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

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Background: Many methods have been proposed to evaluate the efficacy of current and new face mask design for efficacy in both protecting the wearer (primary function) and as a method to reduce viral shedding into community environments (secondary function). Many methods for testing respirators and masks have been proposed however current methods fail address the multitude of variables that are present in a real-world scenario. This newly proposed method attempt to address all of these deficiencies with a more robust testing methodology that closely represent real-world use.

Preliminary testing by ARE Lab's using these newly proposed methodologies has shown substantial deficiencies with the current methods when evaluating a respirators true protective efficacy against viral bioaerosols. Our preliminary investigation aimed to employ these new testing methods to evaluate the effectiveness of various commonly used masks at preventing the inhalation of airborne (aerosol) viral particles.

This proposed methodology differs from current methodologies in these crucial areas: 1). Use a ventilated mannequin and a human model to mimic real-world fit. 2) Breathing human model incorporating the sinusoidal respiratory effects on the fit and flow dynamics 3). Using surrogate virus for the challenge aerosol 4) Exposing the entire human model to a respirable viral bioaerosol of known concentration with known particle size. 5) Evaluating inhaled viral bioaerosol concentrations both with a respiratory in place (test article) and without a respirator (positive control) at specified breathing respiration rate. 6). the ability to quantify total inhaled viral dose for each unique set of conditions.

Method Overview: A ventilated human model was placed in a 1.0 m^3 Lexan dynamic bioaerosol chamber to assess the performance of various masks. The human model is attached to a piston ventilator to a user defined respiratory condition. A respirator was fit to the human model and a fit check performed. A Collison six-jet nebulizer introduced respirable viral bioaerosol (media 0.7um with a GSD of 1.7um (range <100nm-2.4um) into chamber. The bioaerosol concentration was dynamically controlled with continuous nebulization and chamber evacuation to maintain a steady-state viral bioaerosol concentration in the testing environment for the duration of the trial. Midget impingers connected to a sample port on the mannequin throat sampled the inhaled air by the human model. Trial times were 5 min with the respirator in place. The Impinge sample was collected and replaced with fresh sterile impinger then the mask removed (via glove ports access) and the positive control was taken. During this paired trial (test article + positive control) a steady state viral bioaerosol was continuously maintained for the entirety of the exposure. The chamber is rapidly evacuated and the impinger samples recovered. Samples were serially diluted and plated in triplicate using a standard plaque assay technique. Data was collected and analyzed for reduction of inhaled virus. In between sample sets aerosolized hydrogen peroxide is used to decontaminate all surfaces within the chamber. Schlieren imaging was performed prior to bioaerosol testing to check the fit of the mask and show any potential leak points.

Preliminary Method Results: Our results showed that the reported filtration efficiency of standard N95 and N100 mask differ considerably when compared to inhaled viral dose on a breathing human model. For this preliminary study surgical mask, N95 respirator, and N100 respirator showed an average log reduction of inhaled bioaerosol of 0.05 +/- 0.05, 0.22 +/- .09 and 0.74 +/- 0.16 log respectively when compared to the positive controls. This corresponds to a percent net reduction of 11.9% +/-10.2%, 39.2% +/- 19.5%, and 82.0% +/- 30.5% respectively for each mask type.

Conclusions: This newly proposed test method, which was designed to mimic real-world situational use, shows quantifiable and repeatable results are obtained for total inhaled virus and that these data suggest that current respirators, when used in a real-world scenario, may provide little protection from viral bioaerosols when considered from an infectious disease prevention standpoint.

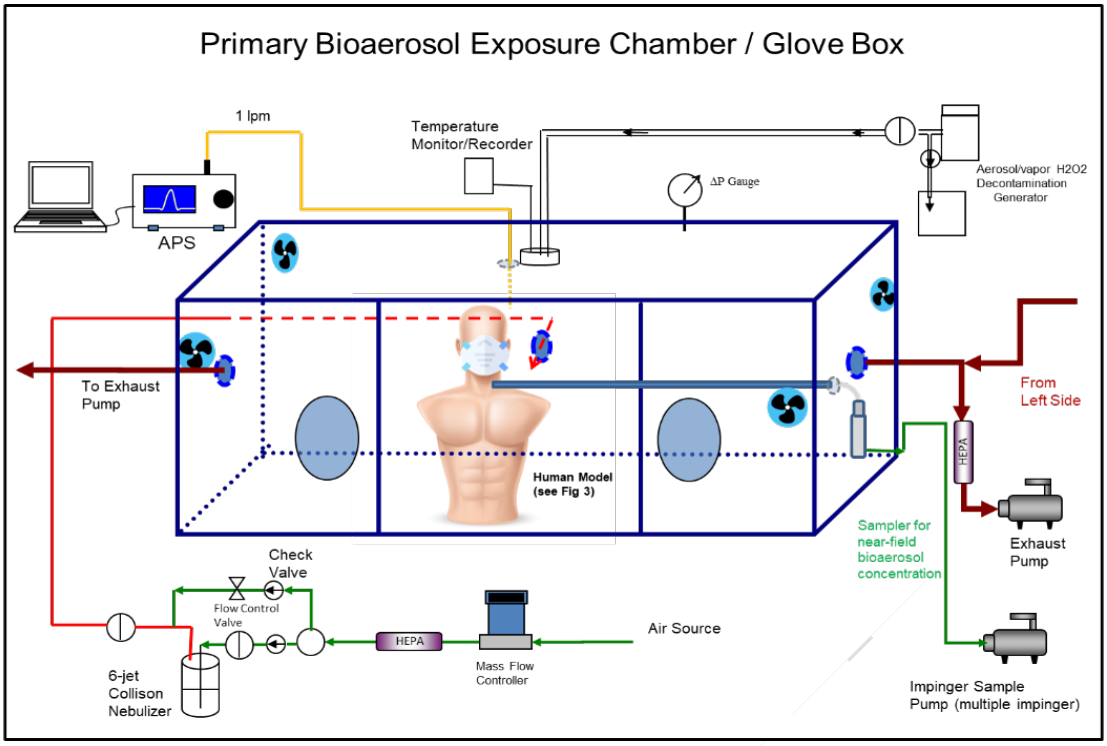


Figure 1: Test chamber flow diagram with breathing manifold, ventilator and impinger samplers for bioaerosol measurement.

TEST METHOD 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 1**. 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.

HUMAN MODEL

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. **Figure 2,** shows the ventilated mannequin with N95 mask fitted.

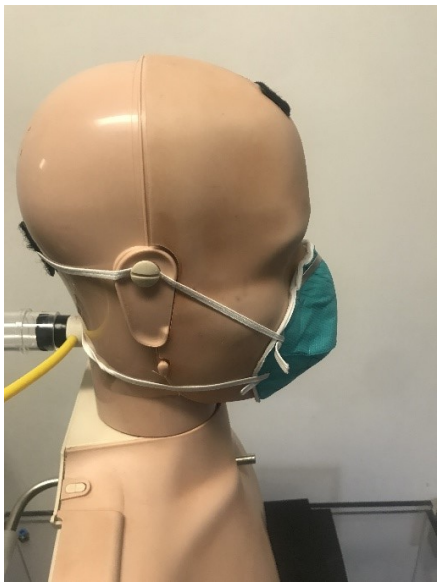


Figure 2: Photo of the ventilated mannequin fitted with the N95 respirator. On the left is the mannequin throat (white) and impinger sampler port (yellow).

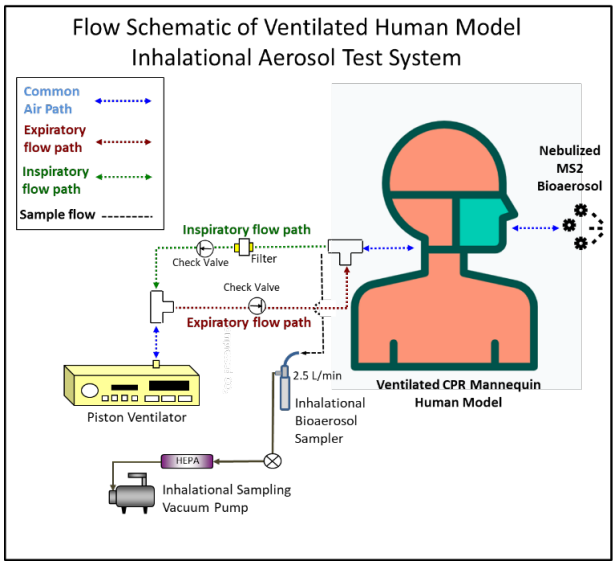


Figure 3: Flow schematic of breathing circuit.

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 viral aerosols. A schematic of the system is shown in **Figure 3.**

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 4.**

RESPIRATORY PARAMETERS

For all mask trial 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.

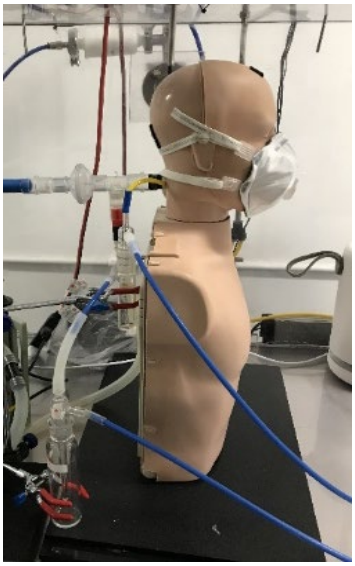


Figure 4: Ventilated mannequin in chamber fitted with a 3M N100 respirator.

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.

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

A ¼ inch sample port located in the mannequin throat is used 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 1.0 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.

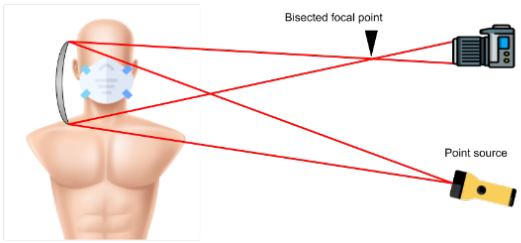


Figure 5: Diagram of the Schlieren imaging experimental setup

FIT TEST & SEAL CHECK WITH SCHLIEREN IMAGERY

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. Our fit test 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 general society.

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. **Figure 5** shows a diagram of the Schlieren imaging experimental setup. **Figure 9** displays as an example Schlieren imagery of the N95 respirator and the surgical mask. Contrast color was added to facilitate visualization of the airflow.

DETAILED 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, midget impingers 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 testing. The chamber was allowed to come to steady-state (determined to be 10 minutes) prior mask testing.

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 removed. 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.

The chamber was evacuated and then impinger samples collected. 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 3 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.

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.

PRELIMINARY TEST METHOD RESULTS

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

Introduction

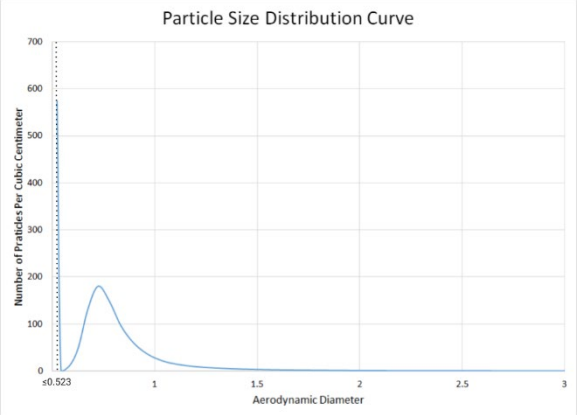
In this study we tested three different types of masks: N100, N95, and surgical-grade. N95 disposable respirators are often recommended for healthcare and those populations with direct contact with individuals suspected or confirmed with COVID-19. **Figure 6**, shows the test masks and **Figure 8**, on the following page, shows the matrix for this preliminary study. 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.

PRELIMINARY TESTING: 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.

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. **Figure 7**, shows the particle size distribution within the chamber for aerosolized MS2 virus (in PBS stock).



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 7: MS2 Particle Size Distribution Values for MS2 Bioaerosol in Chamber

SCHLIEREN IMAGING / FIT EVALUATION

Schlieren imaging was performed to observe the airflow preference for each mask during exhalation. 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. Video recording of the airflow was conducted

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

and frames were overlaid with color gradients to increase contrast. **Figure 9** below some still shots from the imaging technique.

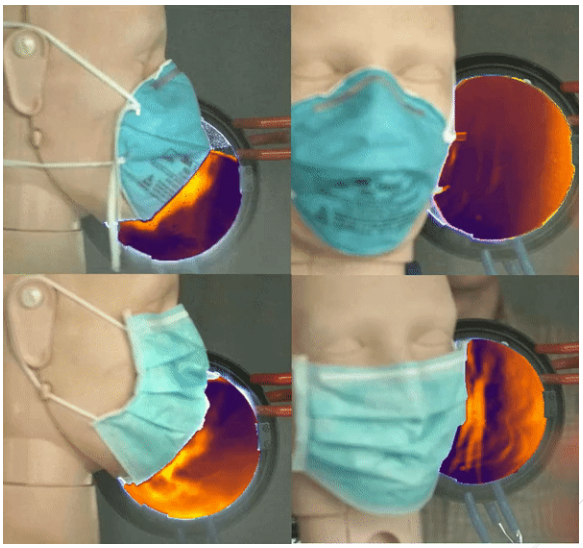


Figure 9: Schlieren imagery of the N95 respirator (top) and the surgical mask (bottom).

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 12** shows a graph of each individual trial results and trial set average +/- standard deviations.

CONCLUSIONS/DISCUSSION

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. 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

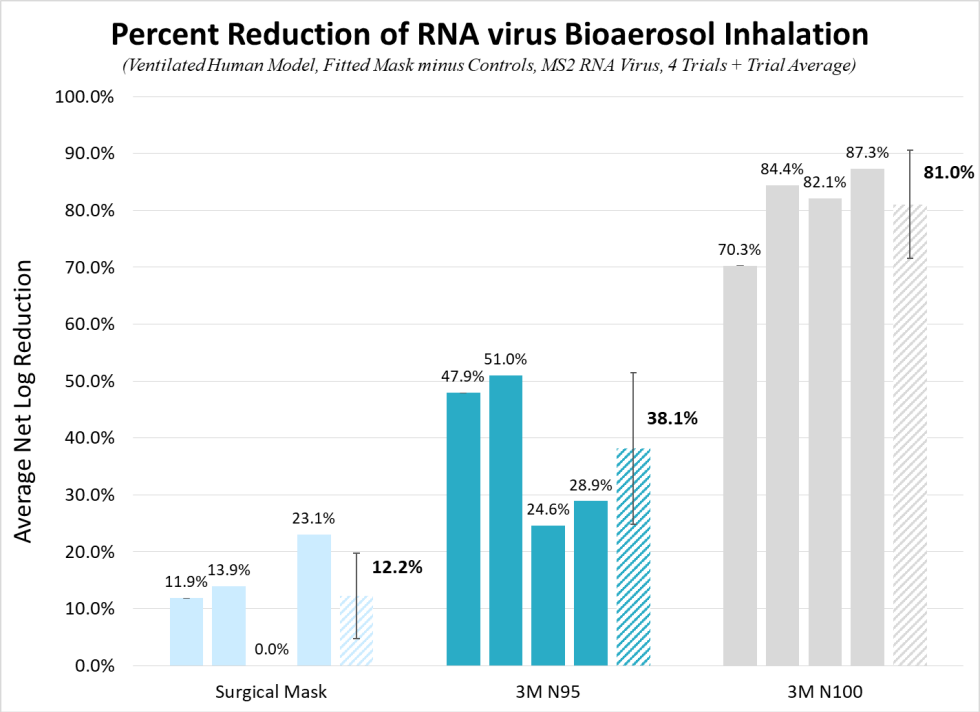


Figure 12: Net log Reduction of all 4 trials plus the trial average for each mask type tested

inhaled viral bioaerosols, falling far below the recommended reduction value. This newly proposed test method, which was designed to mimic real-world situational use, shows quantifiable and repeatable results are obtained for total inhaled virus and that these data suggest that current respirators, when used in a real-world scenario, may provide little protection from viral bioaerosols when considered from an infectious disease prevention standpoint.

This newly proposed test method, which was designed to mimic real-world situational use, does show that quantifiable and repeatable results are obtained for total inhaled virus. Therefore this method would be a more robust and realistic assessment to quantify the protective effect of various respirators. More over this method could be adapted slightly to quantify the viral shedding reduction of personal protective respirators.

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