1-7-2022

Evaluation of Personal Protective Equipment against Bacteriophage Bioaerosols

Jamari Moore
Georgia State University

Evaluation of Personal Protective Equipment against Bacteriophage Bioaerosols

by

Jamari Moore

Under the Direction of Christa Wright, PhD

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Masters of Science

in the College of Arts and Sciences

Georgia State University

2021

ABSTRACT

Infection control of Nosocomial spread of viral illnesses has long been a challenge for the safety of healthcare workers (HCWs) and their patients. The ongoing pandemic caused by the Sars-Cov-2 (COVID-19) virus has highlighted the importance of providing HCWs with the proper personal protective equipment (PPE). This study aims to explore differences between the PPE combination effect on reducing virus penetration at different levels virus concentrations with the use of a novel aerosol generation system. The PPE combinations used in the study: N95 respirator + face shield, surgical mask + face shield, surgical mask + N95, N95 respirator, face shield. The results of the study show that PPE can reduce virus exposures at a higher rate than when no PPE was used. The N95 respiratory was the most effective at reducing viral exposures. The results from the study may offer further insight into the best application of PPE within a medical setting.

INDEX WORDS: Liters per minute (LPM), Personal Protective Equipment (PPE), Healthcare Workers (HCWs), Aerosol Generation System (AGS), Plaque Forming Units (PFU), Mass General Brigham (MGB), Bacteriophage (Phage), Colony Forming Units (CFU)

Evaluation of Personal Protective Equipment against Bacteriophage Bioaerosols

by

Jamari Moore

Committee Chair: Christa Wright

Committee: Lisa Casanova

Electronic Version Approved:

Office of Graduate Services

College of Arts and Sciences

Georgia State University

December 2021

ACKNOWLEDGEMENTS

We would like to thank the Centers for Disease Control and Prevention Epicenters Program for providing the funding (award number U54CK000164) for this research. I would like to express my deepest appreciation to my mentors and committee members Dr. Christa Wright and Dr. Lisa Casanova for providing me with this opportunity. I am thankful for their continuous advice, guidance, and patience throughout this process. I would also like to thank all graduate students within the lab who offered support guidance and assistance with conducting experiments. Lastly, I want to thank my family and friends for their endless support and understanding throughout this journey. I am appreciative of everyone who played a role in this achievement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

Nosocomial spread of viruses can occur in many ways in hospital settings. The fecal-oral, respiratory, and bloodborne routes of transmission are all responsible for the spread of viruses within hospitals (Aitken et al 2001). Of the three major contributors, the respiratory spread of viruses remains to be the most difficult to prevent and track (Aitken et al 2001). This may be due to the short incubation times and infections that have asymptomatic carrier states that allow for spread. The common cold, the flu, and COVID-19 all spread through respiratory droplets. Aerosols can also be produced by medical procedures including intubation, extubation, bronchoscopy, and even CPR can produce aerosols (Jackson et al, 2020). These aerosols can persist in the air and cause infection. These infections can pass from patient to patient and patient to healthcare workers (HCWs) (N. Van Doremalen et al. 2020; A. Fears et al, 2020). This highlights the importance of personal protective equipment or PPE to protect HCWs from respiratory viruses.

PPE in clinical settings helps to prevent occupational infection. Studies have shown that widespread mask use can reduce the spread of viral respiratory illnesses (Wang et al, 2020). PPE comes in many forms. Some PPE can reduce the number of aerosolized viruses inhaled by the user, and the number released by the user into the air (R. B. Patel et al, 2016; Ippolito et al, 2020). Surgical masks are an example of PPE that provide some amount of respiratory protection. Filtering facepiece respirators (FFR) and powered/supplied-air respirators are examples of PPE that offer more protection from inhalation exposures to respiratory aerosols. Numerous types of FFR offer different levels of proception against inhalation exposures. For example, the N95/P95/R95 offers a 95% reduction of aerosols while the N100/N99/P100 offers a 99% reduction of aerosols. (Ippolito et al, 2020; ECDC, 2014). This type of PPE can be given to HCWs who are treating patients with respiratory viral illnesses that are highly contagious, such as COVID-19.

Face shields are another form of PPE that is available to reduce the spread of viral illnesses. Face shields provide a larger surface area to protect against large droplets at close ranges (Lindsley et al 2014). Of the available PPE at the disposal of HCWs, it's important to know which PPE offers the best inhalation protection for the user. Additionally, PPE may be combined to provide extra protection or to keep underlining PPE from getting contaminated (Lindsley et al 2021). Examples of these combinations include combining a face shield with a face covering such as a surgical mask or covering an N95 respirator with a surgical mask. The goal of this work is to identify combinations of PPE that help protect hospital workers during procedures and situations that may expose them to respiratory aerosols containing viruses.

This study aims to answer the following research questions: Using an aerosol generation system with mannequins to simulate viral respiratory exposures, 1) what type or combination of PPE is best at reducing the number of infectious viral particles in an aerosol that can enter the respiratory tract? 2) How does the concentration of particles relate to the number of bioaerosols that enter the respiratory tract while wearing PPE?

We hypothesize that:

1) higher particle concentration will allow more bioaerosols to pass through PPE (lower reduction) across all PPE scenarios.
2) The combination of the N95 respirator and face shield will show the greatest reduction in bioaerosols entering the respiratory tract.

To test this hypothesis, we will employ a novel aerosolization generation system (AGS) capable of creating stable aerosol levels within a glove box chamber fitted with a mannequin head connected to sampling ports. The AGS system possesses a nebulizer or sprayer that will spray a tracer solution that consists of 4ml of a 500 µg/ml iron oxide solution and 1 ml of 10^9 MS2

bacteriophage titer. The mannequin head will don five different PPE combinations throughout the experiment, and there will also be a non-PPE trial that will act as a control. The aerosolized particles/bacteriophages that enter the mannequin's trachea for each of the PPE combinations will be collected. The number of bioaerosols that enter the mannequin's trachea will be measured and log reduction will be used to determine how much each PPE combination reduced the number of bioaerosols.

2 REVIEW OF LITERATURE

2.1 Viral Spread in Hospital Settings

Hospital inquired infections can be a problem for both healthcare workers and their patients. Hospitals are often inundated with various viral, bacterial, or fungal pathogens due to the nature of treating people with illnesses. It's no surprise that pathogens can be spread from patients to healthcare workers and back. Infection control within hospitals is implemented to monitor the types of pathogens that enter the hospital to minimize the spread. In addition to monitoring pathogens, mitigation factors are put into place to reduce the chances of spread. Factors such as frequency of handwashing, isolation of patients with highly infectious illnesses, and appropriate ventilation are important for reducing hospital-acquired infections (Bing-Yuan et al 2018; (Aitken et al 2001).

2.1.1 Potential Transmission Routes

Aitken and colleagues (2001) found that viruses may account for about 5% of all nosocomial infections**.** In a hospital setting, viruses can be spread via contact with infected blood, via the fecal-oral route, or respiratory spread. Accidental needle sticks or blood transfusions put healthcare workers (HCWs) at risk of being exposed to viruses such as hepatitis B and HIV. Viruses that infect the gastrointestinal tract spread via the fecal-oral route within hospital settings. Typically, these viruses are shed in patients' feces, making proper handwashing and PPE wearing important for reducing the spread. Viruses such as rotavirus and enteroviruses spread via the fecal-oral route. In some cases where vomit is produced, viruses such as the Norwalk virus can spread via fomites and aerosols (Aitken et al, 2001). Influenza, rhinovirus, and coronaviruses are examples of viruses that spread via the respiratory route. Respiratory viral

particles spread via small droplets, which can travel far and persist in the air, or larger droplets that are too heavy to travel far thus more commonly infecting a person in proximity. Controlling the spread of respiratory viruses can be difficult due to their ability to spread asymptomatically as well as short incubation times. Early diagnosis and patient isolation are key factors for controlling the spread of respiratory viruses (Aitken et al, 2001).

2.1.1 Processes within Hospitals that Enhance Transmission

HCWs are exposed to numerous pathogens daily due to their proximity to sick patients. The exposure to illnesses can potentially be increased by the types of procedures they perform daily. These procedures are known as aerosol-generating procedures. While some of these procedures are lifesaving, they possess an element of risk to the healthcare workers who perform them daily. Autopsy, intubation and extubating, bronchoscopy, airway suctioning, and CPR are a few procedures that top the list of agreed-upon aerosol-generating procedures based on a literature review done by (Jackson et al, 2020). These procedures are thought to increase the release of aerosols thus increasing the risks of infection for healthcare workers. Klompas, Baker, and Rhee's research suggest four factors contribute to a medical procedure's ability to generate aerosols. The first is forced air. It's believed that any procedure that forcefully blows air into the airways will generate aerosols. The next factors being symptoms and severity of disease suggest that patients with more severe disease/ symptoms will produce more aerosols during a procedure (Luo et al 2020). Distance from the source contributes to the spread as well. Most of these procedures are done near the patient so if a patient is highly infectious, there's a greater chance of becoming infected. Lastly, the duration of exposure can significantly impact the risk of exposure while doing these procedures (2021).

2.2 Preventive Measures to Reduce Spread of Virus

2.2.1 Current Public Health Policies and Recommendations

Governing bodies like the CDC and the WHO and individual countries provide the guidelines for protecting healthcare workers from numerous respiratory viral illnesses. Typically, guidelines for using different types of PPE are based on the risk of exposure and the virulence of the infectious pathogen. These guidelines can vary between different countries and governing bodies. Both the CDC and WHO recommend that healthcare workers who are at high risk of being exposed to SARS should wear an N95 respirator or higher when coming in contact (WHO, 2014; CDC, 2004). For healthcare workers at low risk of being exposed to SARS, the WHO recommends only a surgical mask while the CDC recommends an N95 respirator (Chughtai et al, 2013). This is one example of how guidelines can vary, showing the multitude of guidelines each healthcare system may be subjected to. It's also important to note that resources or lack thereof can have an impact on the types of guidelines of policy that are provided to protect healthcare workers.

There have been numerous public health guidelines and policies that have been implemented to protect healthcare workers from the spread of COVID-19 and other respiratory viruses. During the height of COVID -19, Mass General Brigham (MGB) implemented a universal masking policy and saw the COVID-19 positivity rate within their medical staff fall from 21.32% (pre universal masking) to 11.46% (post universal masking) (Wang et al, 2020). Richterman and colleagues' policy research suggest that these guidelines can aid in public health messaging while allowing medical facilities the ability to maintain an appearance of health and safety for the public (2020). Implementing this type of policy will also provide further evidence

of the efficacy of using masks in indoor settings to reduce some of the political tension surroundings mask use (Richterman et al, 2020).

2.2.2 Personal Protective Equipment (PPE)

There are many forms of PPE from infectious pathogens. Gloves, caps, eye protection face shields, gowns, and face covering (masks/respirators) are essential for healthcare workers to protect themselves in the workplace. The type of PPE worn usually depends on the severity of illness HCWs encounter. Levels of PPE increase as infectivity increases. Masks/respirators are important for protecting against viruses that spread via the respiratory route. Each face-covering provides different levels of protection while also requiring different training for proper use.

2.2.3 Types of Masks worn by Healthcare Professionals

Medical/surgical masks are the most basic form of PPE that protects against respiratory illnesses. These masks are best at protecting against exhaled droplets from the user, but they also offer some protection against larger droplets (Ippolito et al, 2020). They are cheap, single-use, and do not require any special training for proper use. Some disadvantages of surgical masks include their looser fit than other more protective masks and their diminished protection against inhalation exposures (Ippolito et al, 2020).

Filtering Facepiece Respirators (FFP) are a class of face-covering that offer tighter fitting and more secure masks. They offer more protection than surgical masks and they can protect against the inhalation of airborne particles or droplets. This group of respirators breaks down into FFP1 (filters 80% of particles), FFP2 (N95, 95% filtration) and FFP3 (N99, 99% filtration) (Ippolito et al, 2020). These respirators must be fit tested for the proper use and they are also

single-use. It is also important to note that facial hair and glasses can alter the fit of the covering or make the face-covering harder for the user to wear.

Powered and supplied-air respirators offer the best protection from bioaerosols and droplets. These PPE face coverings are technologically advanced. Some feature full-body attachments and are equipped with ventilation for extra protection. Individual who has facial or wear glass would have no problem with a fit while using this face covering (ECDC, 2014). There are some variations of these respirators that can only be used with specialized training. Due to the specialized nature of powered and supplied air respirators, they can be difficult to communicate in as they produce loud noises (Ippolito et al, 2020). Also, it requires special cleaning techniques for safe reuse, which can limit the facilities that can use this type of PPE. The price of this equipment is another limitation of powered and supplied respirators (ECDC, 2014).

2.2.4 Face Shields

Face shields can increase the surface area of protection and allow for fewer particles to reach the face. They also offer eye protection in tandem with nose and mouth protection. The plastic shield can block the fine particles. Ronen and colleagues found that a face shield is better at protecting against frontal exposures as their study showed that exposures from the side can reach the face (2021). The protection provided by face shields is largely dependent on the size of the face shield and the direction in which the exposure is coming from. Larger face shield will provide greater protection from aerosolized particles (Ronen et al, 2021; Ko-Keeney et al 2020).

2.3 Mask Efficacy Testing Strategies and Guidelines

Testing the effectiveness of PPE is generally done using one of the two following methods, randomized control trials, or they are tested using an aerosol-generating machine/ cough simulator. When testing the efficacy of the masks, the masks in question are tested against respiratory viruses such as influenza, SARS, and more recently SARS-cov-2. Some studies use aerosolized bacteriophages to test the effectiveness of PPE. For example, Rengasamy and colleagues used the aerosolized phiX174 bacteriophage model to compare respiratory filtration testing methods to the traditional sodium chloride (NaCl) method (2017).

Loed and colleagues tested the efficacy of surgical mask vs N95 respirator in preventing the flu. The goal of the study was to find out which mask would reduce the number of laboratory-confirmed influenzas among their nurse staff that participated. The results of the study show that there was no significant difference between the incidence of flu amount N95 wearer (22.9% infection rate, 48 nurses) and surgical mask wears (23.6% infection rate, 50 nurses) (2009). In this case, PPE effectiveness was tested using a controlled trial. The use of cough simulators to test the efficacy of PPE is another method used to measure the efficacy of face coverings against each other. Lindsley et al (2021) conducted a study using a cough simulator to test the effectiveness of the N95, medical-grade face mask, face shield, and cloth mask at reducing the aerosols. The results showed that N95 reduced the passage of aerosols the most while face shields allowed the most aerosols to pass through. These study methods are effective at testing different PPE abilities to reduce inhalation exposures and provide further information for HCWs to use when donning PPE for while patients.

In hospital settings, viral shedding of respiratory illness puts HCWs at risk for infection, so it is important to not only measure what PPE can reduce inhalation exposure but also measure what PPE can reduce exhalation exposure or shedding. This factor has become more important in the age of SARS-COV-2 (COVID-19), as hospitals aim to protect their workforce from contracting the highly transmissible COVID-19. A study used patients already ill with influenza and tested the amount of viral aerosol (fine and coarse) they exhaled in their breath. One group had no masks and the other group used surgical masks to reduce the spread. The results from this study found that there was a 3.4-fold reduction in the shedding of viral aerosols when using a surgical mask compared to no mask (Milton et al, 2013). Findings from studies exploring the use of PPE to reduce viral shedding of respiratory illnesses provide evidence for safer practices in medical facilities, as well as evidence that can be used to strengthen policy that supports the use of masks for public safety.

2.4 Use of model Viruses (Bacteriophages)

Modeling the dispersal of Viruses is an important tool for gathering information that can improve worker place safe for healthcare workers. These models provide insight into the dispersion patterns, resistance, viability of various respiratory viruses and can provide the means to test the efficacy of different PPE. Bacteriophages (phages) are typically used to model respiratory viruses due to their ability to be produced fast and they are relatively harmless to humans if the exposure was to occur. Turgeon and colleagues conducted a study that compared five different bacteriophages' survivability and infectivity after being subjected to aerosolization. The goal of the study was to evaluate these phages as potential models for human and animal

respiratory viruses. The bacteriophages used in that study are as follows: MS2, Φ6, Φ X174, PR772, PM2. This study also utilizes a human virus (human influenza A) and a poultry virus (Newcastle disease virus (NDV)). results from the study provided knowledge about the behaviors of the different phages. For example, the results from the study showed that the MS2 phages were the most resistant phage to the aerosolization process and had the highest recovery from quantitative polymerase chain reaction and plaque assays. The researchers concluded that MS2 is an ideal phage to model NDV (Turgeon et al, 2014). The ability to model human respiratory viruses with phages creates opportunities to further study the efficacy of PPE for healthcare workers.

2.5 Bioaerosol Generation Systems Overview

Bioaerosol Generation systems (BGS) are used in this field to simulate the spread of viral respiratory illness. They provide a means to model these viruses as it can be difficult to test their spread without harming people. BGS provides a closed controlled environment in which the spread of this virus can be studied. These systems can be used to test the efficacy of different PPE scenarios, and the data from these studies can be used to protect HCWs as they treat patients.

2.5.1 Evaluation of Other Bioaerosol Generation Systems

Lindsley and collaborators (2013) created a cough simulator to further understand the pattern of disease transmission through aerosolized particles. The system can aerosolize living and nonliving materials. It can also control for the volume of distribution and size of the particle. A defining characteristic of this cough simulator is the stainless-steel bellows. The bellows are

controlled by a linear motor that moves the bellow vertically upward (to release the aerosols) or downward (to load the aerosols). The system is connected to a breathing machine. This area of the system is where aerosols released are collected for analysis via inhalation to simulate breathing.

In addition to testing the effectiveness of PPE, BMS can be used to determine the effectiveness of disinfection techniques on bioaerosols. Lei, Burge, and First (2004) conducted a study using an aerosol-generating system to test UV light disinfection success on aerosolized bacteria. This system was con comprised of an aerosol-generating and drying section, UVGI exposure unit, and a Sampler. In the first part of the system, a nebulizer was used to generate the bioaerosols. In the section of the chamber are structures called baffles that the particles pass through before entering the UV light treatment chamber. These aid in the uniform mixing and drying of aerosol production. The second part of the chamber utilized UV light to deactivate the aerosolized bacteria. The particles would then be pushed through a HEPA filter to the sampler region of the system where the particles were collected.

2.5.2 Evaluation of AGS for this study

The aerosol-generating system used in this experiment was first used to simulate toxic exposures to cosmetics. The system features an air compressor, a master computer for controlling purposes, a glove box, and an animal station. The whole system is controlled by the master computer. The actual exposure occurs within the glove box where a rotating airbrush is responsible for releasing aerosolized products into the chamber. The compressed air pushed the

aerosol through the glove box to the animal chamber where rodents would be exposed to the particles via inhalation through their nose (Pearce et al, 2019).

It's important to note that the configuration of the AGS for this experiment was altered to simulate the parameters needed to assess PPE combinations. The aerosolized particles will no longer be collected via the animal chambers. Instead, the outlet tubes will lead to an impinger system where aerosolized phages will be collected in a fluid within the impinger. Lastly, the compressed air that is forced through the system passes through a HEPA filter before it reaches the airbrush to release the viruses. This ensures that the air is clean and dry before encountering and aerosolizing the test phages.

2.6 Viral Sampling and Detection Methodology

2.6.1 2.6.1 Sampling: Filters vs. Impingers

A review by (Haig et al, 2016), explored best practices for viral aerosol collection instruments. Impingers are made from glass and contain three pieces. A preferred liquid is added to the impinger to catch viruses in suspension this process reduces the amount of desiccation of the aerosols. Factors such as adherence of bioaerosols to impinger walls, evaporation, and re-aerosolization can reduce the amount of aerosol collected. There are two types of filters used to collect aerosolized viruses, fibrous and membrane filters. Fibrous filters are made with layers of fibers that particles become trapped when air is passed through them. Membrane filters are more porous than fibrous filters and contain pore structures within the membrane that trap particles in the filter. After exposure, filters are typically placed on Petri dishes and incubated to analyze what grows. One drawback to filters is that they are prone to sample desiccation due to the lack of moisture used when sampling (Haig et al, 2016).

Both filters and impingers' efficiency are dependent on particle size. Flow rates also are important for collecting samples. Higher flow rates may increase the number of aerosols collected over time. Higher particle concentration also tends to yield a greater number of colony-forming units (CFUs) observed after bacteria (E. Mescioglu et al, 2021). Lastly, both collection methods tend to yield similar amounts of CFU's during culture-dependent experiments and culture-independent (DNA concentrations) experiments (E. Mescioglu et al, 2021).

2.6.2 Detection: Double Agar Assay Procedure

Cormier and Janes (2014) conducted a study to explore methods to optimize the (DAL) assay. This study explores the ratios of bottom agar and top agar, as well as the methods of spreading the sample, in this case, MS2 bacteriophage solution. This study found that the plaque assay yielded the best results with a thin bottom agar layer (about 10ml of bottom agar) and a thin layer of top agar (about 10ml of top agar). They employed a spread plate technique instead of the traditional pour method. Glucose, $CaCl_2$, thiamine was added to the agar as a supplement to the growing E coli host. The study found that these supplements increased the growth and number of available E. coli for the phages to infect. The results from the study showed that the new optimized method in addition to the supplements provided a clear plaque assay as well as more consistent results.

Santos et al (2009) conducted a study to improve phage detection and enumeration while using the DAL assay. Instead of altering the agar, this study experimented with the use of antibiotics and glycerol to improve the visibility of plaque formation. Antibiotics such as ampicillin, penicillin G, rifampicin, and tetracycline were added to the media to see the effects.

The effect of antibiotics was dependent on the dose of antibiotics but also the type of phage that it was exposed to. On the other hand, the effects of glycerol (5%) were seen throughout the experiment. It's thought that glycerol helped the phages progress through the media. They observed larger and more visible plaque for phages that had gram-negative and positive hosts.

2.7 Public Health Relevance

2.7.1 Importance of this Research

The rationale behind this study is to provide necessary evidence in support of masks used to prevent the spread of COVID-19 and other viral respiratory pathogens in hospital settings. It also aims to find out which PPE combination provides the largest reduction in viral levels. This information is important for medical providers who, due to the nature of the job, are at a higher risk of being exposed to respiratory illnesses. This risk is greatly increased when providers perform aerosol-producing procedures such as intubations, extubations, or other ventilation procedures.

2.7.2 Research Question

The focus of this study will be on assessing the effectiveness of personal protective equipment (PPE) ability to reduce the number of bioaerosols present after exposure to aerosolized phages. This research will test the following PPE combinations: 1) (N95 respirator+face shield), (2) (surgical mask + face shield), (3) (surgical mask+N95), (4) (N95 respirator only), (5) (face shield only). In addition to testing the capability to reduce bioaerosols, this study will also explore the effects of viral aerosol concentration on PPE potential to reduce aerosol penetration. This study aims to explore the reduction of bioaerosol transmission via various PPE scenarios.

2.7.3 Experimental Design

The AGS provides a controlled environment in which aerosols are produced to simulate aerosol-generating procedures. An impinger system will be attached to the AGS which will funnel aerosolized phages (that pass through the masks) to a sampler containing phosphate-buffered saline (PBS). The PBS will be analyzed double-layer agar assay to determine how many phages were able to penetrate PPE combinations and reach the trachea of the mannequin. Biosamplers are the recommended method of collecting biogenic particles of bacteriophage MS2 that will be evaluated in this study. In sum, the combination of the AGS system in tandem with the biosampler will enable the simulation of viral exposure scenarios while evaluating PPE effectiveness.

3 METHODS AND PROCEDURES

3.1 Overnight host culture preparation

The overnight host culture consists of 50 ml of tryptic soy broth (TSB) and host bacterium E. coli Famp (ATCC #700891). 50 ml of TSB was placed into a sterile shaker flask. A one ml frozen stock of bacterial host (Famp E. coli) was thawed and pipetted into the shaker flask with TSB. The flask was incubated on a shaking platform at 100 rpm for 24 hours at 37°C.

3.2 Exponential phase culture preparation

The exponential phase culture is prepared on the day of the experiment. It consists of overnight culture and TSB. For every 100 ml of TSB, one ml of overnight host culture was added to a larger shaker flask. It was incubated on a shaking platform at 100 rpm for 2-4 hours at 37°C to allow the development of the exponential growth phase.

3.3 Aerosol Generation System (AGS) parameters

The parameters for the AGS were as follows: sprayer airflow 3.5 =liters per minute (LPM), glove box air flow= 5.5 LPM, Exhaust flow=9 LPM, particle concentration 1.00 mg/m^3, Gain= 2.5, spray duration=2.5 seconds, trigger position 60%, Dataram calibration factor= 1.00. The AGS system must have equal airflow entering and leaving the system for optimal use. This allowed the system to remain at a constant pressure during the exposure run. The sprayer airflow and the glove flow receive their air from the air compressor, which is HEPA filtered. The exhaust flow represents the air being pulled out of the system.

3.4 Bacteriophage titer Preparation

Frozen MS2 bacteriophage stock (approximately 500 µl 10^{11} PFU/mL) was thawed and added to 4.5ml of sterile water. The solution was diluted to a target phage concentration of 10^9 PFU total for use in each experiment. One ml of the diluted phage stock was added to the tracer solution which was added to the AGS for an experimental run.

3.5 Iron Oxide Tracer Preparation

The iron oxide Tracer solution was made using iron (III) oxide, cosmetic, NanoArc 20-40nm APS Powder in combination with cell culture grade water. For this experiment, 500 µg/ml (1:2 ratio: iron oxide/ water) was used. iron (III) oxide was measured out into a 15ml conical tube using Fisher Science Education analytical balance in grams. The ideal range of iron (III) oxide used per tracer solution was about 3-4mg (0.003-0.004 g), as this amount avoids excess waste. To ensure measurements of iron (III) oxide were accurate the conical tube's mass must be recorded first and then scale tared before adding the iron (III) oxide powered. The mass of the iron oxide was determined by taking the difference between the mass of the conical tube with iron oxide and the mass of the conical tube before iron oxide was added. After the preferred mass of iron (III) oxide was determined twice as much cell culture water in milliliters was added to the conical tube. This allows the solution to reach the desired 1:2 ratio. The tracer solution can be vortexed and stored in the fridge until the day of exposure or used the same day. Before starting an exposure run, the tracer solution was sonicated for 4 minutes at 75% amplitude using Fisher Scientific Sonic Dismembrator (model 500) cup sonicator to ensure accurate particle dispersion.

3.6 Procedure

3.6.1 Experimental Setup Procedures

The AGS system was set up with the parameters mentioned. The inlet tubes, outlet tubes, and the Dataram tubes were changed before every experimentally run. The Dataram filter was changed, and the Dataram was zeroed before use. The pressure was released from the system before use as well. 20 ml of PBS is added to three impingers (label A, B, C), one for each trial. The new inlet tube and outlet tube were connected to the impinger.

A 500 µg/ml iron oxide solution was made using cell culture grade water and iron (III) oxide, cosmetic, NanoArc 20-40nm APS Powder. MS2 bacteriophage stock (10^{11}) was diluted to 10^{9} bacteriophage titer using sterile water and 500µl from each dilution. The tracer solution used in the AGS system consisted of 1ml of 10^{9} bacteriophage titer and 7ml of 500 µl/ml iron oxide solution. This solution was added to the sprayer for an experimental run.

3.6.2 Experimental Run

A total of three twenty-minute runs for each experiment was conducted. Each of these runs was performed in duplicate assays (labeled trials). Each run has its impinger with 20 ml of PBS. Before starting a run, the AGS system must be closed, and the air compressor and vacuum was turned on. The fluid in the impinger rotated at a steady pace. After the experiment was started, the Dataram displayed the particle concentration for the duration of the twenty-minute experiment. The peak particle concentration was recorded and checked on the data file produced after the experiment. Once the twenty minutes were up, the air flows to the AGS were turned off. At this time the inlet and outlet tubes were removed from the impinger and a new impinger was

attached to the system. A 20-minute timer was set for the first decay to assess residual particle levels within the glove box chamber after the initial exposure run and to evaluate lower particle concentrations. The starting particle concentration of Decay 1 was recorded at the beginning of the twenty-minute duration and the airflows were turned back on. After the twenty-minute decay duration, the end particle concentration reading on the Dataram was recorded. The air flows were cut off again, and a new impinger was attached to the system to determine the particle concentrations during the second decay period. After the last decay duration, the AGS was cleaned, and the tubes were replaced. All five PPE experiments consisted of 3 duplicate experiments to attain more precise data.

3.6.3 Assay Procedure

Infectious MS2 was measured by the double-layer agar assay after each experimental run. First, the sides of the impinger were rinsed with its own PBS solution five times to ensure there were no bacteriophages stuck to the surface of the impinger. After rinsing, the PBS solution was placed into 15ml conical tubes and labeled. Tenfold dilutions of each sample, as well as the stock solution, were made and assayed.

1ml of exponential phase host was added to 10 mL of top agar (30g of TSB,7.5g of bacto agar per liter). Next, 1 ml of each sample was pipetted into each tube of top agar and host. The mixture was poured into petri dishes. This process was done for each sample that of PBS that comes from an experimental run as well as the bacteriophage titer. Control assays were also performed to ensure that the bacterial host and the agar were not contaminated. This assay consisted of top agar and exponential host culture only.

3.6.4 Analysis of Particle Concentration

As stated above, each experiment has a total of three (runs) and within each run, there were two duplicate trails. During the first run (first twenty minutes), the peak particle concentration was recorded by the Dataram. Each subsequent run was a measurement of particle concentration as it decayed over the twenty-minute exposure. At the beginning of the decay runs, the particle concentration was recorded. At the end of the twenty-minute run, the end particle concentration was recorded. The difference between the starting and ending particle concentration represents the number of particles that were in the system during the decay period.

3.6.5 Plaque Forming Unit analysis (PFU)

Each experiment had a set of plates from each of the three experimental runs (two each run) and a set of plates from the bacteriophage (phage) titer assay. The objective was to count the number of plaque-forming units (PFU) within each plate. Each plaque, regardless of size, will represent a bacteriophage infecting and killing the host E. coli cell. The PFU's from each experimental run were added together and represent the number of bioaerosols that were able to penetrate the PPE. The PFU from each experimental run represents what came out of the system and the PFU's from the phage titer represents what went into the system. Log reduction was calculated using phage titer (into the system) and the PFU from the experimental runs (out of the system).

3.6.6 AGS Cleaning Procedures

The AGS was cleaned using a combination of Cavicide and 70% alcohol. Once the particle concentration on the Dataram reads zero, it was safe to remove the gloves and open the AGS system. At this point, the system was opened. All sides of the AGS were first sprayed with Cavicide wiped down before spraying with 70% alcohol. The mannequin head and stand were sprayed and removed from the system and wiped down. The inside of the gloves was sprayed with Cavicide and wiped down. The inlet, outlet, and Dataram tubes were removed and placed in bleach for cleaning. In addition to the tubes being cleaned, there were pipes within the system that feed into the inlet tube and Dataram tube that must be removed and cleaned. Using soap and water, the inside of the pipes was scrubbed and then rinsed with DI water. After rinsing with water, the pipes were rinsed with 70% ethanol and allowed to air dry before being added back to the system. The top was removed and cleaned.

4 RESULTS

4.1 Viral titer penetrating the mannequin respiratory tract with no PPE

The control experiment did not include the use of PPE. A relationship was observed between the particle concentration and PFU found after exposure. This trend can be seen graphically in Figure 1. As such, the data from this experiment show that higher particle concentrations yielded a higher amount of PFU found within the sample.

The relationship between log reduction and particle concentration can be seen graphically in Figure 2. The trial with the largest log reduction during the control experiments was the Decay 1 trial, the first 20 minutes after initial exposure. The percentage of reduction for the control experiment can be seen in table 2. At peak particle concentrations the no PPE experiment had a reduced rate of 70% and at the lowest particle concentration (decay 2) the reduction rate for this experiment was 98%. The percent of bioaerosol reduction increased as the particle concentration within the system decreased, this trend can be seen in table 2 as well. The result from the control experiment showed how many bioaerosols could be collected without the obstruction of PPE.

4.2 Viral titer penetrating the mannequin respiratory tract with N95

The data from the N95 experiment can be seen graphically in Figure 3. This data displays a similar relationship between the particle concentration and PFU that was observed seen in the No PPE experiment. This means higher particle concentration again resulted in a higher amount of PFUs collected. the average number of particles per trial can be seen in table 1. The average peak of particle concentration across the 3 N95 experiments was 229 (µg/m3) at peak, 174 (µg/m3) during decay 1 trial, and 18 (µg/m3) during decay 2 trial. This data corresponds to the particle concentrations seen in Figure 1.

The inverse relationship was seen between the log reduction and the particle concentration during the N95 experiment. Fewer particles within the glovebox chamber resulted in greater log reduction. Again, you can see that the percent of Bioaerosol reduction increased as the particle concentration within the system decreased table 2. The N95 respirator reduced bioaerosols by 99% across all trials (peak, decay 1, decay 2). This PPE combination resulted in the highest level of bioaerosol reduction among all PPE combinations.

4.3 Viral titer penetrating the mannequin respiratory tract with Face Shield

The association between particle concentration and the PFU was seen again within this data Figure 5. Higher particle concentration was associated with higher amounts of PFUs collected. The average peak of particle concentration across the three face shield experiments was 484 (µg/m3) at peak, 373 (µg/m3) during decay 1 trial, and 74 (µg/m3) during decay 2 trial. This data can be seen in Table 1.

The data from this experiment also showed the inverse relationship between particle concentration and log reduction. This data can be seen in Figure 6. at peak particle concentrations, the face shield reduced bioaerosols by 80%, and at low particle concentrations, the reduction rate was 99%. Face shield had the lowest reduction rate at peak concentrations among all PPE combinations tests.

4.4 Viral titer penetrating the mannequin respiratory tract with Face Shield/N95

The association between particle concentration and PFU was not observed within this data. The number of PFU does not rise as the particle concentration rises. The peak trials PFU/ml

count, which has the highest particle concentration, was slightly lower than the decay 1 trials PFU/ml count. This was different than the trend previously described particle concentrations and can be found in Figure 7. The average particle concentration of the peak trial (296 µg/m3) and the Decay 1 trial (221 µg/m3) reached similar heights as seen in table 1. This may be a reason for similar PFUs observed for both trials.

There was also no inverse relationship seen between the log reduction and particle concentration. The Peak trial and the decay 1 trial were similar log reductions. At peak particle concentrations, the face shield/N95 combination reduced bioaerosols by 84%, and at low particle concentrations, the reduction rate was 99%. The data from this experiment resulted in a bioaerosol reduction that was greater than the face shield-only combination.

4.5 Viral titer penetrating the mannequin respiratory tract with Face Shield/Surgical Mask

The results from this experiment showed a similar relationship between the particle concentration and the PFU counted. A greater number of PFU were found in samples when the particle concentration within the system was at its highest. The average peak of particle concentration across the three-face shield/Surgical Mask experiments was 537 (µg/m3) at peak, 387 (µg/m3) during decay 1 trial, and 73 (µg/m3) during decay 2 trial. This data can be seen in Table 1.

There was an inverse relationship between log reduction and particle concentration. A higher particle concentration resulted in a lower log reduction (Figure 10). This trend was also

seen in other experiments. The Face Shield/Surgical Mask PPE combination reduced bioaerosol by 86% at peak particle concentrations and 99% at low (Decay 2) particle concentrations (table 2). The data showed that the face shield/ surgical mask resulted in a higher reduction rate than the face shield/N95 combination.

4.6 Viral titer penetrating the mannequin respiratory tract with N95/Surgical Mask

The data from this experiment resulted in the same association between PFU and particle concentration. The number of PFU/ml was higher when the particle concentration within the system was high (Figure 11). The average peak of particle concentration across the three N95/Surgical Mask experiments was 309 (µg/m3) at peak, 218 (µg/m3) during decay 1 trial, and 28 (µg/m3) during decay 2 trial. This data can be seen in Table 1.

The relationship between log reduction and particle concentration was not inverse like in previous experiments. The trials Decay 1 and Decay 2 had similar log reductions even though their corresponding particle concentrations were drastically different Figure 12. The N95/Surgical Mask PPE combination reduced bioaerosols by 88% at peak particle concentrations and 99% at low (Decay 2) particle concentrations (Table 2). This PPE combination had the second-highest reduction rate.

4.7 Summary of All Experiments

Figure 13 contains the log reductions for each experiment conducted in this study. The results from this study indicate that having PPE reduces the number of bioaerosols at a higher rate than not having PPE. N95 had the largest log reduction while face shield had the lowest.

Lastly, each PPE combination had a 99% reduction rate the lowest particle concentration (decay 2).

4.8 Statical analysis

GraphPad Prism was used to conduct a Two-way ANOVA test with repeated measures in GraphPad prism to do the statistical analysis. This test allows us to determine if the variables (PPE and particle concentration) affect the outcome (log reduction) was significant. The Tables described in this section provide the source of variation (time, experiment, PPE, and time x variation) and the extent to which each source of variation affects the outcome (percentage). Variation can be described as differences in the data across the variables of the experiment. Time represents the duration of the experiment and corresponding changes in particle concentration. Experiment describes differences between each PPE combination's repeated experiments and PPE describes changes in PPE used.

4.8.1 Variation: Control (No PPE) vs N95

Table 3 compared the data from the control experiment to the data from the N95 trial. The results from this test show that variables time and experiment were significant contributors to variation between these data sets. Time contributed to 36% (P-value: 0.0013) of total variation while experiment contributed to 54% (P-value0.0013) to total variation.

4.8.2 Variation: Control (No PPE) vs Face Shield

Table 4 compared the data from the control experiment to the data from the Face Shield experiment. The results showed that time was the only variable that played a role in differences in the data. Time contributed to 81% (P-value: 0.0025) of total variation.

4.8.3 Variation: Control (No PPE) vs Face Shield/Surgical Mask

Table 5 compared the data from the control experiment to the data from the Face Shield/Surgical Mask experiment. The results showed that time was the only variable that played a role in affecting the differences seen in the data. Time contributed to 78% (P-value: 0.0025) of total variation.

4.8.4 Variation: Control (No PPE) vs Face Shield/N95

Table 6 compared the data from the control experiment to the data from the Face Shield/N95 experiment. The results showed that time was the only variable that played a role in influencing variations within the data. Time contributed to 52% (P-value: 0.0154) of total variation.

4.8.5 Variation: Control (No PPE) vs N95/Surgical Mask

Table 7 compared the data from the control experiment to the data from the N95/Surgical Mask experiment. The results show that the experiment was the only variable that played a role in affecting the differences between the data. Experiment contributed to 12% (P-value: 0.0114) of total variation.

4.8.6 Variation: All PPE Combination

When looking at all the data together, you can see that the variables, time and experiment, contributed to the variation between datasets the most at 37% (P-value: <0.0001) and 33% (P-value: <0.0001) respectively. These two variables were the only variables that had a significant impact on the outcome. This data can be seen in Table 8.

5 DISCUSSION AND CONCLUSION

5.1 Discussion

The results from this study show that the use of PPE reduced the number of bioaerosols penetrating the respiratory system of the mannequin at a higher rate than not using PPE. This may provide further evidence for the use of face coverings to reduce the spread of bioaerosols in medical settings (Milton et al 2013, Ueki 2020). The extent to which transmission is reduced is a constant area of research for scientists. This data could also have implications outside of the medical field. The controversy surrounding mask use has grown through the duration of the pandemic. This data could provide further evidence for policymakers on the efficacy of mask use.

The AGS system was optimized using no PPE as the control experiment. It was important to establish how the AGS would disperse the bioaerosols while also finding a baseline of bioaerosol infiltration to compare with the PPE experiments. The experiment resulted in higher particle concentration within the AGS which was associated with a larger number of plaques forming units (PFU) found in samples. This observation seen within the control experiment provided reassurance for the phages' ability to follow the iron particles throughout the system. The results from the statistical analysis also offer support for this trend seen in the data. Four out of the five PPE types when compared to the control showed that time (particle concentration during that time) had a significant effect on the outcome. This is further evidence that the system is capable of dispersing particles effectively to model these scenarios.

The reduction of viral aerosols at peak particle concentration levels, and decaying particle concentration levels was also greater when using the PPE combination. It appears that the use of

PPE can be effective at reducing inhalation exposures caused by bioaerosols. This result was to be expected as there is current research that suggests the use of any face covering has some capacity to reduce the transmission of aerosolized viral transmission (Lindsley et al 2021, Wang et al 2020). This result not only provides support for mask use in a medical setting but offers support for mask use of any kind for civilians specifically when trying to stop the spread of a pandemic-inducing virus such as the novel Sars-Cov-2 virus.

N95 only showed the largest reduction of bioaerosols across all trials. This may suggest that at high and low particle concentrations, N95 respirators can provide adequate protection against inhalation exposures. The results from this study show that N95 respirators were the best PPE to use when dealing with bioaerosol generating procedures. This result aligns with current research that supports the N95 respiratory as being the most effective face-covering offered (Lindsley et al 2021). N95 needs fit testing for it to work most efficiently (Ippolito et al 2020, ECDC 2014). If the mask is not worn properly, then its ability to reduce the number of bioaerosols can be comprised leaving an unexpecting HCW to be exposed to higher levels of bioaerosols (O'Kelly et al 2021, Reponen et al 2011). This factor could the reason for the differences in the log reduction between the N95 only experiment and the N95/face shield experiment. It's possible that the seal of the N95 to the mannequin was more secure during the N95 trials than N95/face shield. This could be why the N95 alone reduced bioaerosols better than the N95 combined with the face shield. In this study, the face shield offered the lowest amount of protection from bioaerosols. However, data showed that the addition of a face covering (surgical mask or N95) increased the reduction rate. This may suggest the need to combine a face shield with another type of face-covering for it to provide the most protection. The face shield will catch the largest

droplets and some bioaerosols, but the aerosol can travel around the face shield and still leave the user exposed to high levels of aerosols (Ronen et al 2021, Lindsley et al 2014). Ultimately, there is evidence to support the use of PPE to reduce the amount of bioaerosols transmission when compared to no PPE.

The AGS provided a closed system to produce aerosols and test the effectiveness of PPE. To ensure that the system was working effectively there needed to be a way to tell that the aerosolized phages were being collected. Therefore, a tracer was needed so that the system could detect particle concentrations generated in each experiment. Due to the mixture of phages with the iron tracer, it was hypothesized that the phage would follow the particle through the system, and thus the particle concentration would be a reliable indicator of virus concentration. The trend seen in the data shows that the system was working properly. At the highest levels of particle concentration, there were high levels of PFU found in the assays and subsequently, less PFU were found at lower particle concentrations. This finding was significant to the study because it shows that the AGS system can effectively aerosolize and transmit in addition to recording the number of viruses emitted via particle measurement. It also shows that this system can produce bioaerosols at different concentrations which are important for studying aerosol-producing procedures that may produce varying amounts of bioaerosols. In sum, the data herein provides empirical evidence of the efficacy of masks and shows that the AGS system is a reliable tool for additional bioaerosol studies in the future.

Additionally, a relationship between log reduction and particle concentration was observed. The log reduction increases as the particle concentration within the system decrease.

This shows that PPE can reduce bioaerosols transmission at various levels of particle concentration. Each PPE combination reduced bioaerosol transmission by 99% at low particle concentrations. This relationship also ties in well with aerosol-generating procedures and the use of masks to prevent the spread of the bioaerosols they produce. Numerous procedures can produce aerosols, but they may not all produce the same amount of bioaerosols (Macintyre et al 2014, Fowler et al 2004). There was also an increase in percent reduction as the particle concentration inside the system decreased. This suggests that each PPE combination may be capable of reducing penetration at highs and low levels of bioaerosols. This study provides evidence for PPE being safe and effective at preventing bioaerosol transmission that is suspended in the air (Fears et al 2020). In sum, this study could help to bridge the gap between aerosol-generating procedures and the transmission of suspended bioaerosols.

5.2 Challenges and Solutions

While this study was able to provide data in support of mask use, it did not come without some challenges. The ASG used to create the aerosols is a highly advanced automated system that required a steep learning curve to become proficient with its use. Inexperience with the system may have led to variability in the concentration of the particles. This was overcome with experience working with the system as well as having three experiments per PPE combination with duplicate trials. Additionally, not all phages sprayed into the system were collected into the impinger. Phages were also collected in the systems tubbing and potentially on the walls of the chamber. This provided a future direction for optimizing the use of this AGS to account for how many phages were lost in the system.

5.3 Conclusion

In conclusion, the use of PPE to reduce the transmission of bioaerosols is more effective than not using PPE. PPE is also capable of this reduction factor at low, medium, and high levels of PPE. this is an important finding of the study due to the nature of aerosol-generating procedures. These procedures tend to produce different levels of PPE and this study has shown that PPE, at different levels of effectiveness, can help protect HCWs when performing these procedures. The most proficient PPE combination used in this study was found to be the N95 respiratory. As such, this face-covering may offer adequate protection from bioaerosol transmission from most medical procedures and patients sick with viral respiratory illnesses. Face shields offer protection but may need additional face covering to provide the most protection. Lastly, data from this study can provide evidence to support mask use not just for HCWs but also everyday people as we continue to try to stop the spread of Covid-19.

WORKS CITED

Aitken, C. and D. J. Jeffries. “Nosocomial Spread of Viral Disease”. Clinical Microbiology Reviews, vol. 14, no. 3, Clinical Microbiology Reviews, 2001, pp. 528–546.

Bartoszko, J. J., Farooqi, M. A. M., Alhazzani, W., & Loeb, M. (2020). Medical masks vs N95 respirators for preventing COVID-19 in healthcare workers: A systematic review and meta-analysis of randomized trials. Influenza and Other Respiratory Viruses, 14(4), 365–373. https://doi.org/10.1111/irv.12745

Bing-Yuan, Zhang, Y.-H., Leung, N. H. L., Cowling, B. J., & Yang, Z.-F. (2018). Role of viral bioaerosols in nosocomial infections and measures for prevention and control. Journal of Aerosol Science, 117, 200–211. https://doi.org/10.1016/j.jaerosci.2017.11.011

Chughtai, Abrar Ahmad et al. “Availability, consistency and evidence-base of policies and guidelines on the use of mask and respirator to protect hospital health care workers: a global analysis.” BMC research notes vol. 6 216. 31 May. 2013, doi:10.1186/1756-0500-6-216

Cormier, J. And M. Janes. “A double layer plaque assay using spread plate technique for enumeration of bacteriophage MS2”. Journal of Virological Methods, vol. 196, Journal of Virological Methods, 2014, pp. 86–92.

European Centre for Disease Prevention and Control. Safe use of personal protective equipment in the treatment of infectious diseases of high consequence. Stockholm: ECDC; 2014.

Fears, A. C., Klimstra, W. B., Duprex, P., Hartman, A., Weaver, S. C., Plante, K. S., Mirchandani, D., Plante, J. A., Aguilar, P. V., Fernández, D., Nalca, A., Totura, A., Dyer, D., Kearney, B., Lackemeyer, M.,

Haig, C. W., et al. “Bioaerosol sampling: sampling mechanisms, bioefficiency and field studies”. Journal of Hospital Infection, vol. 93, no. 3, Journal of Hospital Infection, 2016, pp. 242–255.

Ippolito, M., et al. “Medical masks and Respirators for the Protection of Healthcare Workers from SARS-CoV-2 and other viruses”. Pulmonology, vol. 26, no. 4, Pulmonology, 2020, pp. 204–212.

K. Pearce, W.T. Goldsmith, R. Greenwald, C. Yang, G. Mainelis & C. Wright (2019) Characterization of an aerosol generation system to assess inhalation risks of aerosolized nano-enabled consumer products, Inhalation Toxicology, 31:9-10, 357-367, DOI:10.1080/08958378.2019.1685613

Klompas, M., et al. “What Is an Aerosol-Generating Procedure?”. JAMA Surgery, vol. 156, no. 2, JAMA Surgery, 2021, p. 113.

Lai, Ka M et al. “Size and UV germicidal irradiation susceptibility of Serratia marcescens when aerosolized from different suspending media.” Applied and environmental microbiology vol. 70,4 (2004): 2021-7. doi:10.1128/AEM.70.4.2021-2027.2004

Lindsley, W. G., Blachere, F. M., Law, B. F., Beezhold, D. H., & Noti, J. D. (2021). Efficacy of face masks, neck gaiters, and face shields for reducing the expulsion of simulated cough-generated aerosols. *Aerosol Science and Technology, 55*(4), 449–457. https://doi.org/10.1080/02786826.2020.1862409

Lindsley, William G., et al. “Efficacy of Face Shields against Cough Aerosol Droplets from a Cough Simulator.” Journal of Occupational and Environmental Hygiene, vol. 11, no. 8, 2014, pp. 509–518., doi:10.1080/15459624.2013.877591.

Infection Prevention and Control of Epidemic- and Pandemic-Prone Acute Respiratory Infections in Health Care. Geneva: World Health Organization; 2014. Available from: https://www.ncbi.nlm.nih.gov/books/NBK214359/

"Supplement I: Infection Control in Healthcare, Home, and Community Settings." *Cdc.gov*, 8 Jan. 2004, https://www.cdc.gov/sars/guidance/i-infection/healthcare.pdf.

Loeb, M., et al.. "Surgical Mask vs N95 Respirator for Preventing Influenza Among Health Care Workers". JAMA, vol. 302, no. 17, JAMA, 2009, p. 1865, doi:10.1001/jama.2009.1466.

Luo, Lei et al. "Contact Settings and Risk for Transmission in 3410 Close Contacts of Patients With COVID-19 in Guangzhou, China: A Prospective Cohort Study." Annals of internal medicine vol. 173,11 (2020): 879-887. doi:10.7326/M20-2671

Rengasamy, S., Shaffer, R., Williams, B., & Smit, S.. (2017). A comparison of facemask and respirator filtration test methods. *Journal of Occupational and Environmental Hygiene, 14*(2), 92–103. https://doi.org/10.1080/15459624.2016.1225157

Mescioglu, E., et al.. "Efficiency of bioaerosol samplers: a comparison study". Aerobiologia, vol. 37, no. 3, Aerobiologia, 2021, pp. 447–459.

Milton, D. K., et al. "Influenza Virus Aerosols in Human Exhaled Breath: Particle Size, Culturability, and Effect of Surgical Masks". PLOS Pathogens, vol. 9, no. 3, PLOS Pathogens, 2013, p. e1003205, doi:10.1371/journal.ppat.1003205.

Milton, D. K., et al. "Influenza Virus Aerosols in Human Exhaled Breath: Particle Size, Culturability, and Effect of Surgical Masks". PLOS Pathogens, vol. 9, no. 3, PLOS Pathogens, 2013, p. e1003205, doi: 10.1371/journal.ppat.1003205.

O'Kelly, E., Arora, A., Pirog, S., Ward, J., & Clarkson, P. J. (2021). Comparing the fit of N95, KN95, surgical, and cloth face masks and assessing the accuracy of fit checking. PLOS ONE, 16(1), e0245688. https://doi.org/10.1371/journal.pone.0245688

Patel, R. B., Skaria, S. D., Mansour, M. M., & Smaldone, G. C. (2016). Respiratory source control using a surgical mask: An in vitro study. *Journal of Occupational and Environmental Hygiene*, *13*(7), 569–576. https://doi.org/10.1080/15459624.2015.1043050

Reponen, T., Lee, S.-A., Grinshpun, S. A., Johnson, E., & Mckay, R.. (2011). Effect of Fit Testing on the Protection Offered by N95 Filtering Facepiece Respirators Against Fine Particles in a Laboratory Setting. Annals of Occupational Hygiene, 55(3), 264–271. https://doi.org/10.1093/annhyg/meq085

Ronen, A., et al.. "Investigation of the protective efficacy of face shields against aerosol cough droplets". Journal of Occupational and Environmental Hygiene, vol. 18, no. 2, Journal of Occupational and Environmental Hygiene, 2021, pp. 72–83.

Turgeon, Nathalie, et al. "Comparison of Five Bacteriophages as Models for Viral Aerosol Studies." Applied and Environmental Microbiology, vol. 80, no. 14, 2014, pp. 4242–4250., doi:10.1128/aem.00767-14.

Ueki H, Furusawa Y, Iwatsuki-Horimoto K, Imai M, Kabata H, Nishimura H, Kawaoka Y. 2020. Effectiveness of face masks in preventing airborne transmission of SARS-CoV-2. mSphere 5: e00637-20. https://doi.org/10.1128/mSphere.00637-20.

Van Doremalen, N., Bushmaker, T., Morris, D. H., Holbrook, M. G., Gamble, A., Williamson, B. N., Tamin, A., Harcourt, J. L., Thornburg, N. J., Gerber, S. I., Lloyd-Smith, J. O., De Wit, E., & Munster, V. J. (2020). Aerosol and Surface Stability of SARS-CoV-2 as

Compared with SARS-CoV-1. *New England Journal of Medicine*, *382*(16), 1564–1567. https://doi.org/10.1056/nejmc2004973

Wang, X., et al.. “Association Between Universal Masking in a Health Care System and SARS-CoV-2 Positivity Among Health Care Workers”. JAMA, vol. 324, no. 7, JAMA, 2020, p. 703.

6 TABLES

Table 1 ALL PPE Summary: Average Particle Decay Concentration (µg/m3) in system

No PPE	
Peak	324.6
Decay 1	245.94
Decay 2	63.44
N95 Only	
Peak	229.1667
Decay 1	174.2333
Decay 2	18.43333
Face Shield Only	
Peak	484.3333
Decay 1	373.7667
Decay 2	74.2
Face Shield/N95	
Peak	296.5333
Decay 1	221.7667
Decay 2	31.43333
Face Shield/ Surgical Mask	
Peak	537.6667
Decay 1	387.5
Decay 2	73.26667
N95/Surgical Mask	
Peak	309
Decay 1	218.6333
Decay 2	28.96667

Table 2 ALL PPE Summary: Percent Reduction

No PPE	
Peak	70.82397
Decay 1	79.13858
Decay 2	98.44075
N95 Only	
Peak	99.57383279
Decay 1	99.94918567
Decay 2	99.99953094
Face Shield Only	
Peak	80.06896552
Decay 1	86.82758621
Decay 2	99.89448276
Face Shield/N95	
Peak	84.26356589
Decay 1	95.66666667
Decay 2	99.93255814
Face Shield/Surgical Mask	
Peak	86.10778443
Decay 1	94.66467066
Decay 2	99.94790419
N95/ Surgical Mask	
Peak	88.41269841
Decay 1	98.84126984
Decay 2	99.98698413

Table 3 Variation: No PPE vsN95

Source of variation	% of total variation	P value	Significant?
Time x experiment	2.297	0.1141	No
Time	36.92	0.0013	Yes
experiment	54.21	0.0013	Yes
PPE	3.387	0.1691	No

Table 4 Variation: No PPE vs Face Shield

Source of variation	% of total variation	P value	Significant?
Time x experiment	0.2914	0.8939	No
Time	81.46	0.0025	Yes
experiment	2.496	0.2493	No
PPE	5.505	0.4292	No

Table 5 Variation: No PPE vs Face Shield/Surgical Mask

Source of variation	% of total variation	P value	Significant?
Time x experiment	1.142	0.5849	No
Time	78.49	0.0025	Yes
experiment	8.13	0.051	No
PPE	4.275	0.4294	No

Table 6 Variation: No PPE vs Face Shield/N95

Source of variation	% of total variation	P value	Significant?
Time x experiment	0.3981	0.9342	No
Time	52.96	0.0154	Yes
experiment	13.45	0.081	No
PPE	9.986	0.5264	No

Table 7 Variation: No PPE vs N95/Surgical Mask

Source of variation	% of total variation	P value	Significant?
Time x experiment	8.758	0.5531	No
Time	21.44	0.2794	No
experiment	12.4	0.0114	Yes
PPE	2.529	0.9822	No

Table 8 Variation: All PPE

Source of variation	% of total variation	P value	Significant?
Time x experiment	6.816	0.5893	No
Time	37.02	<0.0001	Yes
experiment	33.16	<0.0001	Yes
PPE	3.742	0.9546	No

7 FIGURES

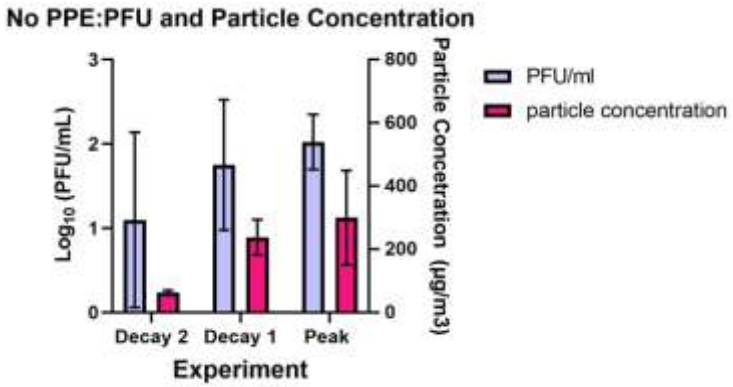

Figure 1 No PPE: PFU and Particle Concentration Relationship

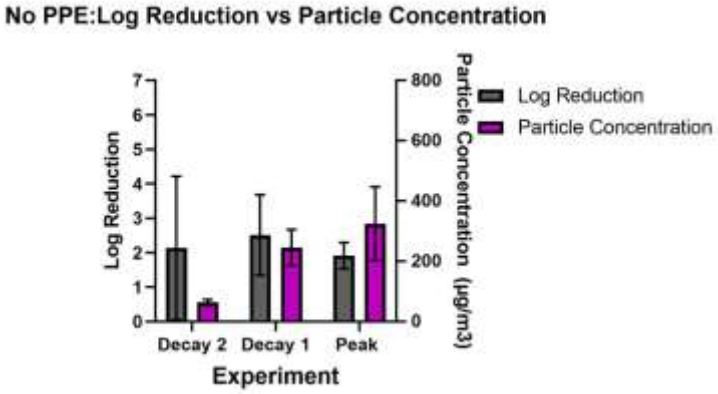

Figure 2 No PPE: Log Reduction and Particle Concentration Relationship

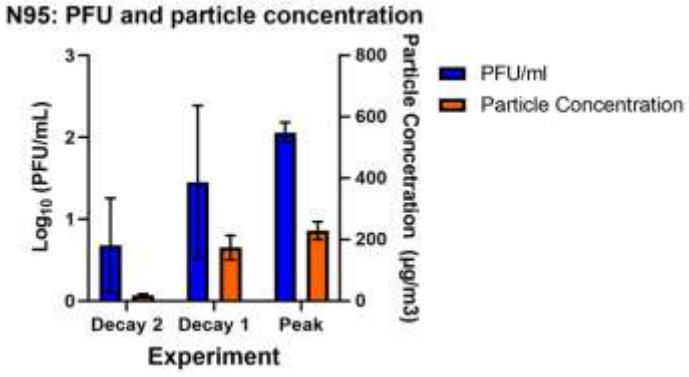

Figure 3 N95: PFU and Particle Concentration Relationship

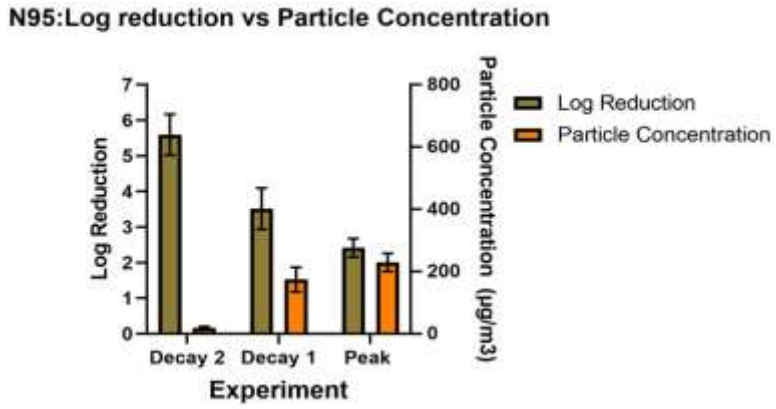

Figure 4 N95: Log Reduction and Particle Concentration Relationship

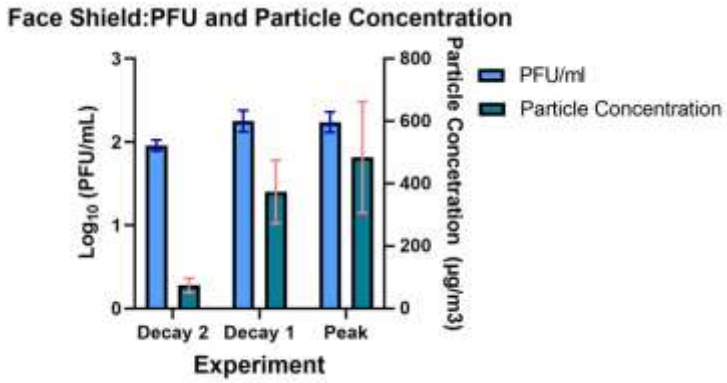

Figure 5 Face shield: PFU and Particle Concentration Relationship

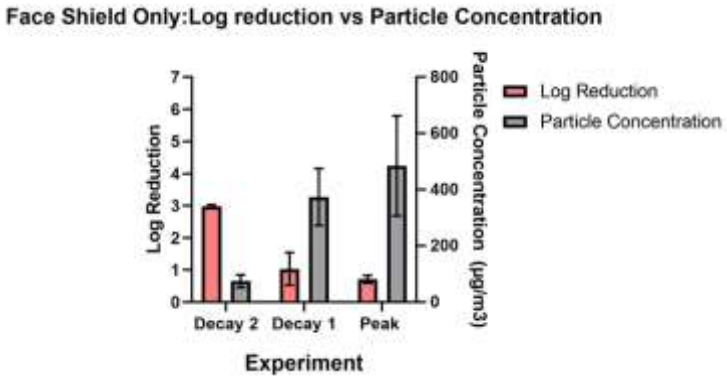

Figure 6 Face shield: Log Reduction and Particle Concentration Relationship

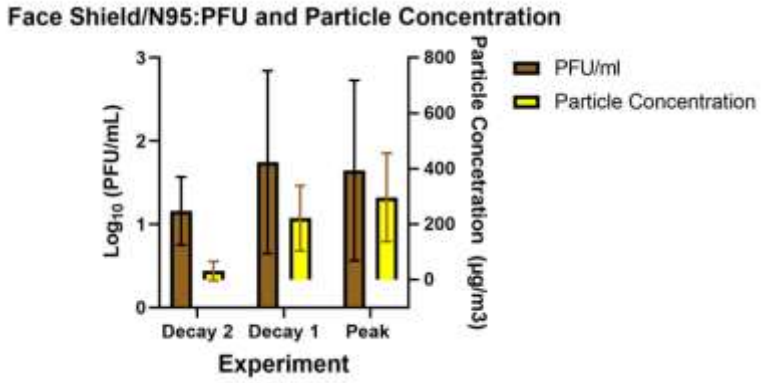

Figure 7 Face Shield/N95: PFU and Particle Concentration Relationship

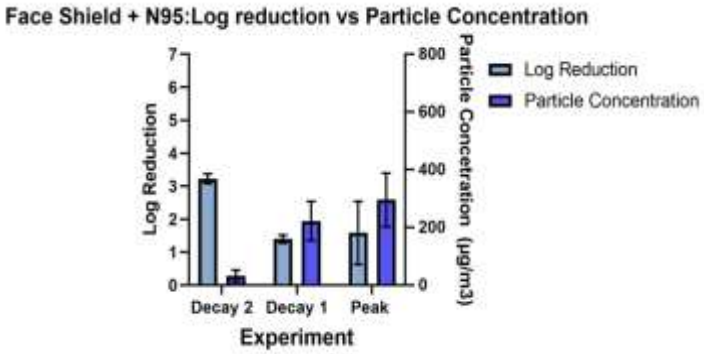

Figure 8 Face Shield/N95: Log Reduction and Particle Concentration Relationship

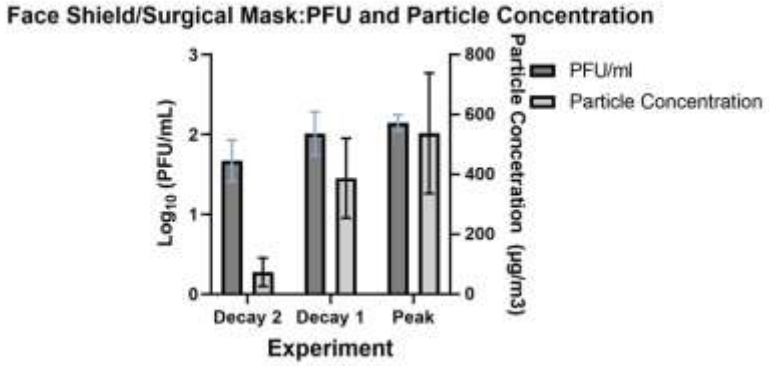

Figure 9 Face Shield/Surgical Mask: PFU and Particle Concentration Relationship

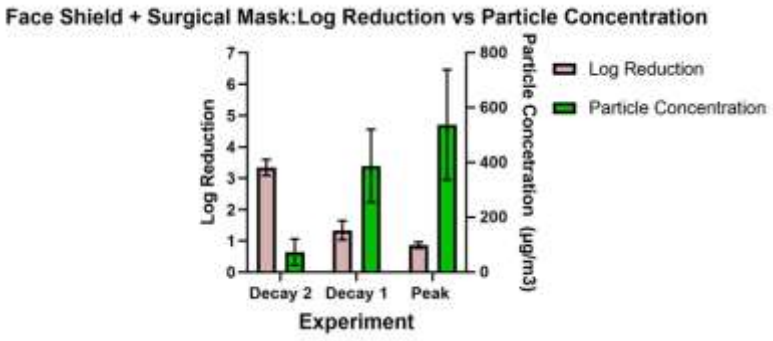

Figure 10 Face Shield/Surgical Mask: Log Reduction and Particle Concentration Relationship

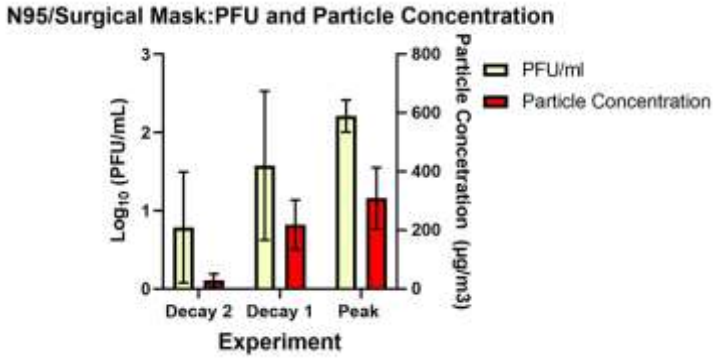

Figure 11 N95/Surgical Mask: PFU and Particle Concentration Relationship

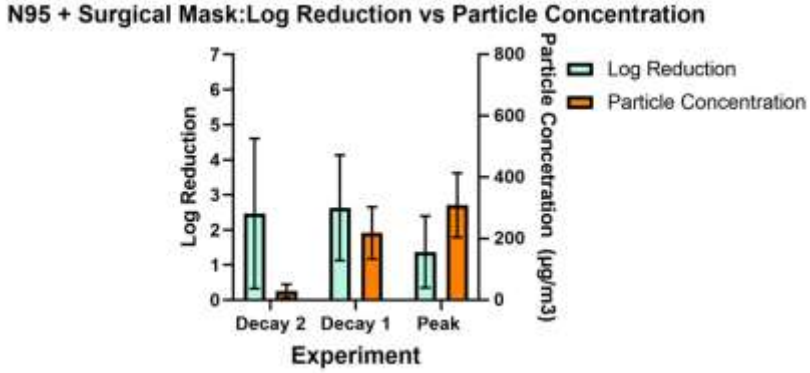

Figure 12 N95/Surgical Mask: Log Reduction and Particle Concentration Relationship

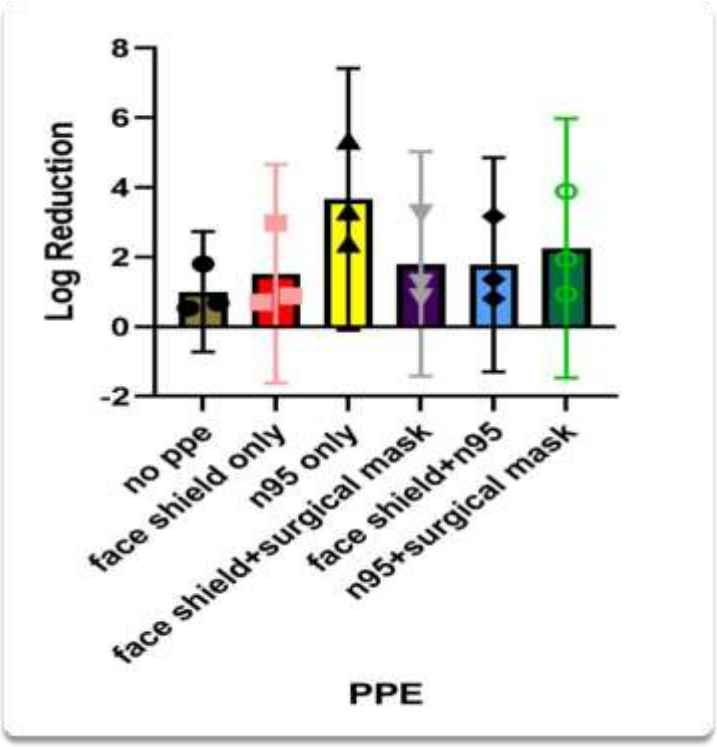

Figure 13 Summary: Log Reduction All PPE