Facemasks and Respirators as a Non-Pharmaceutical Protective Measure in Preventing Viral Infection: *Quantification of Inhaled Virus Using a Ventilated Human Model*

Aerosol Research and Engineering Laboratories Inc. July 2020.

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Background: There has been an increase in interest in the use of facemasks and respirators as a protective measure against the transmission of SARS-CoV2. Our investigation aimed to characterize the effectiveness of various commonly used masks at preventing the inhalation of airborne (aerosol) viral particles.

Methods: This study evaluated and compared the effectiveness of various masks at reducing inhaled viral particulates. A ventilated human model was placed in a 1.0 m^3 Lexan dynamic bioaerosol chamber to assess the performance of various masks. A ventilated mannequin was placed in the chamber and attached to a piston ventilator to simulate breathing. A Collison six-jet nebulizer introduced viral bioaerosol into chamber. The bioaerosol concentration was dynamically controlled to maintain a steady-state environment while the human model simulated breathing. Midget impingers connected to a sample port on the mannequin throat sampled the inhaled air. Samples were serially diluted and plated in triplicate using a standard plaque assay technique. Data was collected and analyzed for reduction of inhaled virus. Schlieren imaging was also performed to characterize exhalation dynamics.

Results: Our results showed that the surgical mask, N95 respirator, and N100 respirator showed an average reduction of inhaled bioaerosol of 0.05 +/- 0.05, 0.22 +/- .09 and 0.74 +/- 0.16 log respectively. This corresponds to a percent reduction of 11.9% +/-10.2%, 39.2% +/- 19.5%, and 82.0% +/- 30.5% respectively.

Conclusions: Our results indicate *th*at surg*i*cal ma*s*ks do not *p*rotect we*a*rers from inhaling infectious airborne viral *p*artic*l*e*s*. The N95 and N100 masks showed some deg*r*ee of protection from resp*i*rable viru*s* i*n*halati*on*, though reduction wa*s* not *en*ough to prevent inhaling infectiou*s* dos*e*s of airborne viral pathogens. While it is known that the N95 and N100 mask material does provide effective filtration, when placed on a face the performance decreases significantly. The poor performance is most likely linked to the leak rate at the seal between the face and the mask. During testing, the masks were securely fixed to the smooth faced mannequin, however, Schlieren imaging revealed that it was not possible to eliminate all leak points. As the majority of pathogens may induce infection at extremely low concentrations, the reduction of infectious bioaerosols required to prevent infection is tremendously high. The importance of a proper seal then lies in ensuring the filtration of the mask is that of the material it is composed of, else the mask exhibit reduced reduction potential than that of the material. As such, this study emphasizes the importance of a proper fit test.

Introduction

In this study we tested three different types of masks: N100, N95, and surgical-grade. N95 disposable respirators are often recommended for healthcare and those populations with direct contact with individuals suspected or confirmed with COVID-19. N95 respirators are regulated by the Food and Drug Administration (FDA). These masks are intended to reduce the spread of the virus through exhaled droplets. The American Society for Testing and Materials (ASTM) standards are referenced by the Food and Drug Administration (FDA) as the endorsed standard in the US for medical face mask production.

A confirmation of asymptomatic transmission coupled with the lengthy gestation period of this virus outlines the primary public health imperative: Indiscriminate control of the virus's spread must be facilitated by reducing the probability of uptake and transmission from both symptomatic and symptomatic individuals, accounting for those asymptomatic individuals unknowingly acting as source points. Viral RNA shedding is higher at the time of symptom onset and declines after days or weeks.

Currently available evidence indicates that the human coronavirus's primary transmission mode is through respiratory droplets generated by breathing, sneezing, coughing, etc., as well as contact (direct contact with an infected subject or indirect contact, trough hand-mediated transfer of the virus from contaminated fomites to the mouth, nose, or eyes).[1] Studies on influenza suggest

Mask Study Bioaerosol Challenge Test Matrix

Mask Type	Manufacturer	Challenge Organism	Surrogate for	ATCC #	# of Trials	Sampling	Impinger Sample Times (min)	Total Trial Time (min)
N100	*3M*	*Escherichia virus MS2*	*nCov-SARS2(COVID19)*	*15597-B1*	4	Midget & AGI Impingers	With Mask: 5; No Mask: 5; Chest: 10	10
N95	*3M*	*Escherichia virus MS2*	*nCov-SARS2(COVID19)*	*15597-B1*	4	Midget & AGI Impingers	With Mask: 5; No Mask: 5; Chest: 10	10
Surgical	*CareMates by Sheperd Medical Products*	*Escherichia virus MS2*	*nCov-SARS2(COVID19)*	*15597-B1*	4	Midget & AGI Impingers	With Mask: 5; No Mask: 5; Chest: 10	10

Figure 1*:* Mask Study Test Matrix of the bioaerosol microbe used in our comparative mask investigation.

the concentration of viral particles within aerosolized droplets of 5 microns or less is much greater than that within large droplet sprays.[3] Furthermore, these sub 5 micron particles can remain aloft in the atmosphere for several hours and correspond to the respirable particle size range, as sub 10 micron particles can pass through the human respiratory system, and sub 5 micron particles can reach the alveoli.

Our investigation sought to compare three different types of commonly used personal protective masks: A surgical mask, N95 respirator, and N100 respirator. These masks were tested in their ability to filter bioaerosol to help assess their use as a preventative measure against airborne virus. **Figure 1** shows our experimental test matrix. **Figure 2** shows the mask types used in our investigation.

STUDY DESIGN

TEST CHAMBER

Our investigation used a 1.0 m^3 Lexan bioaerosol test chamber with glove box access. A flow diagram of the chamber set up is shown in **Figure 3**. The test chamber was built at ARE Labs. The human model was set up within the test chamber. The test chamber is designed to operate dynamically with continual aerosol introduction and evacuation for precise bioaerosol challenge control over time.

A Collison 6-jet nebulizer is used to generate the respirable bioaerosol and mixed with an additional 60 lpm of HEPA filtered dilution air during all exposures. Four (4) internal mixing fans within the chamber were used to ensure homogeneity of the bioaerosol with the in the chamber. The chamber is operated dynamically for all trials with continual introduction of bioaerosols and a high flow rotary-vane vacuum pump used maintain the chamber at -0.5 in H2O during the operation.

A valved Gast rotary-vane vacuum pump (Gast Manufacturing; Benton Harbor, MI) was as used for impinger sampling the inhalation stream from the mannequin and an additional impinger used to sample the bulk chamber environment.

Tested Masks	Surgical Mask	N95 Respirator	N100 Respirator
Picture:			
Mask Features			
Manufacturer:	Sheperd Medical Products	3M	3M
Face Seal Fit:	Loose Fitting	Tight Fitting	Tight Fitting
Features*:	ATSM Level 1 (Fluid Resistance), Soft 3-Layer non woven material	Filters out at least 95% of all airborne particles and non-oil aerosols	Filters out at least 99.7% of all airborne particles and non-oil aerosols
Testing & Approval:	Cleared by the FDA	Evaluated, tested, and approved by NIOSH as per the requirements in 42 CFR Part 84	Evaluated, tested, and approved by NIOSH as per the requirements in 42 CFR Part 84
* Partial listing of the Major Features.			

Figure 2: A comparison of the different masks investigated in this study.

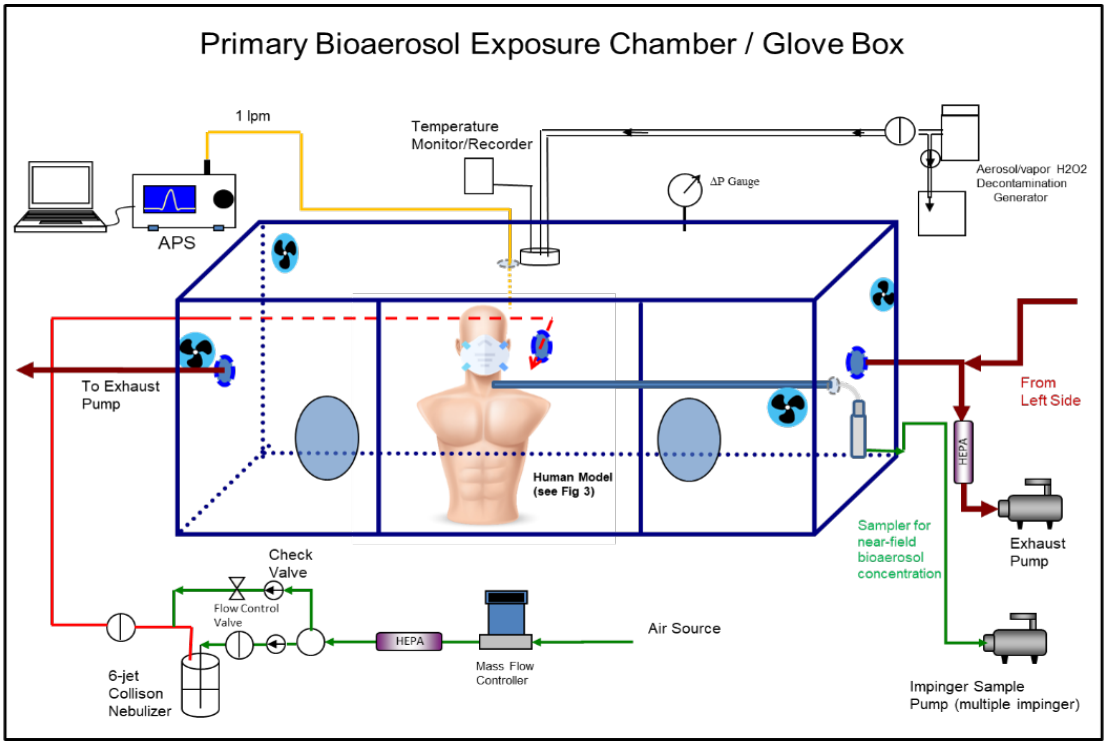


Figure 3: Test chamber flow diagram with breathing manifold, Lifecare® ventilator and impinger samplers for bioaerosol measurement.

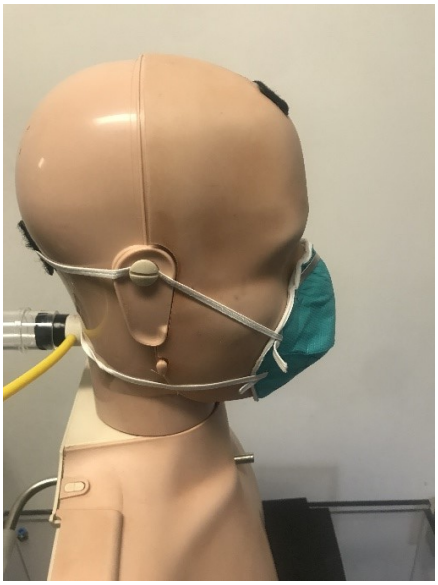


Figure 4: Photo of the ventilated mannequin fitted with the N95 respirator. On the left is the mannequin throat (white) and impinger sampler port (yellow).

The mannequin was fitted with the mask to be tested and sealed within the chamber. Bioaerosol challenge was started and the chamber was allowed to come to equilibrium for 10 minutes before the mask trial began. The ventilator and inhalational sampler was turned on and the sample was allowed to run for 5 minutes. The midget impinger was removed and a new impinger attached to the mannequin throat and the mask was removed from the face. A second five (5) sample was then taken to assess the inhalation viral dose for the mannequin without the mask. The chamber was evacuated, opened and both samples collected for quantification of inhalation viral bioaerosol. In additional a third impinger was used to quantify the bulk bioaerosol challenge level (pfu/L) with in the chamber

MANNEQUIN

A standard first aid training waist-high adult mannequin was used as the human model. A 5" x 3/4" inch PVC trachea was attached to the inside of the mannequin's mouth and sealed to fit the mouth opening. This allowed air flow to occur in and out of the mannequin's mouth.

FIT TEST & SEAL CHECK

A fit test scrutinizes the seal between the respirator's face piece and your face. When preparing to

wear an N95 or N100 respirator to protect from hazardous aerosol, rigorous fit testing is usually carried out in order to ensure that there are no leaks at the seal between the skin of the face and the margin of the respirator. OSHA has fit testing standards for respirators when someone is working in an environment with hazardous aerosol exposure such as in hospitals during an aerosol generating procedure. In our investigation, our fit testing was limited due to the use of a mannequin model. It involved careful analysis of the seal between the respirator and the surface of the mannequin. Given the mal-compliance or lack-there-of among the general populous in proper fitting of these types of respirators, we believe our fit test as rigorous as what would be seen in society, with the exception of careful OSHA regulated circumstances such as healthcare settings. A picture of the fit of the N95 mask is shown in **Figure 4.**

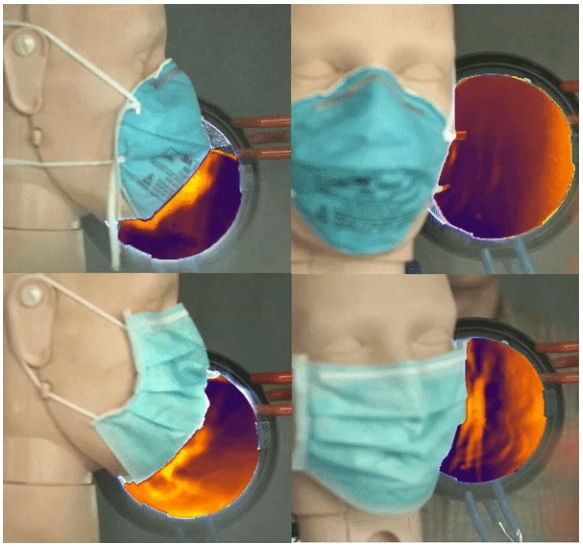


Figure 5: Schlieren imagery of the N95 respirator (top) and the surgical mask (bottom).

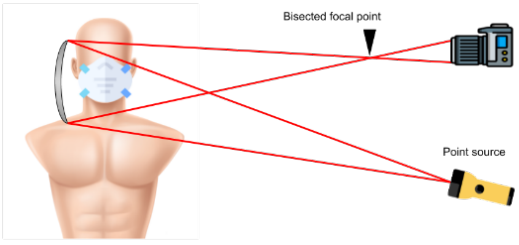


Figure 6: Diagram of the Schlieren imaging experimental setup

SCHLIEREN IMAGERY

Respirators and surgical facemasks display preferential airflow through the mask and side leakage points for each mask respectively. Schlieren imaging was performed to assess exhalation airflow associated with wearing masks, as well as to strengthen our fit testing method for our mannequin model. This method involved positioning the mask-wearing mannequin in a profile orientation in relation to a digital camera and point light source. A 114 mm parabolic mirror of a 1100 mm focal point was placed behind the mannequin's head to reflect light from the point source. A heat gun facilitated a temperature discrepancy between the exhaled air and the ambient atmosphere, thus creating a change in the refractive index. Light traveling through the exhaled air therefore alters its course and is blocked by a razor blade that bisects the image at the focal point. Blocking this light creates a contrast in the image corresponding to the density gradient. **Figure 5** displays Schlieren imagery of the N95 respirator and the surgical mask. Contrast color was added to facilitate visualization of the airflow. **Figure 6** shows a diagram of the Schlieren imaging experimental setup.

The N95 respirator showed preferential airflow through the mask. A limitation of our study in using a mannequin, included the inability to perform a proper fit test. However, careful attention was placed on achieving a proper respirator and mask fit throughout our investigation. Despite much time and effort placed into mask adjustment and with nearly all of the airflow occurring through the mask, a small amount of air leaked. This occurred along the respirator's seal at the position where the upper strap attaches to the respirator cup. This result emphasizes the difficulty in achieving 100% seal of masks and respirators, an achievement that is necessary in the context of airborne pathogen transmission.

BREATHING CIRCUIT

The breathing circuit used in our chamber testing consists of the custom trachea breathing and sampling manifold, respiratory particle filter, connecting tubing and a Lifecare® PLV-100 mechanical piston ventilator (Respironics, Inc. Murrysville, PA). The Lifecare® mechanical piston ventilator was used to control the respiration/exhalation frequency and tidal volumes of the model mannequin during each test. The breathing and aerosol sampling manifold, connected to the Lifecare® mechanical piston ventilator, is equipped with a circuit incorporating two check valves and an absolute filter to capture inhaled aerosols and prevent exhalation

of previously inhaled/captured aerosols. A schematic of the system is shown in **Figure 7.**

The inhalation/exhalation and APS sample manifold was connected to the ventilator Inhalation/exhalation ports and the APS particle size analyzer with flexible non-kink tubing for unimpeded movement of the mannequin during the bioaerosol testing. A picture of the mannequin with the custom treachea manifold incuding the: inhalation/exhalation ports, tubing, inhalation filter and APS sample port is shown in **Figure 8.**

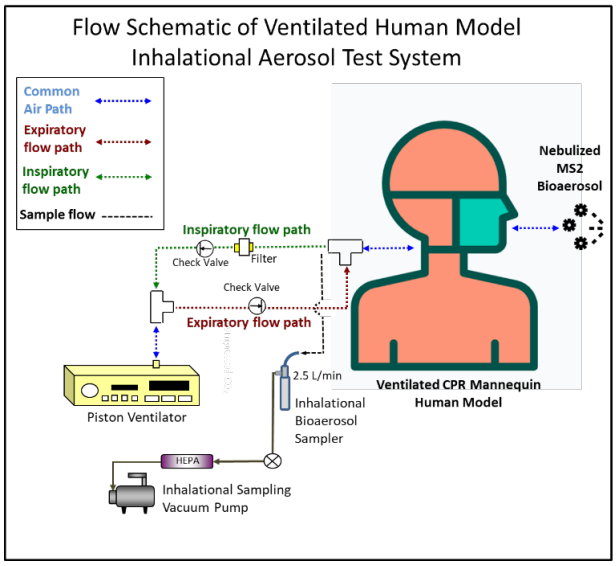


Figure 7: Flow schematic of breathing circuit.

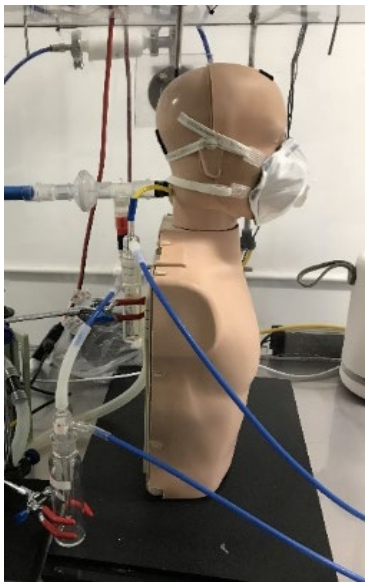


Figure 8: Ventilated mannequin equipped with a trachea and sampling system. The trachea manifold is connected to the Lifecare piston ventilator in exposure chamber.

RESPIRATORY PARAMETERS

For all mask trial sets, the Lifecare® PLV-100 mechanical piston ventilator was set to mimic the respiration frequency, tidal volume, and minute-volume of a typical adult during light activity. The ventilator test operation settings were controlled and set as follows: Tidal volume was set at 0.70 L/min. The breaths-per minute were set to 16 bpm. The Inspiration-to-Expiration (I:E) ratio was set to 1:2.5 with a peak inspiratory flow rate of 60 L/min.

VIRAL CULTURE & PREPARATION

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown in a similar fashion to the vegetative cells in an appropriate liquid media. The liquid media was infected during the logarithmic growth cycle with the MS2 bacteriophage. After an appropriate incubation time the cells were lysed and the cellular debris separated by centrifugation. MS2 stock yields were greater than 1 x 10^{11} plaque forming units per milliliter (pfu/ml) with a single amplification procedure. This stock MS2 viral solution was then diluted with PBS for use in the 6-jet Collison nebulizer.

BIOAEROSOL GENERATION SYSTEM

Test bioaerosols were disseminated using a Collison 6-jet nebulizer (BGI Inc. Waltham MA) driven by HEPA filtered house air supply. A pressure regulator allowed for control of disseminated particle size, use rate and sheer force generated within the Collison nebulizer.

Prior to testing, the Collison nebulizer flow rate and use rate were characterized using an air supply pressure of 35 psi. The Collison nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul MN). All trials operated the nebulizer @ 35 psi.

During testing, our impinger sample taken at the chest was used as a means to assess the average chamber concentration of the bioaerosol. We quantified this challenge concentration in pfu per liter. It was taken over the 10 minute trial time. It ran concurrently with the “mask” and “no mask” impinger samples, which were both taken in sequence at 5 minutes each.

Throughout testing, our average challenge concentration in our chamber were approximately 1 x 10^{6} pfu/L. This concentration was chosen to allow for a 5-6 log reduction while still being within the limits of detection of the setup.

IMPINGER SAMPLING

Immediately beyond the neck of the mannequin, a ¼ inch sample port was made on the PVC throat in order to collect inspiratory samples as air flows into the mouth of the mannequin with or without a mask on. Midget impingers were connected at this point, collecting air at a fixed flow rate of 2.5 L/min and taking inspiratory samples both while a mask or respirator was on and off. An additional midget impinger was connected to a port that sampled the air in front of the mannequin at the level of the sternum.

VIRAL SPECIES SELECTION

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for BSL3 pathogenic organisms. *MS2* is a viral RNA bacteriophage that is commonly used as a surrogate for the influenza virus, and is now being considered as a possible surrogate for other RNA viruses such as SARS-COV-2. This is due to SARS-COV-2s similar size to influenza and RNA genome. One major difference is the enveloping of SARS-COV-2 that influenza does not possess.

ADDITIONAL MATERIALS

An Aerodynamic Particle Sizer (APS, model 3321) was used at the beginning of testing to ensure the proper introduction of viral particles by the nebulizer and to characterize the bioaerosol's particle size distribution. A MERV-15 air filter was also placed into the chamber during testing to remove aerosol from the chamber after the completion of each trial. Lab stands were used to secure the impingers and tubing. Flow meters and valves were used to control the flow of air into and out of the chamber.

To prepare for plating of each sample, 1.5mL micro centrifuge tubes were used for serial dilution of the samples. For each dilution tube, 800uL of PBS and 100uL of an overnight E. coli stock were added. 100uL of the sample was then added to each dilution tube. Three dilutions per sample series each in triplicate were plated. A large drop plaque assay technique was used, utilizing a 500uL drop of the selected diluted sample on a tryptic soy agar plate, and incubated overnight prior to enumeration.

TESTING METHOD

For each trial, the Collison nebulizer was filled with approximately 50 mL of biological stock and operated at 35 psi for a period of 10 minutes. For all samples, the midget impinger was filled with 5 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection.

The chamber mixing fans were turned on during bioaerosol generation to ensure a homogeneous bioaerosol concentration in the test chamber prior to the first impinger sample. Mixing fans remained on for the full duration of each trial.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each trial sampling with a midget impinger attached to a port located at the mannequin's sternum. This sample collected for 10 minutes in each trial. Mask and no mask samples were collected for 5 minutes in each trial during the same exposure.

Aliquots of impinger samples were collected and then used for plating. Impingers were rinsed 6x with sterile filtered water between each sampling interval, and re-filled with PBS using sterile graduated pipettes for sample collection.

For each mask or respirator, the sampling began at a time 0 baseline and operated for 5 minutes. The inspiratory impinger sample was then replaced with a new impinger to sample for 5 additional minutes with the mask off. The two 5 minute sampling intervals were separated with a 2 minute pause to change impingers and remove the mask or respirator. This resulted in a total trial time of approximately 12 minutes.

Samples were plated and enumerated for viable concentration to measure the effective viable bioaerosol reduction between the sample with no mask and the sample using a mask. All samples were plated in triplicate on tryptic soy agar media over a minimum of a 2 log dilution range. Plates were incubated and enumerated for viable plaque forming unit (pfu) counts to calculate bioaerosol challenge concentrations in the chamber and reduction of viable microorganisms between the no mask (controls) and mask trials.

This testing method was designed to assess the viable bioaerosol reduction in the test chamber, it did not directly assess the kill or collection of the microorganisms due to the mask or respirator.

POST-TRIAL DECONTAMINATION

Following each test, the chamber was air flow evacuated/purged for a minimum of 10 minutes between tests and analyzed with the APS for particle

concentration decrease to baseline levels between each test. This prevented possible cross contamination between trials. This process was facilitated by a MERV-15 air filter. The chamber was decontaminated at the conclusion of the trials with a solution of 50/50 3% peroxide/Isopropanol. The Collison nebulizer and impingers were cleaned at the conclusion of each day of testing by soaking in a 5% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

SAMPLE PLATING

Once the trial was complete and the chamber was cleared of airborne particles the three (3) impinger samples were removed. A total of three samples were made per trial run. The trial samples were placed in sterile 50mL conical tubes and labeled as MASK, NO MASK, and CHEST.

SCHLIEREN IMAGING

Schlieren imaging was performed to observe the airflow preference for each mask during exhalation. This employed a point source light, 114 mm parabolic mirror with a focal length of 1125 mm, and a razor blade to bisect the image at the focal point. A heat gun was used to direct warm air through the throat of the mannequin and out the mouth. The difference in temperature between the ambient air and the warm air exhaled by the mannequin translated to a density gradient and thus a variable index of refraction. Such a difference resulted in a portion of the light rays traveling through the exhaled air to be blocked by the razor at the focal point, thereby contrasting the exhaled air with the ambient air and allowing qualitative analysis of airflow. Video recording of the airflow was conducted and frames were overlaid with color gradients to increase contrast.

ANALYSIS

PLAQUE COUNTS

After proper agar plate incubation, agar plated samples were analyzed for bacteriophage plaques. These plaques were enumerated, recorded, and readied for analysis. The Mask, No Mask, and Chest samples are plotted showing log reduction in viable bioaerosol.

Results from trials were graphed and plotted to show natural viability loss over time in the chamber. To analyze the reduction of bioaerosol attributed to the mask, the bioaerosol concentration (pfu/L) was calculated for both the Mask and No Mask impinger samples. Log and percent reduction values were achieved by dividing the bioaerosol concentration of the Mask samples by the values from the No Mask samples. These values were plotted showing log or percent reduction of viable bioaerosol attributed to wearing the mask. All data is normalized using the No Mask sample data and their enumerated concentrations. All trials show group average +/- standard deviations for net log reduction on a per trial basis.

Median (µm)	Mean (µm)	Geo. Mean (µm)	Mode (µm)	Geo. Std. Dev. (µm)	Total Conc. (#/cm³)
0.757	0.801	0.785	0.723	1.208	1355.850

Figure 9: Key Particle Size Distribution Values for MS2 Bioaerosol in Chamber

BIOAEROSOL PARTICLE SIZE DATA

The APS used in this study exhibits a measurement range of 0.5 to 20µm and was programmed to record particle sizes within the chamber at one minute intervals. Data was logged in real time to an Acer laptop computer, regressed, and plotted. The size distribution curve generated from this data can be observed in **Figure 10,** and **Figure 9** displays the statistical values of the plot.

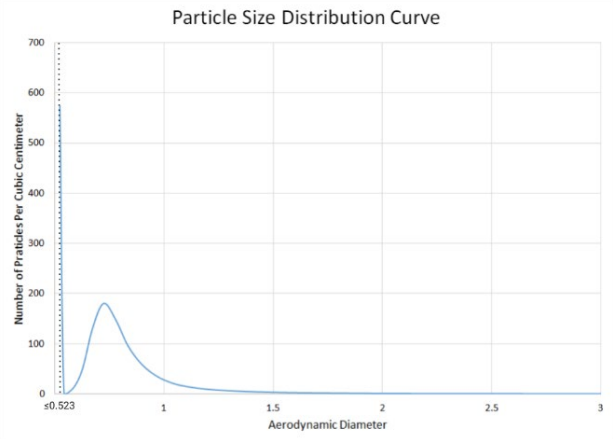


Figure 10: MS2 Particle Size Distribution in Test Chamber.

Mask Viral Bioaerosol Challenge Summary				Results							
	Mask Type	Virus Used	ATCC #	T1	T2	T3	T4	Average Net LOG Reduction	Stand. Dev	Average % Reduction	Stand. Dev
1	*3M N100*	*Escherichia virus MS2*	*15597-B1*	*0.53*	*0.81*	*0.75*	*0.90*	*0.74*	*0.16*	*82.0%*	*30.5%*
2	*3M N95*	*Escherichia virus MS2*	*15597-B1*	*0.28*	*0.31*	*0.12*	*0.15*	*0.22*	*0.09*	*39.2%*	*19.5%*
3	*Surgical Mask*	*Escherichia virus MS2*	*15597-B1*	*0.05*	*0.00*	*0.06*	*0.11*	*0.05*	*0.05*	*11.9%*	*10.2%*

Figure 11: Summary of Results showing each trial and the net log reduction.

The low geometric standard deviation suggests the generated bioaerosols were relatively monodispersed. The particle size distribution suggests the size of bioaerosols within the chamber was representative of highly respirable aerosol, median 0.75 µm, which display at least 30% penetration of the alveoli.[7]

RESULTS

When tested against the *MS2 bacteriophage,* our investigation found different levels of reduction in bioaerosol inhalation for each mask that was tested.

When compared to having no surgical mask, the average log reduction in of bioaerosol inhalation was 0.05 ± 0.05 with an average percent reduction of 11.9% ± 10.2%. The surgical mask showed no significant log reduction between trials with the mask and without the mask. This was the only mask to show no appreciable reduction in bioaerosol inhalation.

When compared to having no respirator fitted to the mannequin, the 3M N95 respirator showed an average log reduction of 0.22 ± 0.09 with an average percent reduction of 39.2% ± 19.5%.

When compared to having no respirator fitted to the mannequin, the 3M N100 respirator showed an average log reduction of 0.74 ± 0.16 with an average reduction of 82% ± 30.5%. The N100 respirator saw the largest reduction of inhaled bioaerosol. **Figure 11** shows a summary of our data results. **Figure 12** shows a graph comparing masks by net log reduction. See **Appendix A** for the data displayed as percent reduction.

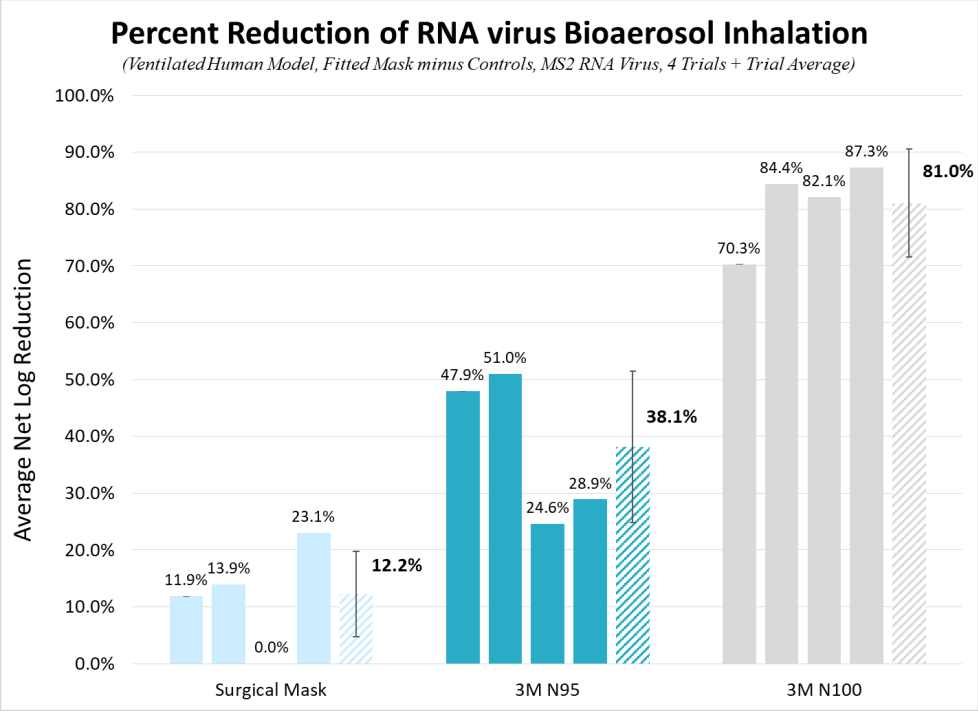


Figure 12: Net log Reduction of all 4 trials plus the trial average for each mask type tested

CONCLUSIONS/DISCUSSION

Protection against an airborne pathogen requires a clear understanding of how it is aerosolized and by whom it is emitted. Our study focused on the presence of bioaerosol within a controlled chamber surrounding a mannequin-breathing model. Our results bring into question the idea of using masks as a non-pharmaceutical public health measure in the prevention of transmitting airborne bioaerosol.

During the COVID19 pandemic, communities across the US have implemented mask mandates requiring citizens to wear face coverings when in public or outside of their home. Our results show that masks do not fully protect wearers from inhaling infectious airborne viral particles. Particularly, surgical masks provide negligible protection from bioaerosol inhalation, with a percent reduction of 11.9% ± 10.2% and an exhalation airflow preference for the lateral leakage points rather than through the mask material. It is important to note that the FDA states surgical masks are inadequate for the filtration of bioaerosols and are intended to fit loosely, rendering them ineffective at preventing bioaerosol inhalation. The N95 masks prove more effective, with 39.2% ± 19.5% reduction and an exhalation airflow preference through the mask material. The reduction of inhaled bioaerosols through the N95 mask, while superior to the surgical mask, does not meet the 4 log reduction recommended by the FDA for air purifying devices.[8] Likewise, the N100 mask displayed a 0.74 ± 0.16 log, or 82% ± 30.5%, reduction of inhaled bioaerosols, falling far below the recommended reduction value. More importantly, the N100 respirator cannot be expected to prevent bioaerosol spread during exhalation as the exhalation valve contributes no filtration value. This applies to all masks possessing exhalation valves.[6]

This study primarily addresses the use of masks for inhibiting uptake of respirable bioaerosols, which does not measure the efficacy of masks for source control. While the data from the chamber tests suggests face masks are ineffective for preventing uptake of infectious airborne particles, the Schlieren imaging provides only qualitative analysis of their efficacy in capturing exhaled bioaerosols. From such qualitative analysis, one can observe the critical impact fit has on mask effectiveness, as loose fitting masks direct the majority of their airflow through the large leakage points rather than through the filtration material. It cannot, however, provide an analysis of the quantitative relationship between unfiltered and filtered bioaerosol concentrations during exhalation through the masks. The mannequin was removed from the setup for mask fitting in an effort to reduce changes in position of the setup elements, though the small scale of the setup and its elements, namely the diameter of the parabolic mirror, posed limits on attenuation. The sensitivity of the system is largely dependent on the amount of during which the reflected light is allowed to diverge. While the angular displacement of light rays remains constant, an increase in the diameter and focal length of the mirror would allow an increase in linear displacement of the light rays upon reaching the focal point, thereby increasing the contrast between air densities in the recorded image.

The study also employed the growth of virus on *E. coli* coated agar plates for a determination of viral particle reduction. While PCR techniques provide a record of all viral RNA captured post inhalation, the plaque assay method is limited to quantification of viable viral particles, thereby underreporting the total unfiltered bioaerosol concentration due to die-off.

APPENDICIES

Appendix A: Percent Reduction of Bioaerosol Inhalation

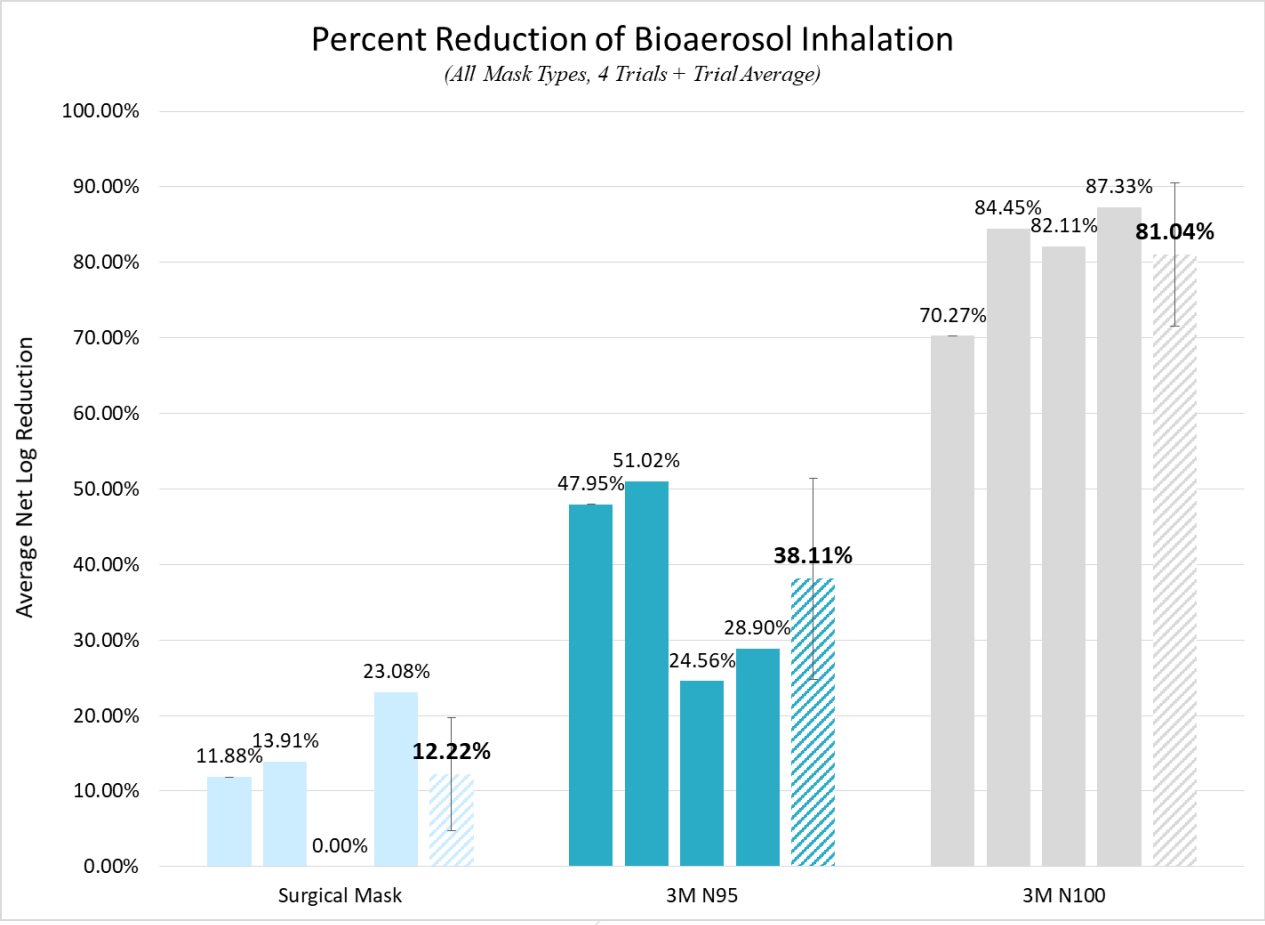
Appendix B: Net Log Reduction Charts of by Mask Type

REFERENCES

1. Centers for Disease Control and Prevention. (2020, June 6). How Coronavirus Spreads. Retrieved August 25, 2020, from https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fprepare%2Ftransmission.html

2. Bischoff WE, Swett K, Leng I, Peters TR. Exposure to influenza virus aerosols during routine patient care. J Infect Dis. 2013;207(7):1037-1046. doi:10.1093/infdis/jis773ISO 27427 (Nebulizing Systems and Components), 2009
3. Milton, D. K., Fabian, M. P., Cowling, B. J., Grantham, M. L., & Mcdevitt, J. J. (2013). Influenza Virus Aerosols in Human Exhaled Breath: Particle Size, Culturability, and Effect of Surgical Masks. *PLoS Pathogens, 9*(3). https://doi.org/10.1371/journal.ppat.1003205
4. Tellier R. (2006). Review of aerosol transmission of influenza A virus. Emerging infectious diseases, 12(11), 1657–1662. https://doi.org/10.3201/eid1211.060426
5. Nikitin N, Petrova E, Trifonova E, Karpova O (2014) Influenza Virus Aerosols In the Air and Their Infectiousness. Advances in Virology, Volume 2014, Article ID 859090. https://doi.org/10.1155/2014/859090
6. Center for Devices and Radiological Health. (2020, August 20). N95 Respirators, Surgical Masks, and Face Masks. Retrieved August 25, 2020, from https://www.fda.gov/medical-devices/personal-protective-equipment-infection-control/n95-respirators-surgical-masks-and-face-masks
7. Tellier, R. (2009). Aerosol transmission of influenza A virus: A review of new studies. *Journal of The Royal Society Interface, 6*(Suppl_6). https://doi.org/10.1098/rsif.2009.0302.focus
8. U.S. Food and Drug Administration. (2020). Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency. Guidance for Industry and Food and Drug Administration Staff. Retrieved August 25, 2020, from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/enforcement-policy-sterilizers-disinfectant-devices-and-air-purifiers-during-coronavirus-disease

Appendix A: *Percent Reduction of Bioaerosol Inhalation*


Percent Reduction of Bioaerosol Inhalation
(All Mask Types, 4 Trials + Trial Average)
Average Net Log Reduction
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
11.88%
13.91%
0.00%
23.08%
12.22%
47.95%
51.02%
24.56%
28.90%
38.11%
70.27%
84.45%
82.11%
87.33%
81.04%
Surgical Mask
3M N95
3M N100