Adjusted				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	0.00E+00	0.00E+00	3.00E+05	3.00E+05
2	4.00E+04	0.00E+00	5.00E+04	5.00E+04
3	2.00E+04	1.00E+05	1.50E+05	2.50E+05
4	0.00E+00	4.00E+04	5.00E+04	9.00E+04
5	0.00E+00	8.00E+04	2.50E+05	3.30E+05
6	4.00E+04	6.00E+04	5.00E+04	1.10E+05
7	0.00E+00	2.00E+04	1.00E+05	1.20E+05
8	0.00E+00	0.00E+00	5.00E+04	5.00E+04
9	6.00E+04	2.00E+04	5.00E+04	7.00E+04
10	0.00E+00	6.00E+04	1.50E+05	2.10E+05
11	2.00E+04	6.00E+04	1.50E+05	2.10E+05
12	0.00E+00	2.00E+04	1.50E+05	1.70E+05
Average	1.50E+04	3.83E+04	1.25E+05	1.63E+05
Stan. Dev	2.11E+04	3.35E+04	8.39E+04	9.67E+04
Stan. Error	6.09E+03	9.68E+03	2.42E+04	2.79E+04

Appendix K Raw Data After Replating Selected Points in the Case Runs (Surrogate Viruses)

Table K 1 Raw and Adjusted Data for *Φ-121* after Replating Selected Points

Φ-121				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	2.00E+03	1.00E+02	2.00E+03	2.10E+03
2	0.00E+00	3.00E+03	1.00E+02	3.10E+03
3	1.00E+03	3.00E+03	4.00E+03	7.00E+03
4	1.00E+03	3.00E+03	5.00E+03	8.00E+03
5	1.00E+02	1.00E+03	5.00E+03	6.00E+03
6	1.00E+03	2.00E+03	2.00E+02	2.20E+03
7	0.00E+00	3.00E+03	1.00E+03	4.00E+03
8	7.00E+03	4.00E+03	0.00E+00	4.00E+03
9	5.00E+03	5.00E+03	3.00E+03	8.00E+03
10	0.00E+00	3.00E+02	0.00E+00	3.00E+02
11	4.00E+02	2.00E+02	5.00E+03	5.20E+03
12	1.00E+03	0.00E+00	1.00E+03	1.00E+03
Average	1.54E+03	2.05E+03	2.19E+03	4.24E+03
Stan. Dev	2.21E+03	1.70E+03	2.10E+03	2.63E+03
Stan. Error	6.38E+02	4.90E+02	6.06E+02	7.59E+02

Φ-121 Adjusted				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	4.00E+04	2.00E+03	4.00E+04	4.20E+04
2	0.00E+00	6.00E+04	2.00E+03	6.20E+04
3	2.00E+04	6.00E+04	8.00E+04	1.40E+05
4	2.00E+04	6.00E+04	1.00E+05	1.60E+05
5	2.00E+03	2.00E+04	1.00E+05	1.20E+05
6	2.00E+04	4.00E+04	4.00E+03	4.40E+04
7	0.00E+00	6.00E+04	2.00E+04	8.00E+04
8	1.40E+05	8.00E+04	0.00E+00	8.00E+04
9	1.00E+05	1.00E+05	6.00E+04	1.60E+05
10	0.00E+00	6.00E+03	0.00E+00	6.00E+03
11	8.00E+03	4.00E+03	1.00E+05	1.04E+05
12	2.00E+04	0.00E+00	2.00E+04	2.00E+04
Average	3.08E+04	4.10E+04	4.38E+04	8.48E+04
Stan. Dev	4.42E+04	3.40E+04	4.20E+04	5.26E+04
Stan. Error	1.28E+04	9.80E+03	1.21E+04	1.52E+04

Table K 2 Raw and Adjusted Data for *Φ-S1* after Replating Selected Points

Φ-S1				
Sample	Impinger	Filter	Filter Flask	Captured By Mask
1	2.00E+03	4.00E+02	9.00E+02	1.30E+03
2	1.00E+03	1.10E+03	1.00E+03	2.10E+03
3	4.00E+02	3.00E+02	0.00E+00	3.00E+02
4	7.00E+02	2.00E+03	4.00E+03	6.00E+03
5	2.00E+03	3.00E+03	2.00E+02	3.20E+03
6	1.00E+03	1.00E+03	0.00E+00	1.00E+03
7	4.00E+03	1.00E+03	2.00E+03	3.00E+03
8	1.00E+03	1.00E+02	0.00E+00	1.00E+02
9	3.00E+03	1.00E+03	2.00E+03	3.00E+03
10	0.00E+00	1.00E+03	3.00E+03	4.00E+03
11	4.00E+03	7.00E+02	2.00E+03	2.70E+03
12	0.00E+00	3.00E+03	0.00E+00	3.00E+03
Average	1.59E+03	1.22E+03	1.26E+03	2.48E+03
Stan. Dev	1.42E+03	9.63E+02	1.34E+03	1.66E+03
Stan. Error	4.11E+02	2.78E+02	3.88E+02	4.78E+02

Φ-S1 Adjusted				
Sample	Imp Adjust	Fil Adjust	F.F Adjust	Captured By Mask
1	4.00E+04	8.00E+03	1.80E+04	2.60E+04
2	2.00E+04	2.20E+04	2.00E+04	4.20E+04
3	8.00E+03	6.00E+03	0.00E+00	6.00E+03
4	1.40E+04	4.00E+04	8.00E+04	1.20E+05
5	4.00E+04	6.00E+04	4.00E+03	6.40E+04
6	2.00E+04	2.00E+04	0.00E+00	2.00E+04
7	8.00E+04	2.00E+04	4.00E+04	6.00E+04
8	2.00E+04	2.00E+03	0.00E+00	2.00E+03
9	6.00E+04	2.00E+04	4.00E+04	6.00E+04
10	0.00E+00	2.00E+04	6.00E+04	8.00E+04
11	8.00E+04	1.40E+04	4.00E+04	5.40E+04
12	0.00E+00	6.00E+04	0.00E+00	6.00E+04
Average	3.18E+04	2.43E+04	2.52E+04	4.95E+04
Stan. Dev	2.84E+04	1.93E+04	2.69E+04	3.32E+04
Stan. Error	8.21E+03	5.56E+03	7.76E+03	9.57E+03

Table K 3 Raw and Adjusted Data for *PR-772* after Replating of Selected Points

PR-772				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	2.00E+03	6.00E+02	2.00E+03	2.60E+03
2	4.00E+02	1.00E+02	1.00E+03	1.10E+03
3	4.00E+02	1.00E+03	3.00E+03	4.00E+03
4	3.00E+02	2.00E+03	4.00E+03	6.00E+03
5	1.00E+03	1.00E+03	1.00E+03	2.00E+03
6	2.00E+03	1.00E+03	4.00E+03	5.00E+03
7	2.00E+02	1.00E+03	0.00E+00	1.00E+03
8	1.00E+03	2.00E+02	4.00E+03	4.20E+03
9	0.00E+00	1.00E+03	1.00E+03	2.00E+03
10	1.00E+03	0.00E+00	2.00E+03	2.00E+03
11	2.00E+03	1.00E+03	3.00E+03	4.00E+03
12	1.00E+03	1.00E+02	3.00E+03	3.10E+03
Average	9.42E+02	7.50E+02	1.37E+03	3.08E+03
Stan. Dev	7.27E+02	5.78E+02	1.37E+03	1.57E+03
Stan. Error	2.10E+02	1.67E+02	3.96E+02	4.53E+02

PR-772 Adjusted				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	4.00E+04	1.20E+04	4.00E+04	5.20E+04
2	8.00E+03	2.00E+03	2.00E+04	2.20E+04
3	8.00E+03	2.00E+04	6.00E+04	8.00E+04
4	6.00E+03	4.00E+04	8.00E+04	1.20E+05
5	2.00E+04	2.00E+04	2.00E+04	4.00E+04
6	4.00E+04	2.00E+04	8.00E+04	1.00E+05
7	4.00E+03	2.00E+04	0.00E+00	2.00E+04
8	2.00E+04	4.00E+03	8.00E+04	8.40E+04
9	0.00E+00	2.00E+04	2.00E+04	4.00E+04
10	2.00E+04	0.00E+00	4.00E+04	4.00E+04
11	4.00E+04	2.00E+04	6.00E+04	8.00E+04
12	2.00E+04	2.00E+03	6.00E+04	6.20E+04
Average	1.88E+04	1.50E+04	4.67E+04	6.17E+04
Stan. Dev	1.45E+04	1.16E+04	2.74E+04	3.14E+04
Stan. Error	4.20E+03	3.33E+03	7.91E+03	9.07E+03

Table K 4 Raw and Adjusted Data for *MS2* after Replating of Selected Points

MS2				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	1.00E+02	0.00E+00	6.00E+03	6.00E+03
2	2.00E+03	1.00E+03	1.00E+03	2.00E+03
3	1.00E+03	5.00E+03	3.00E+03	8.00E+03
4	0.00E+00	2.00E+03	1.00E+03	3.00E+03
5	0.00E+00	4.00E+03	5.00E+03	9.00E+03
6	2.00E+03	3.00E+03	1.00E+03	4.00E+03
7	0.00E+00	1.00E+03	2.00E+03	3.00E+03
8	0.00E+00	5.00E+02	1.00E+03	1.50E+03
9	3.00E+03	1.00E+03	1.00E+03	2.00E+03
10	2.00E+02	3.00E+03	3.00E+03	6.00E+03
11	1.00E+03	3.00E+03	3.00E+03	6.00E+03
12	4.00E+02	1.00E+03	3.00E+03	4.00E+03
Average	8.08E+02	2.04E+03	2.50E+03	4.54E+03
Stan. Dev	1.02E+03	1.54E+03	1.68E+03	2.44E+03
Stan. Error	2.93E+02	4.46E+02	4.85E+02	7.06E+02

MS2 Adjusted				
Sample	Impinger	Filter	Filter Flask	Capture By Mask
1	2.00E+03	0.00E+00	1.20E+05	1.20E+05
2	4.00E+04	2.00E+04	2.00E+04	4.00E+04
3	2.00E+04	1.00E+05	6.00E+04	1.60E+05
4	0.00E+00	4.00E+04	2.00E+04	6.00E+04
5	0.00E+00	8.00E+04	1.00E+05	1.80E+05
6	4.00E+04	6.00E+04	2.00E+04	8.00E+04
7	0.00E+00	2.00E+04	4.00E+04	6.00E+04
8	0.00E+00	1.00E+04	2.00E+04	3.00E+04
9	6.00E+04	2.00E+04	2.00E+04	4.00E+04
10	4.00E+03	6.00E+04	6.00E+04	1.20E+05
11	2.00E+04	6.00E+04	6.00E+04	1.20E+05
12	8.00E+03	2.00E+04	6.00E+04	8.00E+04
Average	1.62E+04	4.08E+04	5.00E+04	9.08E+04
Stan. Dev	2.03E+04	3.09E+04	3.36E+04	4.89E+04
Stan. Error	5.87E+03	8.92E+03	9.69E+03	1.41E+04

Appendix L Breakthrough of Surrogate Viruses from the Mask by Dilution

Table L 1 Breakthrough Concentration by Dilution

Dilution	Φ-121	Φ-S1	PR-772	MS2
0	1.00E+05	6.00E+04	1.20E+05	1.40E+05
-1	6.00E+04	6.00E+04	4.00E+04	1.20E+05
-2	4.00E+04	4.00E+04	6.00E+04	8.00E+04
-3	4.00E+04	2.00E+04	6.00E+04	4.00E+04
-4	2.00E+04	2.00E+04	2.00E+04	4.00E+04
-5	2.00E+04	2.00E+04	0.00E+00	2.00E+04
-6	0.00E+00	2.00E+04	0.00E+00	2.00E+04
-7	0.00E+00	0.00E+00	0.00E+00	2.00E+04
-8	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Appendix M Raw and Adjusted Data for the Surrogate Viruses Recovered from the HEPA Filters

Table M 1 Raw Data for the HEPA Filter Runs

Φ-121		
Sample	Filter	Filter Flask
1	2.00E+03	1.00E+03
2	2.00E+03	0.00E+00
3	1.00E+03	0.00E+00
4	5.00E+02	0.00E+00
Average	1.38E+03	2.50E+02
Stan. Dev.	7.50E+02	5.00E+02
Stan. Error	2.17E+02	1.44E+02

Φ-S1		
Sample	Filter	Filter Flask
1	4.00E+03	1.00E+03
2	3.00E+03	0.00E+00
3	7.00E+03	0.00E+00
4	3.00E+03	1.00E+03
Average	4.25E+03	5.00E+02
Stan. Dev.	1.89E+03	5.77E+02
Stan. Error	5.46E+02	1.67E+02

PR-772		
Sample	Filter	Filter Flask
1	3.00E+03	1.00E+03
2	5.00E+03	0.00E+00
3	3.00E+03	0.00E+00
4	2.00E+03	0.00E+00
Average	3.25E+03	2.50E+02
Stan. Dev.	1.26E+03	5.00E+02
Stan. Error	3.63E+02	1.44E+02

MS2		
Sample	Filter	Filter Flask
1	4.00E+03	2.00E+03
2	4.00E+03	1.00E+03
3	6.00E+03	1.00E+03
4	3.00E+03	0.00E+00
Average	4.25E+03	1.00E+03
Stan. Dev.	1.26E+03	8.16E+02
Stan. Error	3.63E+02	2.36E+02

Table M 2 Adjusted Data for the HEPA Filter Runs

Φ-121		
Sample	Filter	Filter Flask
1	4.00E+04	2.00E+04
2	4.00E+04	0.00E+00
3	2.00E+04	0.00E+00
4	1.00E+04	0.00E+00
Average	2.75E+04	5.00E+03
Stan. Dev.	1.50E+04	1.00E+04
Stan. Error	4.33E+03	2.89E+03

Φ-S1		
Sample	Filter	Filter Flask
1	8.00E+04	3.00E+04
2	6.00E+04	0.00E+00
3	1.40E+05	0.00E+00
4	6.00E+04	3.00E+04
Average	8.50E+04	1.50E+04
Stan. Dev.	3.79E+04	1.73E+04
Stan. Error	1.09E+04	5.00E+03

PR-772		
Sample	Filter	Filter Flask
1	6.00E+04	3.00E+04
2	1.00E+05	0.00E+00
3	6.00E+04	0.00E+00
4	4.00E+04	0.00E+00
Average	6.50E+04	7.50E+03
Stan. Dev.	2.52E+04	1.50E+04
Stan. Error	7.26E+03	4.33E+03

MS2		
Sample	Filter	Filter Flask
1	8.00E+04	6.00E+04
2	8.00E+04	3.00E+04
3	1.20E+05	3.00E+04
4	6.00E+04	0.00E+00
Average	8.50E+04	3.00E+04
Stan. Dev.	2.52E+04	2.45E+04
Stan. Error	7.26E+03	7.07E+03

VITA

VITA

Joshua Lee Horton
School of Health Sciences, Purdue University

EDUCATION & TRAINING

Undergraduate:

		Major Subject
2007-11	University of Tennessee, Martin, TN Bachelors of Science	Cell Biology

Graduate:

		Discipline & Advisor
2012-14	Purdue University, West Lafayette, IN Masters of Science Candidate	Industrial Hygiene James McGlothlin, PhD

RESEARCH EXPERIENCE

School of Health Sciences, Industrial Hygiene M.S. Program — May 2012-2014
Graduate Research Assistant to Dr. James McGlothlin

SEMINARS & INVITED TALKS

Laboratory Study of a Scavenging Mask System to Evaluate and Control Airborne Pathogens for Healthcare Workers in the Post Anesthesia Care Unit (PACU) and Intensive Care Unit (ICU), School of Health Science Student Seminar, West Lafayette, IN, April, 2014.

POSTERS PRESENTED

Horton, J.L., McGlothlin, J.D. (2013) Development of ASAP-VEM System for Rapid Detection and Monitoring of Airborne Pathogens in the Workplace. Presented at American Industrial Hygiene Association Student Night in Chicago, IL on February 20, 2013.

Horton, J.L., McGlothlin, J.D. (2013) Development of ASAP-VEM System for Rapid Detection and Monitoring of Airborne Pathogens in the Workplace. Presented at American Industrial Hygiene Association Conference in Montreal, Canada on May 21, 2013.

Horton, J.L., McGlothlin, J.D., Applegate, B.M., Schweitzer, J.F., (2014) Evaluation and Control of Airborne Pathogens for Health Care Workers in the Post Anesthesia Care Unit (PACU) and Intensive Care Unit (ICU). Presented at American Industrial Hygiene Association Student Night in Chicago, IL on February 19, 2014.

Horton, J.L., McGlothlin, J.D., Applegate, B.M., Schweitzer, J.F., (2014) Laboratory Study of a Scavenging Mask System to Evaluate and Control Airborne Pathogens for Healthcare Workers in the Post Anesthesia Care Unit (PACU) and Intensive Care Unit (ICU). Presented at American Industrial Hygiene Association Conference in San Antonio, TX on June 4, 2014.

HONORS & AWARDS

Eli Lil Industrial Hygiene Award	Spring 2014
First Place in AIHA Chicago Graduate Poster Competition	Spring 2014
Eli Lily Industrial Hygiene Award	Spring 2013
Neil J. Zimmerman Student Service Award	Spring 2013
Second Place in AIHA Chicago Graduate Poster Competition	Spring 2013
NIOSH Graduate Fellowship Recipient	2012 – 2014
Order of Omega – An honor society for greek organization members	Fall 2011
Dean's List	Fall 2009
	Spring 2010
	Fall 2011

TEACHING EXPERIENCE

Contemporary Issues in International Public Health: Study Abroad in Ireland Spring 2014
Teaching Assistant to Dr. James McGlothlin

Occupational Safety and Ergonomics HSCI 58000 Fall 2013
Teaching Assistant to Dr. James McGlothlin

LEADERSHIP EXPERIENCE

Purdue Industrial Hygiene Student Association
Executive Committee – Graduate Advisor 2013-2014

Sigma Chi Fraternity
Member of the Executive Committee 2010-2011
House Manager 2010-2011
Assistant Risk Management 2010-2011
Assistant in Ritual and Education 2009-2011
Pledge Class President 2009

University of Tennessee at Martin Rugby Club
Captain 2007-2008

PROFESSIONAL AFFILIATIONS

American Conference of Governmental Industrial Hygienists (ACGIH)
American Industrial Hygiene Association (AIHA)
AIHA Indiana Chapter

CONFERENCES ATTENDED

American Industrial Hygiene Association Conference; Montreal, Canada 2013
American Industrial Hygiene Association Conference; San Antonio, Texas 2014

RESEARCH INTERESTS

Industrial Hygiene
Prevention through Design
Ergonomics
Root Cause Analysis
Risk Assessment
Pharmaceutical and Biotechnology
Infection Control