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Decontamination of *E. coli* and *S. epidermidis* on Cloth Masks with Ultraviolet Germicidal Irradiation

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ABSTRACT

Spurred by a global pandemic, precautions have been taken by the general public to slow the spread of the respiratory virus called COVID-19. As Cloth Masks have become staples in protection during this time, it is important to understand any limitations that could arise when using a product. Several studies have found that certain methods of decontaminating Cloth Masks, so they may be reused safely, can result in slight degradation. This study analyzes the decontamination method of Ultraviolet Germicidal Irradiation (UVGI) on Cloth Masks that have different ply counts. Using both *Escherichia coli* and *Staphylococcus epidermidis*, three different masks were contaminated with bacterial colonies then put through a session of UVGI to sterilize the fabric. Once the session was over, masks were collected and analyzed for effectiveness of decontamination and changes in fabric pore size. It was found that when decontaminating the Cloth Masks, regardless of the bacteria they have been contaminated with, there was a positive correlation between the number of plies a mask had to the amount of bacteria able to be decontaminated. Additionally, there was a negative relationship between the number of plies a mask had and the degradation of the material over time. UVGI provides an effective method of decontamination for Cloth Masks now that its benefits and limitations have been understood.

Introduction

Since early 2019, the COVID-19 pandemic has greatly contested the world. With over 100 million cases reported in early February 2021, precautions are being taken to slow the virus's spread [18]. Many nations have emphasized using Personal Protective Equipment (PPE) to minimize exposure to hazards such as the virus. For example, it has become mandatory for health care workers to wear hospital-grade PPE such as N95 respirators. These respirators ensure that 95% of particles are blocked from passing through the filter media. Filtration efficiency is very important for not only health care workers but for any mask user who places importance on keeping viruses or bacterium from entering their mouth or nasal cavity. High demand for masks, from both health care workers and the general public has led to immense shortages of N95s and other PPE. The Center for Disease Control and Prevention (CDC) has stated that they do not recommend that the general public wear masks that "are intended for healthcare workers, including N95 respirators or surgical masks" to ensure available masks are supplied to frontline workers[17]. To further prevent these shortages from worsening, the CDC has recommended the general public to use "non-valved multi-layer cloth masks" despite these masks having a lower filtration efficiency than hospital-grade PPE [17, 15]. Unfortunately, due to the aforementioned shortages, hospitals have resorted to reusing PPE meant for single use. Without enough access to new single-use respirators, such as N95s that are meant to be disposed of after one use, healthcare workers are left with the difficult choice of reusing them.

Over time, wearing an N95 respirator can decrease its filtration efficiency, which can become unsafe for the wearer. As explained by researchers Bhattacharjee et al. from the Biosecurity Program at the Kirby Institute in Australia, N95s "are usually positively charged", which allows them to "attract aerosols and particles (which are

negatively charged) by electrostatic force"[2]. If single-use respirators are used for prolonged periods, their layers will lose this positive charge, rendering them less effective. In addition to charge, excessive donning and doffing of N95 respirators can result in "head strap breaks and nosepiece breaks" according to a 2012 fit-testing study of consecutively used face respirators by researchers Bergman et al. from the National Institute for Occupational Safety and Health[1]. To ensure that hospitals are not risking the spread of the virus, or using compromised PPE, many researchers and hospitals have proposed the decontamination of single-use respirators to allow multiple uses with limited reduction in filtration efficiency and fit.

Review of Literature

Decontamination Methods

Researchers Torres et al., at the Photomedicine and Photobiology Unit at the Henry Ford Health System, conducted a meta-analysis on the various decontamination methods that have been studied, accessing the limitations and benefits of each [16]. The review covered the most commonly discussed sterilization methods, "microwave-generated steaming," "dry heating," "hydrogen peroxide vaporization," and "ultraviolet germicidal irradiation." It was stated that while Microwave-generated steaming or MGS is readily available, fast, and resulted in minimal degradation after three cycles, the delivery was not uniform, and after more than five cycles there was "reduced filtration capacity." Dry heating decontamination was found to be readily available, left no chemical residue, and maintained filtration after 20 cycles. The limitations of this method were that the respirators require direct supervision during the decontamination process and could degrade FFR, or filtering facepiece respirators. Torres et al. cited that at the time of their study there was insufficient efficiency data regarding whether dry heating is an applicable method [16]. It was found that dry heating must be customized to the specific material being decontaminated, therefore, this method may not be the most effortless to apply in a hospital setting. For example, according to researchers Oh et al., while for N95 respirators, "dry heat treatment at 100 °C for 50 min is an appropriate method", they feel that different respirator models made with different materials could require a separate regime [14]. Another method that has been used by healthcare professionals and studied by Torres et al. is Hydrogen Peroxide Vaporization (HPV). This method has also proven to be an effective sterilization method; its set-up allows for sterilization of large quantities, uniform delivery, and protects the FFR strength for up to 30 cycles. The limitations of this method are that it is not readily available, requires an enclosed space, possibly producing off-gassing, and can result in degradation after 50 cycles. The last method Torres et al. discussed was Ultraviolet Germicidal Irradiation (UVGI). This method kills germs and viruses by shining a short-wavelength ultraviolet light (UV-C light) over the masks and has been advantageous because of its short treatment duration. The main restraint for this method is that it is not readily available, and is not very penetrative, which could decrease the strength of FFR straps. Another group of researchers, Kierat et al., attempted to solve the accessibility problem with UVGI by constructing a UVGI device with common materials that can be used in low-income areas [10]. Their findings show that these decontamination methods, especially UVGI as Torres et al discussed, can be amendable to any circumstance.

Cloth Mask Efficacy

As discussed before, the shortage of clean N95 respirators and the lack of readily available decontamination methods have led the CDC to advise the public against wearing N95 respirators and instead using cloth masks since they are "inexpensive, locally available, and reusable after washing." The CDC website says the most effective cloth masks are "tightly woven fabrics, such as cotton and cotton blends" and "two or three fabric layers" masks [19]. According to a 2010 study, researchers Rengasamy et al., study the filtration efficiency of cloth masks compared to N95 respirators. While N95 respirators usually only allow less than five percent of nano-sized particles to pass through, other cloth

masks and fabrics tested "had 40–90% instantaneous penetration levels" [15]. This means that cloth masks were much less effective at protecting the wearer from the harmful agents masks are meant to keep out; despite the paper's age this information is important to consider when evaluating decontamination methods.

Pore Size of Cloth Masks

In addition to poor filtration efficiency, washing, drying, and stretching cloth masks may increase the fabric's pore size. A study done by researchers Neupane et al. quantified these limitations by viewing the pore sizes on cloth masks following stretching, washing, and drying [13]. A common method of wearing a mask discussed in this paper was tying a loop on the mask straps to attain a better fit. Neupane et al. feel it can be "inferred that efficiency will decline if a mask with a knotted ear-loop is used due to changes in pore morphology" [13]. Stretching fabric over long periods of time will stretch its pores, allowing more or larger particles to pass in and out. Furthermore, while the CDC recommended washing cloth masks with laundry detergent "using a washing machine" and drying in a warm dryer, Neupane et al. found evidence negating this [9, 13]. Studying the filtration efficiency after washing and drying cycles, it was found that "after the 4th washing and drying cycle there was ~20% drop in filtering efficiency." While the public may not be aware of this statistic, it could pose be problematic. The CDC, a highly trusted organization may recommend a cleaning regimen that could degrade filtration of cloth masks after only four cycles of cleaning. While wearing a mask has proven to decrease the spread of the virus, wearing masks that have low filtration efficiency can allow larger particles to pass through, putting the wearer and people who surround them in danger of transmitting COVID-19. Another study by researchers Chughtai et al. also found that cloth masks were much less effective than hospital-grade devices and should be used with caution. Chughtai et al. stress that cloth masks should "not [be] reuse[d]…unless washed and cleaned" especially if wet or soiled [3]. In agreement with Neupane et al, these researchers felt that due to the existing limits of washing and drying cloth masks more options should be analyzed [13]. In the "Future Research Directions" portion of their study, Chughtai et al. state that "various [other] methods for decontaminating cloth masks should be tested" [3].

Bacteria on Cloth Masks

Prolonged reuse of respirators and masks can cause them to collect bacteria and viruses that may be dangerous if they enter the body. While the current reason for wearing masks is to protect from the COVID-19 virus, masks and respirators can also collect and stop bacteria such as *Escherichia coli* and *Staphylococcus epidermis*.

According to researchers at the American College of Healthcare Sciences, Gram-positive bacteria like *S. epidermidis* have thick cell walls made of a compound called peptidoglycan. Contrastingly, Gram-negative bacteria, like *E. coli*, have thinner peptidoglycan cell walls. As discussed by Torres et al., UVGI, a method that utilizes UV-C light, is able to kill bacteria by breaking down these cell walls. Decontamination methods can ensure that these bacterial strains do infect humans if used on respirators and masks.

Both *E. coli* and *S. epidermidis* are known to be found on the human body. *S. epidermidis*, according to researchers Cogen et al., is a "major inhabitant" on human skin and "mucosa"[4]. The mucosa is the mucus membrane of the body, which lines the inner parts of major organs. Since *S. epidermidis* is commonly found in and on the human body, there is a definite possibility this bacterium could contaminate a cloth mask. *E. coli* is also found on the human body. In a 2020 study evaluating *E. coli* 's role in the human gut, researchers Martinson et al. explain that this bacterium is a common "member of the healthy human gut" flora[12]. While these bacteria are found in the body, if ingested, different stains can cause ailments such as *E. coli* poisoning.

Due to the discoveries that current cleaning methods of cloth masks utilized by the general public could result in degradation of filtration efficiency, there is a gap in the body of knowledge on other methods of decontamination that can be harnessed by the public without degrading the pore size of cloth masks. Taking inspiration from the aforementioned methods of decontaminating N95 masks used by hospitals, it can be conjectured whether these methods

can be adapted for sterilizing cloth masks. As mentioned by Torres et al., UVGI technology can be applied despite having some limitations, such as cost and accessibility[16]. One can inquire if cloth masks contaminated with *E. coli* and *S. epidermidis* may be decontaminated using an affordable, commercial UVGI technology to preserve filtration efficiency. The public needs a method that will be cost-efficient and accessible like the usual washing and drying method but will concurrently not stretch or enlarge pores like the predecessor.

Methods

To test whether commercial UVGI technology is a plausible method of decontamination that limits degradation of pore size in cloth masks, an experimental procedure was developed that pulled from the ideas of multiple studies in the literature review. During testing, maintaining safety for myself and others in my vicinity was also a factor in design. The experimentation was conducted in February and March of 2021, during the COVID-19 pandemic, so access to the lab was limited. Because of this, some days during my testing I experienced delays in attending the lab as the lab did not let students enter the building. Additionally, as mentioned before, testing decontamination of the COVID-19 virus was not accessible to my experiment as it is a very infectious virus. The setting of my experiment also limited my use of bacteria to nonpathogenic strains.

Experimental Design

To collect the scientific data this study requires an experimental method of inquiry was approached. The research method that my study design was modeled after is one commonly used by researchers who aim to analyze all results that arise from experimentation: the quasi-experimental method. A 2010 study by researchers Diab-Elschahawi et al., whose study design greatly influenced my own, used a similar methodology when analyzing the efficacy of decontamination methods on cleaning cloths[5]. This method allows for the manipulation of independent variables so conditions and results can be analyzed. In the case of this experiment, the independent variable I manipulated was the cloth mask type and the bacteria contaminant. The dependent variables which I analyzed were colony growth with and without UVGI decontamination and changes in pore size. Quasi-experimental studies rely on not using randomized testing due to the ethicality of the experiment's goal. For example, I was not able to collect used cloth masks from participants to test the decontamination of any existing bacteria because that would add additional uncontrollable variables to the experiment. Instead, I contaminated masks by inoculating them with *E. coli* and *S. epidermidis* colonies. These bacterial species were chosen to replicate bacteria that would be found on a used-cloth mask while maintaining a controlled experiment and independent variables. I chose to test both bacteria's because they represented two of the main groups of bacteria and could commonly be found on respirators and masks. Understanding how UVGI impacts these two bacteria will also signify if UVGI responds different to different Gram types. Additionally, neither bacteria are pathogenic, so they were relatively safe to handle [8]. In addition, quasi-experimental experiments use both secondary and primary data to synthesize results and draw conclusions. Much of the information from previous scientific studies in the literature review will be synthesized with the findings of this study to find connections between UVGI technology, cloth masks, decontamination, and pore size.

Material Explication

A majority of the materials used in this experiment were provided by the lab I conducted the experiment in, but some required additional purchases. The materials required were as follows: 1-ply, 2-ply, and 3-ply cotton masks, commercial UVGI device, *E. coli,* and *S. epidermidis* colonies, Petri-dishes, multiple sterilized pipettes, micropipettes with sterilized tips, eight beakers, six glass beaker lids, graduated cylinder, shake table, nutrient agar, microscope, glass slides, gram staining dyes, Bunsen burner, Eppendorf tubes, two metal forceps, nutrient broth, foil and two metal

racks, sterilization mediums like bleach and ethanol, gloves, autoclave, and 37°C incubators. Table 1 shows additional information about the products that were purchased.

Table 1. Itemized list of materials that were purchased.

Product Name	Company	Quantity	Cost
3 Pack Face Covering, 3 Ply Black Face Coverings	NiUB5	3 pack	$4.99
2-Ply 100% Cotton Facemask	New Republic	3 pack	$11.99
Reusable Masks -100% Cotton - Hand Made -Single Layer	TUFF	2 pack	$9.99
Vemingo Portable UV Light Sanitizer Bag	Vemingo	1 device	$49.99

Cloth Mask Samples

The three mask types were purchased through Amazon.com but from third-party retailers. They were chosen to view how UVGI technology performed or impacted different plies. A study conducted by researchers Konda et al. analyzed the filtration efficiency of multiple commonly used mask fabrics[11]. Their findings support the CDC's recommendations for cloth masks which state that two and three plied fabrics are more effective to stop the spread of aerosols [16]. As a parallel to their study, which also viewed different plies, I chose to test 1-ply, 2-ply and 3-ply cotton masks to see if any changes to pore size were observed, and to account for the fact that most people wear different types of masks. Many options are available to the public ranging from blue surgical varieties to reusable cloth masks.

The 1-ply mask used in this experiment is a navy blue, 100% cotton material with three pleats, as shown in Figure 1. The mask is manufactured by company called 'TUFF' and produced in the USA. The 2-ply mask is made of two layers of 100% cotton fabric and shown in Figure 2. The masks are sold through Amazon.com by a seller called 'New Republic', they are produced in Los Angeles, California. The 3-ply mask, shown in Figure 3, consists of two layers of 100% cotton fabric; however, the middle layer is Ethylene-Propylene, which is another variety of fabric similar to cotton. This mask was also sold through Amazon.com by a seller company called 'NiUB5'. These masks were chosen as they are all made from relatively the same materials, the main difference between the three being the number of layers. Choosing these specific masks allowed for the testing of how pore size changes but for different plies.

Figure 1. 1-ply mask

Figure 2. 2-ply mask

Figure 3. 3-ply mask

Selection of UVGI device

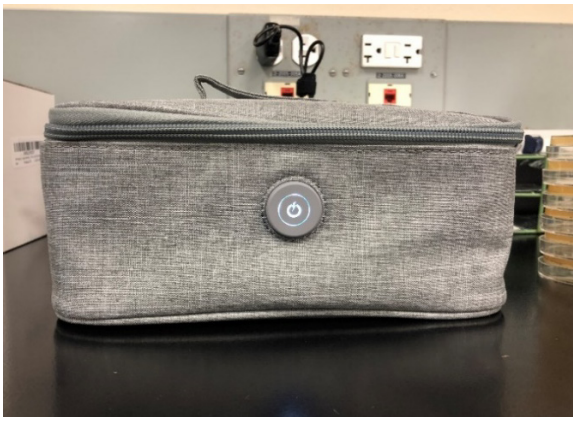

Figure 4. Front view of Vemingo device. **Figure 5.** Side view of Vemingo device. The right is the side view of the device when it is on. The small window shows when the device is in use, as the blue UV-C light can be seen on.

The premise of this study was to test the effectiveness of UVGI treatment on cloth masks from the perspective of the general public. Because of this, the tool chosen for UVGI decontamination must be accessible too most. UVGI devices used in hospital settings are very costly. For example, installation of UVGI towers in hospitals can cost more than "$20,000" USD, according to a study by researchers Gilbert et al [7]. A device that is used by the public should be efficient and yet economical. The device used for this experiment was purchased for $49.99. The name of the device is the Vemingo Portable UV Light Sanitizer Bag as seen in Figures 4 and 5. The device was purchased through Amazon.com through the seller company named 'Vemingo'. The box is marketed as being small and easily portable, having dimensions of 8.25 by 4.75 by 3.25 inches. Featuring an 'on' button that will only function if the device is plugged in and the magnet clasp is attached to the lid, the box's features protect the user from any exposure to the light. For example, the UV-C light will turn off if the lid is opened at an angle of more than 45°.

According to scientific literature review by researchers Torres et al., the peak emission of UV-C waves suited for bacterial killing is "265 nm" [16]. However, this device has a UV-C emission of 270-280 nm. While this emission is higher than the amount found to be most suitable, it is still considered a short-wave UV-C amount that is effective against viruses and bacteria by Fischer et al., who found that the effective emission was "260-285 nm" [6].

Experiment Set-up

Throughout the experiment in order to maintain control over all variables and warrant that no bacterial contamination will occur, proper aseptic technique was followed. Many common laboratory procedures like heat sterilization, the use of sterile equipment, autoclaving materials, and the use of bleach and ethanol allowed the experiment's tools and location to stay sterile while ensuring the safety of those near the site. Between uses of the UVGI device the machine was allowed one session where it ran empty to ensure no traces of bacteria from the samples were left. Repeating certain techniques also helped keep the experiment controlled; I made sure to use a

hinge-method of opening agar plates to prohibit vertical columns of air that may bring unwanted bacteria or air microflora on to the plate. Any plates, beakers, or graduated cylinders that held nutrient broth or bacteria were covered at all times with glass lids or Parafilm, a clear film that clings over labware.

Additionally, separate containers with ethanol were used to store and sterilize the tweezers used in this experiment. The tweezers were labelled according to the bacteria they were used to handle. Labeling and color coding proved to be very useful during the experiment. Tools that corresponded with *E. coli* trials were labeled red or simply with tape that read the scientific name of that bacteria. Similarly, *S. epidermidis* was labeled using purple. Another technique used to prevent contamination of the test materials was using constructed racks made from test tube holders and tin foil. As seen in Figure 6, the structures created shelfs where control materials and test materials could be separated. Two racks were made, one for *E. coli* control and trial samples, and another for *S. epidermidis* control and trial samples.

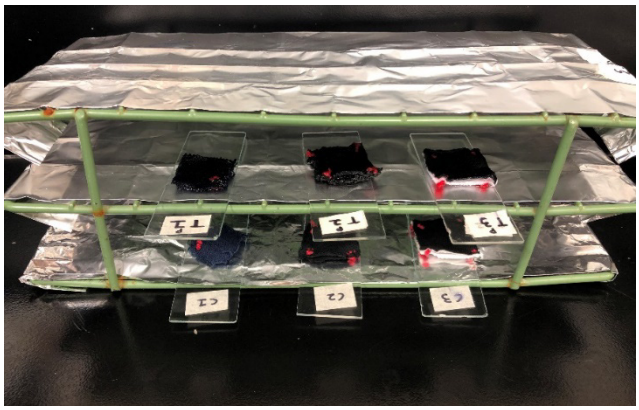

Figure 6. *E. coli* drying rack

For both ease of transportation and sake of separation materials, the cloth mask samples were stored on glass slides which were labelled to signify the differences between the control cloth samples and the trial samples. All samples were cut into 3 cm by 3 cm squares so they could fit on these slides. In order to keep all of the cloth layers of the samples together, following cutting them, embroidery thread was used to make knots on four corners. This was especially important for the 2-ply and 3-ply layers. The thread, as said before, was colored red and purple to signify which bacterium will be used on each cloth sample. Figure 7 shows all cloth samples used. The experiment required 12 different cloth mask samples: four 3-ply samples, four 2-ply samples, and four 1-ply samples. Within these four samples of each ply, one sample is a sample devoted to the experiment trials using *E. coli,* and one sample is the *E. coli* control sample. The other two are for *S. epidermidis* trials, and the other for control testing.

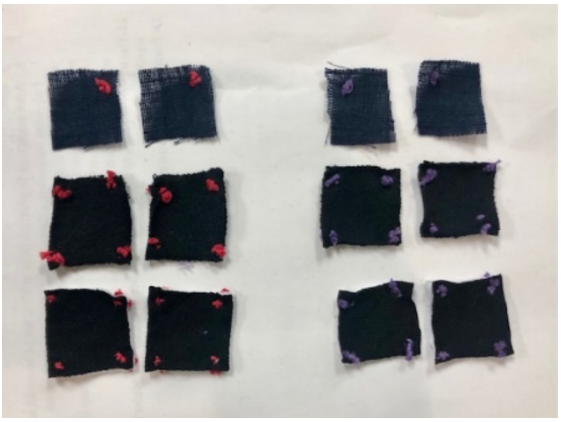

Figure 7. Cloth mask samples

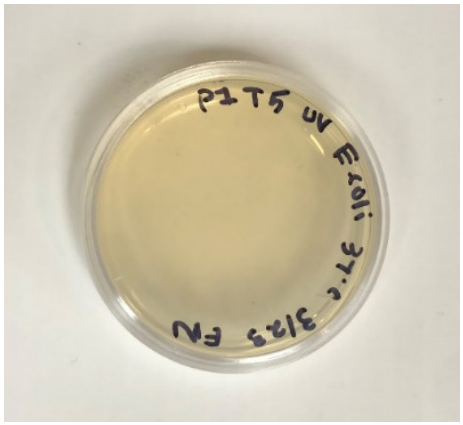

Figure 8. Example nutrient agar plate, labelled

Throughout the experiment, counting both control tests and trials, 60 nutrient agar plates were made. To differentiate between these plates labelling was very important. Each plate had a code that indicated what ply and trial it corresponded too. The coding told if it was a control or had been processed through the UVGI device, the bacteria species it tested, the temperature it must be incubated at, the date it was placed in the incubator, and my initials. As

seen in Figure 8, the plate reads "P1 T5 UV" at the top, meaning the plate will contain bacteria collected from the 1-ply sample in the fifth test trial. Since each trial also had a control test, the control plate that corresponded would have read "P1 T5 C." Control testing allowed for the comparison between the samples that had been processed through the UVGI device and those that had not.

Procedural Process

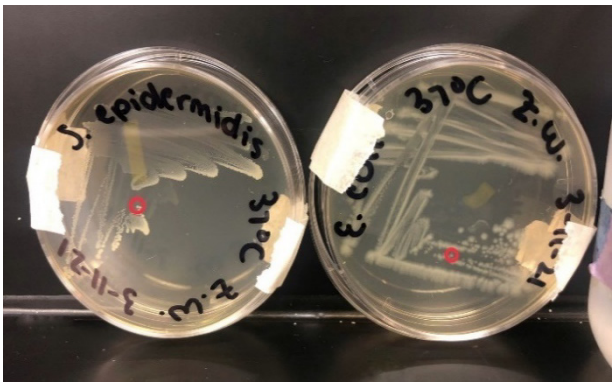

Figure 9. (Right) Quadrant-streaked plates As shown in the Figure above, the red circles show the size of one colony of bacteria. **Figure 10.** (Left) Covered Beakers on Orbital Shaker.

The first step of the experiment was control testing. To begin this process nutrient agar for the petri dishes was prepared. This agar allows bacteria to grow and form colonies for data collection. Before the experiment began colonies of *E. coli* and *S. epidermidis* were isolated and quadrant streaked. Using sterilized inoculating loops, small amounts of each bacterium were collected and streaked on two plates in four directions. Once these plates were incubated 37°C for 24 hours specific sections will be less or denser. In areas where bacteria colonies were less dense, singular colonies were able to be collected. Additionally, before any experimentation began, all cloth mask samples were dry autoclaved to ensure no outside bacterium contaminated results. Another procedural step done at the beginning and end of the tests was gram staining, or the process of viewing the morphology and classification of the bacterial species at hand. Gram staining the colonies collected from the trials and controls ensured that there was a transfer of the same bacteria. Gram testing also allowed for me to see if any contamination had occurred.

The procedure created allowed for multiple trials to be conducted at once, which allowed for the experimentation time to be reduced and kept the experiment controlled. Despite conducting trials at the same time, *E. coli* and *S. epidermidis* trials were done following each other to certify no contamination would occur. After using a Bunsen burner flame to sterilize an inoculating loop, a singular colony was chosen from the quadrant-streaked plate.

As shown in Figure 9, the quadrant-streaked plates show an area where most colonies are relatively the same size. The red circle shows an example of the size of colonies I chose to use during the experiment. Since *E. coli* and *S. epidermidis* are very different bacteria, the size of colonies cannot be comparable between the species; however, to remain controlled, while different species cannot be compared, colony sizes can be compared within the species. This allowed roughly the same number of bacteria to be used in each trial. Using a graduated cylinder, I measured out 10 mL of nutrient broth and poured it into a glass beaker, making sure to cover the beaker with a glass lid after. The chosen colony from the quadrant-streaked plate was then stirred thoroughly into the beaker with the inoculating loop. Then using sterilized tweezers, one cloth mask sample was inserted into the bacteria-broth solution and let to absorb the liquid contents. Once saturated, the samples were removed from the beakers and placed on labelled glass slides, which have been cleaned using ethanol. For control samples these labels read "C1", "C2", and "C3" —depending on

the ply of the control sample. These glass slides that held the samples were then placed on the dry storing rack shown in Figure 6. The samples were left to dry for 24 hours at room temperature.

Following 24 hours, the samples are then placed in clean beakers holding 10 mL of nutrient broth. I then placed these beakers with glass lids on an orbital shaker. This device, as shown in Figure 10 was used to shake the solutions to separate the bacteria that had been saturated from the cloth. A similar approach was taken by researchers Diab-Elschahawi et al. who utilized a shake table or orbital device to separate *E. coli* from microfiber cleaning cloth samples they had been testing [5]. The orbital shaker in my experiment was used at a setting of 3.5 speed and left to shake for five minutes. Once ready, the cloth samples were again removed from the beakers using sterilized tweezers and stored in the dry strong rack on glass slides. Using a micropipette, sterilized tips, and labelled Eppendorf tubes, a dilute was created for the solution left over in the beaker. To view the number of bacteria that were removed using the orbital shake, the bacterial-broth solution must be absorbed into an agar plate and incubated. To do this a dilution was created using 500 µl or 0.05 mL of the broth-bacteria solution and 0.45 mL of nutrient broth. This created a 9:1 ratio of broth to bacteria solution, diluting the solution allows for easier counting of colonies following incubation. The dilutions were made in 0.5 mL Eppendorf tubes, which are small, graduated snap tubes. The contents of the Eppendorf tubes were pipetted onto labelled nutrient agar plates and left agar side down so the solution could absorb into the agar. After the solution had absorbed, plates were incubated agar-side-up for 24 hours at 37°C. These steps were repeated five times for both bacteria and all three plies, in conjunction with trial testing that used the UVGI device. The control tests did not use the UVGI device so there was comparative data between the use of no sterilization tool and the use of one.

Figure 11. Cloth samples in device

Five trials were conducted because of the findings of researchers Neupane et al., who conducted an optical microscopic study of the changes in pore size and filtration efficiency of face masks [13]. Their study found that following "the 4th washing and drying cycle" the filtration efficiency of face masks "dropped by 20%" because on a change in pore size that the contributed to the stretching of the cloth masks. To access whether UVGI sterilization has any similar effects on pore size, this study will conduct five trials; one more than the number of trials Neupane et al. used.

Subsequent to the first control test was the trial testing. The procedure for the trial testing was very similar to the control testing except after cloth samples were placed into a beaker holding 10 mL broth and one species of bacteria and left to dry, they were put through one session of UVGI treatment. As instructed by the device's instructive manual, the session lasted three minutes. The samples, that were left on the glass slides were places on a metal rack on the inside of the device and place in such a way to reduce any overlap or shadowing. The positions of the cloth samples can be seen in Figure 11. Three samples were able to be sterilized at a time, only one species of bacteria was placed in the device at a time to reduce chances of combination.

After decontamination using UVGI, the samples were placed in clean beakers holding 10 mL more of nutrient broth, covered with glass lids and placed on the orbital shaker for five minutes at 3.5 speed. Following these steps, the steps that occurred on the control testing procedure were followed: samples placed to dry on rack on glass slides, the creation of a 9:1 broth to bacteria solution dilution and then absorption of solution onto nutrient agar plate. The plate was again incubated at 37°C for 24 hours. These steps were also repeated five times, for both bacteria and all three plies. After the plates had been incubated, they were removed from the incubator and the visible colonies were counted.

Before any testing occurred, the pore sizes of the mask samples were measured using a microscope at 100X magnification. Measuring the pore size before and after the experiment will provide the data needed to see if UVGI

has any effect on mask pore size changes. The scientific procedure that I followed is also shown in more detail in Appendix A.

Results

Following 24 hours of incubation, plates were examined, and colonies were counted. To evaluate the effectiveness of the UVGI device the number of colonies grown from the control test and the number of colonies grown from the trial tests must be found. Shown in Figure 12 is an example of a control plate and its corresponding trial plate, both with bacterial growth. The colonies on the trial plate are clearly denser, while the colonies on the control plate are very depressed and easily countable. To categorize the plates with large amounts of growth two methods are approached. If the plate is very full but singular colonies are still identifiable, a grid counting method can be used. When the plate is broken up into a grid, only the colonies in one square must be counted. This way the number of colonies in that square can then be multiplied by the number of squares to estimate the total amount of colonies found on the entire plate. The second method would be to categorize the plate as having lawn growth. Lawn growth can be viewed as a field of colonies where little agar is showing, and most of the surface area is covered. The colony numbers for *E. coli* and *S. epidermidis* before and after decontamination for each ply are shown on Tables 2, 3, and 4.

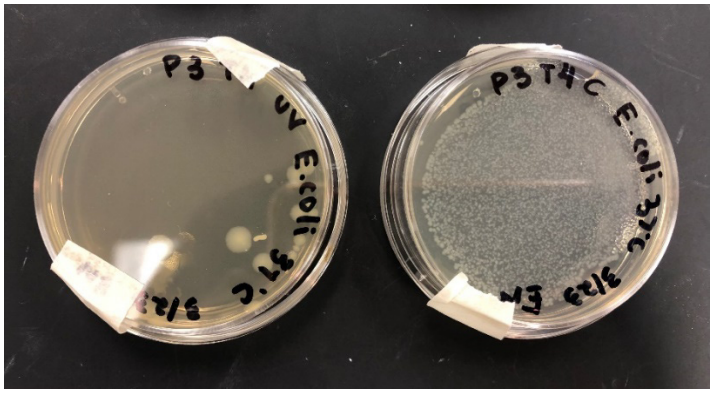

Figure 12. Trial and Control plates. The plate shown on the left contains bacteria collected from a cloth sample following UVGI. The plate on the right is a control test, and the growth can be categorized as 'lawn growth'.

Table 2. 1-ply Colony numbers before and after decontamination with UVGI for *E. coli* and *S. epidermidis*

E. coli	**Control Test Colony Count**	**Trial Tests Colony Count**
Trial 1	Lawn growth	~387
Trial 2	Lawn growth	~12
Trial 3	3 large colonies	~0
Trial 4	Lawn growth	~25
Trial 5	Lawn growth	~0

S. epidermidis	**Control Test Colony Count**	**Trial Tests Colony Count**
Trial 1	Lawn growth	~41
Trial 2	Lawn growth	~14

Trial 3	6 large colonies	~1
Trial 4	Lawn growth	~0
Trial 5	Lawn growth	~0

Table 3. 2-ply Colony numbers before and after decontamination with UVGI for *E. coli* and *S. epidermidis*

E. coli	**Control Test Colony Count**	**Trial Tests Colony Count**
Trial 1	Lawn growth	~347
Trial 2	Lawn growth	~21
Trial 3	15 colonies	~6
Trial 4	Lawn growth	~38
Trial 5	Lawn growth	~3

S. epidermidis	**Control Test Colony Count**	**Trial Tests Colony Count**
Trial 1	Lawn growth	~87
Trial 2	Lawn growth	~17
Trial 3	6 large colonies	~3
Trial 4	Lawn growth	~2
Trial 5	Lawn growth	~0

Table 4. 3-ply Colony numbers before and after decontamination with UVGI for *E. coli* and *S. epidermidis*

E. coli	**Control Test Colony Count**	**Trial Tests Colony Count**
Trial 1	Lawn growth	~1125
Trial 2	Lawn growth	~34
Trial 3	3 large colonies	~9
Trial 4	Lawn growth	~10
Trial 5	Lawn growth	~11

S. epidermidis	**Control Test Colony Count**	**Trial Tests Colony Count**

Trial 1	10530	~243
Trial 2	Lawn growth	~77
Trial 3	6 large colonies	~15
Trial 4	Lawn growth	~10
Trial 5	Lawn growth	~5

As discussed in the methods portion, Gram staining was done to ensure no contamination occurred and the same

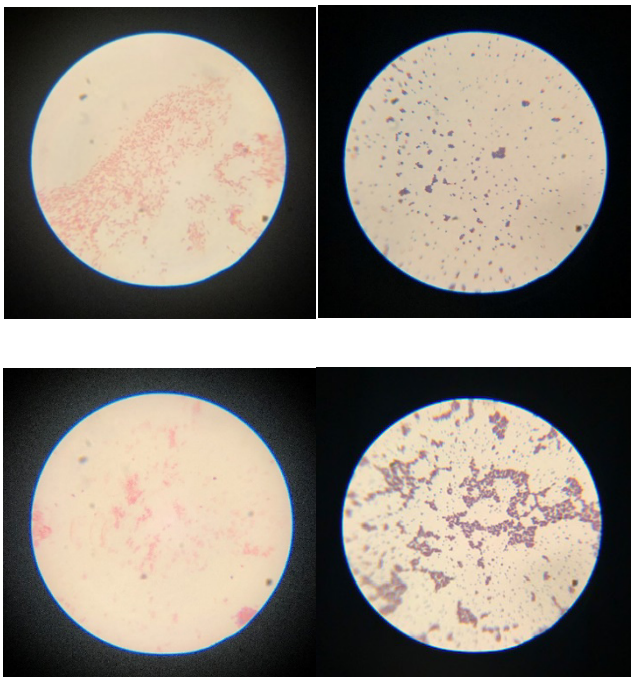

Figure 13. Gram Stains. The images above are gram stains from the experiment taken on 400X magnification. The top two (from left to right) are the control test gram stains for E. coli and S. epidermidis. The bottom two (from left to right) are the trial tests for E. coli and S. epidermidis.

Ratio used to find pore size from microscope images:

$$\frac{(\text{measured value in mm})}{47\ \text{mm}} = \frac{X\ \text{mm}}{2\ \text{mm}} \qquad X\ mm\ \times 1000 = \text{pore size in } \mu\text{m}$$

bacteria was being used at the beginning of the experiment and the end. Figure 13 shows the gram stains for the control test *E. coli* and *S. epidermidis*, and the trial test *E. coli* and *S. epidermidis*. Gram-negative bacteria stain red while Gram-positive bacteria stain purple. The images in Figure 13 show that throughout the experiment, for both the control and trial tests the type of bacteria remained the same.

Figure 14. Field of view conversion equation

In addition to the colony count, another factor of the experiment was the pore size. Before and after the experimentation the sizes of the cloth mask sample pores were analyzed. To view the pore size mask samples were observed at 100X magnification. Shown in Figures 15 and 16 are images of the mask samples under the microscope. The equation used to find the actual size of the pores took the microscope's field of view (FOV) and the FOV of the images taken into account. Since the diameter of the image taken was 47 mm and the diameter of the microscopes FOV was 2 mm, I formulated a ratio so I could measure the tangible image taken rather than measuring using very small nanometer increments on the microscope. The equation is shown in Figure 14. I modeled my method of examining pore size after a study conducted by researchers Neupane et al. who examined the morphology and filtering efficiency of face masks. They measured pore size by the "longest dimension of each pore." Using a ruler, I measured the longest dimension of each pore on the microscope images and converted these values into nanometers using the aforementioned ratio. The pore size values are shown in Table 5.

Table 5. 1-ply, 2-ply, and 3-ply mask pore size before and after decontamination with UVGI for *E. coli* and *S.epidermidis*

Mask Samples	Original Sample Pore Sizes	Test Organism	Control Sample Pore Sizes	Averages of Control Sample Pore Sizes	Trial Tests Pore Sizes	Averages of Trial Test Pore Sizes	Percent Change in Pore Size from UVGI
1-ply	680.9 µm	*E. coli sample*	617.0 µm	648.9 µm	851.1 µm	808.5 µm	41.58%
		S. epidermidis sample	680.8 µm		765.9 µm		
2-ply	382.9 µm	*E. coli sample*	425.5 µm	468.1 µm	489.4 µm	479 µm	25.75 %
		S. epidermidis sample	510.6 µm		468.6 µm		
3-ply	297.8 µm	*E. coli sample*	255.6 µm	276.9 µm	340 µm	319 µm	7.12%
		S. epidermidis sample	298.2 µm		298 µm		

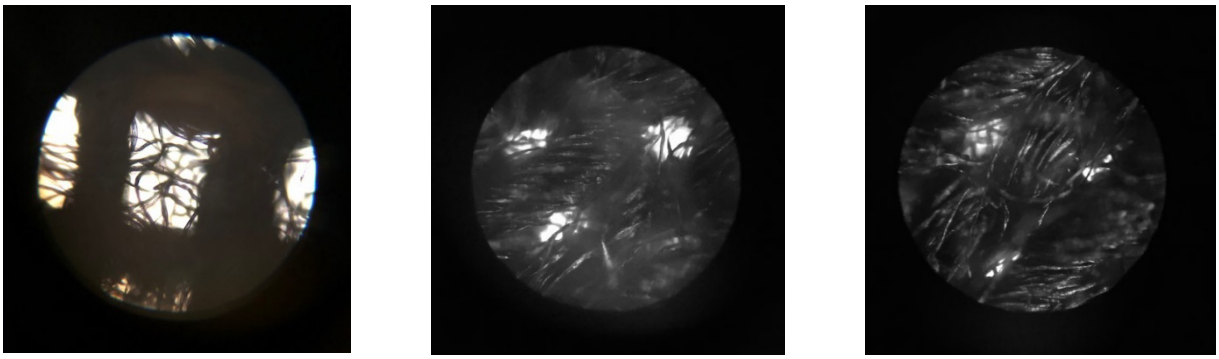

Figure 15. Cloth Mask samples before decontamination. The images shown above were taken using 100X magnification of a microscope. Pictured from left to right is the 1-ply sample, 2-ply sample and the 3-ply sample. These images were taken before any experimenatation and were used with the equation in Figure 14 to find the pore sizes.

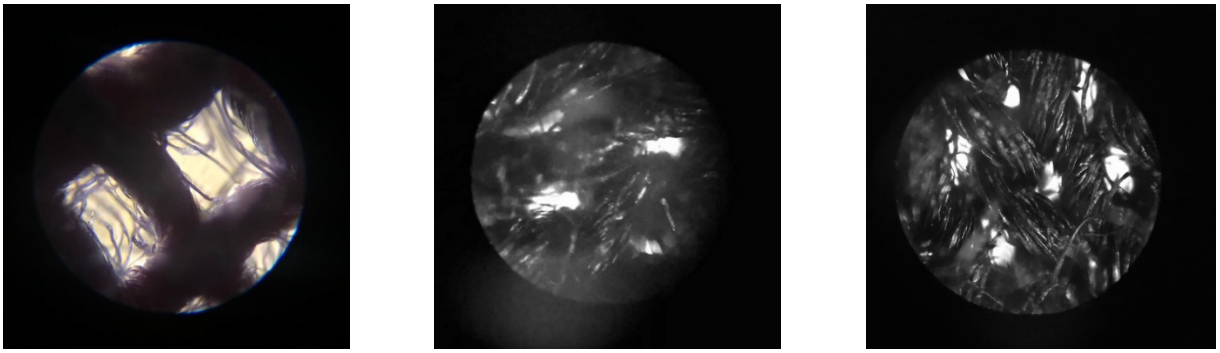

Figure 16. Cloth Mask samples after decontamination. The images shown above were taken using 100X magnification of a microscope. Pictured from left to right is the 1-ply sample, 2-ply sample and the 3-ply sample. These images were taken after the experimenatation and were used with the equation in Figure 14 to find the pore sizes.

Discussion

When looking at the data shown on Tables 1, 2 and 3, the number of colonies allow for insight on whether the UVGI device is a plausible solution to the degradation of filtration efficiency and pore size that washing cycles cause in cloth masks. In the 1-ply trials while most control tests began with lawn growth, the UVGI device reduced this growth from tens of thousands to 387±0 colonies in the *E. coli* tests. Similarly, the *S. epidermidis* tests showed a reduction of mostly lawn growth in the control tests to 41±0 colonies in the trial testing. The data from the second trials show a slightly different patter, while the control tests show mostly lawn growth again, the trial tests seemed to have larger colony numbers. Larger colony numbers in the trial testing correlates to poorer decontamination as more amounts of bacteria were left on the samples following the UVGI session. Similarly, to the 2-ply trials, the 3-ply trials had on average, larger trial test colony counts compared to the other two plies. Unlike the 1-ply and 2-ply trial test colony results which both had trials that resulted in zero colonies being grown following UVGI decontamination, the lowest number of colonies counted from both *E. coli* and the *S. epidermidis* tests was 9 and 10 colonies.

As shown on Figure 17 and 18, the graphs show the number of colonies comparing the trial test colony counts for all three plies. While a pattern is most visible in Figure 17, it is apparent between both bacterium types. Overall, the data consistently shows a connection between the ply number and number of bacteria that was left on the cloth samples following decontamination using the UVGI device. In Figure 17, while the 2-ply trials show some outlying results, such as the colony count for the fourth and second trial, the overall pattern showed consistently the 3-ply had more growth. Within this graph, the first trial results were not used because during experimentation, the before mentioned 9:1 dilution was not performed. Because of this the trial count result numbers were too high to analyze with the following trials. However, the first trial results did show a reduction in colony growth from before and after UVGI decontamination, so the results were still included within the data table.

In addition to the colony count following UVGI and plies, there is also a clear connection between the pore size and number of trials. Drawing connections with a study by Neupane et al. and this experiment's results, there is also a connection between filtration efficiency and pore sizes of the fabric. As shown on Table 4, which lists the percent changes and pore sizes of both the original samples and the cloth samples following testing, there is a correlation between number of plies and change in pore size. Interestingly, while 3-pyl sample's pore size only increased by 7.12% over the course of five trials, the 1-ply mask has an increase in pore size of 41.58%. Additionally, the 2-ply mask has an increase in pore size of 25.75%. These findings not only prove that in the long-run 3-ply masks may be more durable than 1-ply and 2-ply masks but also may be reinforced by Neupane et al.'s findings that uncovered the

connection between filtration efficiency and pore size. Figure 19 includes a graph showing the relationship between pore size and filtration efficiency can be used to estimate the filtration efficiency of the masks used in this experiment.

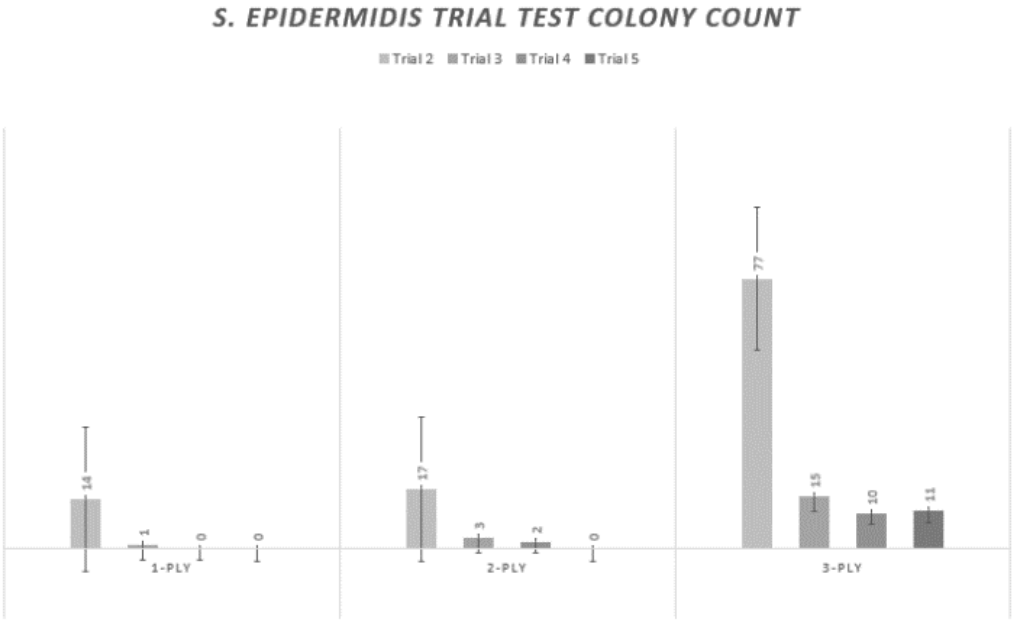

Figure 17. Bar graph showing colony count for *S. epidermidis* This graph shows the results of trials 2-5 for all three plies for *S. epidermidis.*

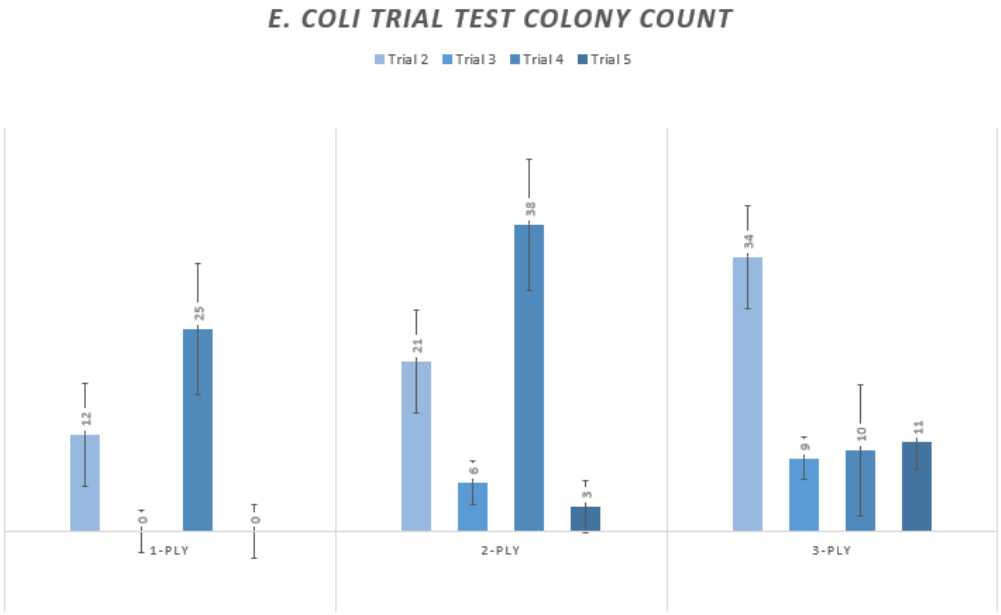

Figure 18. Bar graph showing colony count *E. coli.* This graph shows the results of trials 2-5 for all three plies for *E. coli.*

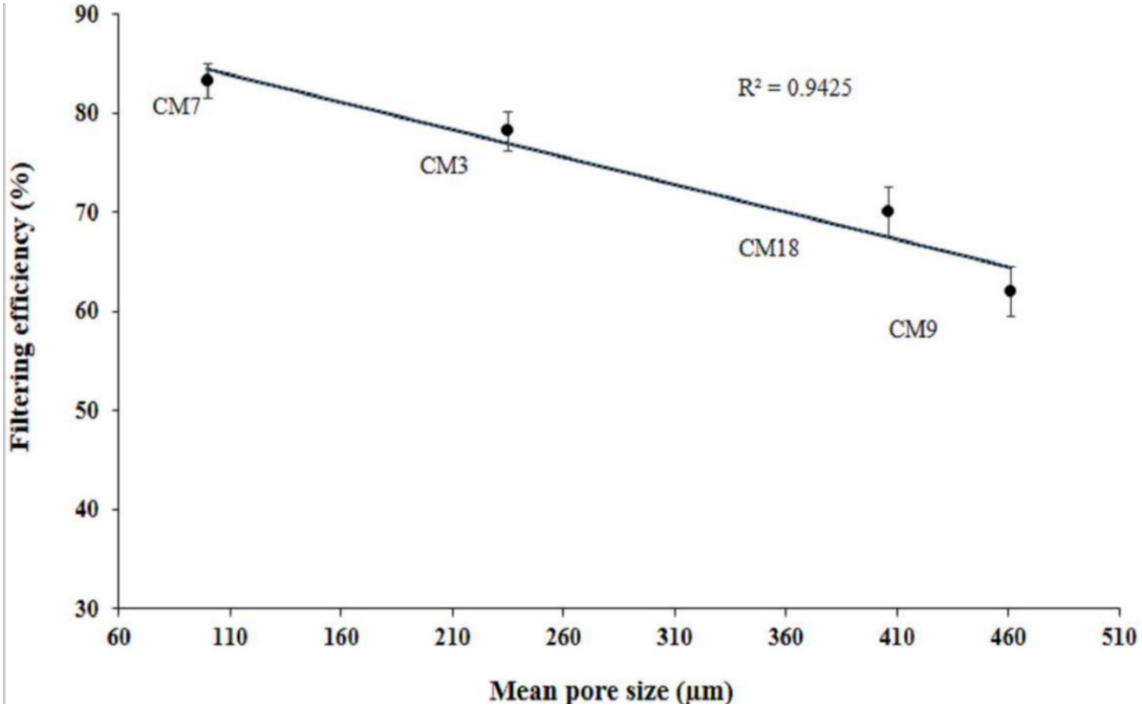

Figure 19. Graph from Neupane et al.[13.] This graph was taken from Neupane et al.'s study to show the relationship between filtration efficiency and mean pore size of the cloth masks. While this masks only shows the linear relationship for pore sizes 60-510 µm, the relationship still shows that the larger the pore size the less efficient it is at filtering particles.

Comparing the results of this experiment to the reuslts of Neupane et al.'s over the course of the trials, multiple repeated uses of the UVGI there was a very small reduction in filtration efficiency for the 3-ply mask material; however, the change in filtration efficiency for the 1 and 2-ply samples was much greater, and lower than the filtration efficiency of masks recommended by the CDC[17].

Conclusion

As initially hypothesized, the use of a commercial UVGI device did result in the reduction of bacteria. Through experimentation I found that, though some decrease in filtration efficiency did occur, there was less decrease in pore size for some plies compared to the decrease that would occur through washing and drying. Additionally, it was found that using a 3-ply mask is more durable against succumbing to increases in pore size.

If I were to conduct additional research or experiments to broaden the scope on this topic, I would have analyzed the emission amounts of UV-C light and how different dosages can be utilized by the general public. I would also like to compare UVGI technology to other accessible methods of mask sterilization discussed previously such as microwave-generated steam sterilization which uses a microwave and water to decontaminate masks. For future directions within this body of knowledge, I would advise other researchers to analyze how exactly the general public can begin using UVGI devices and create a methodology that most efficiently sterilizes masks while maintaining aspects of cost, time efficiency, and other factors of personal need.

This research contributes the body of knowledge of public health solutions and information pertaining to the global pandemic as it could potentially aid the public in choosing more effective ways of decontaminating their cloth masks. The implications of my findings connect to public health situations beyond the current pandemic. As time progresses cloth masks have greatly affected how we interact. The use of cloth masks can be applicable from times of national health emergency to times when we feel sick. Knowing how to care for our materials in ways that prevent the

degradation and extend the longevity of their use is important for these reasons. Showing the community, the unknown dangerous that arise from habits that are thought to be the norm will help make many more aware about their choices. Hopefully, being more aware can both slow the spread of viruses while incorporating efficient, accessible tools, like UVGI, into our routines.

Limitations

Throughout the course of the experiment there were a few limitations that may have impacted the results of the experiment. For example, the first trial conducted no dilution was created when the bacteria-broth solution was plated. This caused the growth of the bacterium to be too large to individually count. On Figures 17 and 18, the reason only trials 2 to 5 are shown because of this. While the pattern of less bacteria being decontaminated from the samples with more plies, the colony numbers were much large than the other trials. Because of this a dilution was conducted for the remaining four trials. Additionally, some trials had contamination where *S. epidermidis* colonies grew on an *E. coli* plate. However, since the premise of the experiment was to test the disinfecting abilities of UVGI, the number of colonies is more important that the species that had been gown. Both bacteria tested responded to decontamination the same, so contamination was disregarded and had little to no effect on the studies overarching results.

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