Assessment of mask efficiency for preventing transmission of airborne illness through aerosols and water vapor [version 1; peer review: 1 not approved]

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Abstract

Background: Currently the Center for Disease Control has advised the use of face coverings to prevent transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to those who are unvaccinated. This study seeks to evaluate if cloth masks have increased efficiency with the addition of a filter material.

Methods: An adult airway and test lung model were exposed to nebulized 'coarse' aerosol droplets (0.5-11 µm) and humidified 'fine' water vapor particles (0.03-0.05 µm). Aerosol was quantified based on particles deposited on the face, airway and lung model. Tracheal humidity levels characterized fine particle permeability. Both phases of testing were conducted by evaluating the following testing conditions: 1) no mask; 2) cloth mask; 3) cloth mask with Swiffer™ filter; 4) cloth mask with Minimum Efficiency Reporting Value (MERV) 15 filter; 4) cloth mask with PM2.5 filter 5) surgical mask and 6) N95 respirator.

Results: All mask conditions provided greater filtration from coarse particles when compared to no mask (P<0.05). All cloth mask with filter combinations were better at stopping fine particles in comparison to no mask. A cloth mask without a filter and surgical mask performed similarly to no mask with fine particles (P<0.05). The cloth mask with MERV 15 filter and the surgical mask performed similarly to the N95 with coarse particles, while the cloth mask with Swiffer™ performed similarly to the N95 with the fine particles (P<0.05).

Conclusions: Respiratory viruses including SARS-CoV-2 and influenza are spread through exposure to respiratory secretions that are...
aerosolized by infected individuals. The findings from this study suggest that a mask can filter these potentially infectious airborne particles.

**Keywords**
COVID 19, SARS-CoV-2, PPE, Cloth mask, N95, Surgical mask

This article is included in the **Coronavirus (COVID-19)** collection.
Introduction

The coronavirus disease 2019 (COVID-19) pandemic’s first cases were reported in China in January of 2020 (World Health Organization, 2021). As of June 15, 2021, the World Health Organization has reported a total of 175,987,176 confirmed cases globally and 3,811,564 deaths. Many communities across the globe have shut down schools, businesses and travel in attempts to decrease the number of new infections. The virus can cause a range of symptoms including cough, shortness of breath or difficulty breathing, fever, chills, repeated shaking with chills, muscle pain, headache, sore throat, new loss of taste or smell and severe acute respiratory failure (Centers for Disease Control and Prevention, 2020). In addition, there is a portion of the population who are asymptomatic with the infection or infectious prior to any symptoms (Li et al., 2020; McMichael et al., 2020; Pan et al., 2020; Rothe et al., 2020). The ability for this novel strand of the virus to spread through respiratory secretions and other airborne media, even when carriers are asymptomatic or have mild symptoms, has made it difficult to contain.

There are multiple components involved in transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for COVID-19. This inhaled pathogen is transmitted from person to person by way of expelled airborne particulate or ‘aerosols’ excreted from respiratory secretions through coughing, sneezing, singing etc. It may also be transmitted through contact with respiratory secretions on surfaces, although it is estimated that droplet and inhalation transmission routes outweigh contact transmission. (Asadi et al., 2020; Jones, 2020) Aerosols from infected persons pose an inhalation threat even at considerable distances and in enclosed spaces, particularly if the area is poorly ventilated. Expelled aerosol may carry further than the recommended six feet of distancing with a strong cough or sneeze (Jones & Brosseau, 2015; Verma et al., 2020).

The size of the SARS-CoV-2 virus is documented with a diameter ranging from 0.06-1.4 µm with spikes extending 0.009-0.012 µm (Zhu et al., 2020). Respiratory droplets can be of various sizes and are commonly classified as aerosols (<5 µm) and droplets (greater than 5 µm) (Konda et al., 2020). A systematic review of 26 studies reporting particle sizes generated from breathing, coughing, sneezing and talking, demonstrated that healthy individuals generate particles between 0.01 and 500 µm and individuals with infections produce particles between 0.05 and 500 µm (Gralton et al., 2011). There is also evidence that sneezing can expel sheet-like layers of respiratory fluids which break apart into various sized particles and liquid which falls quickly (Scharfman et al., 2016). This indicates that expelled particles carrying pathogens, such as SARS-CoV-2 likely do not exclusively disperse by airborne or droplet transmission but by both methods simultaneously (Gralton et al., 2011).

Apart from COVID-19, respiratory droplets are the primary means of transmission for other illnesses including the common cold, influenza, tuberculosis, SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) (Verma et al., 2020). Inhaled droplets and aerosol particles have different sites of deposition in the recipient. Large inhaled droplets are deposited on the face or in the upper regions of the respiratory tract. In contrast, inhaled droplets and viral particles <5.4 µm can penetrate to the depths of the lungs, where they may then be deposited in the bronchial tubes and alveoli. Due to the airborne nature by which SARS-CoV-2 virus is transmitted, it is highly contagious and has potential for rapid spread, morbidity, and mortality.

Previous studies have demonstrated some benefits of homemade masks, however their filtration was not equivalent to surgical masks or other commercially available respirators (Asadi et al., 2020; Davies et al., 2013; Konda et al., 2020; van der Sande et al., 2008; Verma et al., 2020). Therefore, we sought to explore if adding a filter to a cloth mask could improve the performance. This in vitro study was designed to evaluate mask efficiency and prevention of potentially infectious airborne particles between no mask, cloth mask (with/without filter), surgical mask, and N95 respirators to prevent inhaled airborne exposure of ‘coarse’ aerosols and ‘fine’ water particles. The study sought to test the hypotheses that there is no difference in the filtration of aerosol particles and water vapor delivered to an adult 3D printed airway model in a spontaneously breathing test lung between cloth mask with/without a filter, surgical mask, and a N95 respirator.

Methods

Study design

The study was performed at the Seattle Children’s Research Institute, Seattle, WA from April 2020- Oct. 2020. Studies in vitro were carefully designed to evaluate protective attributes based on the efficiency and permeability between simple cloth face masks, commonly worn in the community, and those applied in hospital settings. We also measured efficiency with the addition of a filter to the cloth mask. Initially, we evaluated efficiency on prevention of inhaled ‘fine’ particles (0.03–0.05 µm) by assessing mask permeability of water vapor generated by a humidifier. These water vapor particles, also capable of transmitting airborne viruses, are generated in the exhaled breath of infected individuals while talking and present a significant risk for infection. We then characterized albuterol particle size generated by a nebulizer with a particle impactor. Lastly, we applied the nebulizer to a spontaneously breathing 3D printed airway affixed to an adult lung model and quantified particle deposition to the masks and filters (when applicable) and at the level of the face, airway model, and lung model filter. For this study, we considered nebulized aerosols as “coarse” particles within the range of particle sizes known to generate infectious airborne droplets generated through coughing or when performing aerosol generating procedures (AGPs) by caregivers in clinical settings.

Masks and filters

Figure 1 A–H show the different mask and filters used with each of the experimental conditions. The cotton or “cloth”
facemasks, unisex fashion stretch lightweight cotton covering face and mouth (Getien Biotech, Inc; Nanjing, China), were chosen as they had elastic ear loops, a filter pocket and nose wire that allowed for fit adjustment (Figure 1 C, D, E). An N95 respirator (Figure 1A) (3M™; St. Paul, Minneapolis) and surgical mask (Figure 1B) (Wujin Economic Development Zone; Changzhou, Jiangsu) are commonly used in the hospital setting for respiratory precautions were also used.

Prior to testing, masks and mask filters were preconditioned using a vacuum warmer (Labconco 7982010 CentriVap Aqueous System, 115V, 60Hz) at 40°C for 30 minutes. Three commercially available filter materials were used including PM2.5 filters (Getien Biotech, Inc; Nanjing, China) (Figure 1F), MERV 15 (Capital air filter; Raleigh, NC) (Figure 1G), and unscented Swiffer™ Sweeper Dry™ sweeping cloth (Procter & Gamble; Cincinnati, OH) (Figure 1H). Both the MERV 15 filter and the Swiffer™ dry cloth were cut to the same dimensions as the PM2.5 filters (12cm x 8cm) (see Figure 1F, G, H).

Conditions
Both phases of testing were conducted by evaluating 5 separate runs at each of the following conditions: 1) no mask; 2) cloth mask; 3) cloth mask with Swiffer™ filter; 4) cloth
mask with MERV 15 filter; 4) cloth mask with PM2.5 filter and 5) surgical mask. Owing to a critical shortage of N95 masks in our region, at the time of testing, we were only able to test 3 each of the N95 respirator. For the fine particle testing we repeated testing on two of the masks after drying for a total of 5 runs (N=5). These same three masks were used for course particle testing for a total of 3 runs (n=3).

Fine particle permeability

The first phase of testing evaluated mask efficiency based on ‘permeability’ of fine water vapor particles. A 3D printed face and nasopharyngeal model was used. This airway was replicated from the Computed Tomography (CT) scans of a 17-year-old male’s head (printed with PLA black material on Creality CR 10 printer with embedded nasa airway printed with FormLabs Clear V4 resin on the Form 2 printer). Spontaneous breathing was simulated using a Harvard Large Animal Ventilator (Model #613, Harvard Apparatus; Holliston, Massachusetts).

The model simulated normal breathing through different mask conditions (Figure 2A).

The adult test lung was configured with pre-set tidal volume of 700 mL, respiratory rate of 20 breaths/min, and inspiratory: expiratory (I:E) ratio of 1:1 with half sinusoidal inspiratory and expiratory flow profile to mimic normal spontaneous breathing. (Li et al., 2019) All values were monitored prior to testing with the TSI 5200 analyzer. A traceable hygrometer (Model #4185, Fisher Scientific, Pittsburg, Penn) was placed within the simulated trachea in series between the 3D printed airway and the lung model to measure relative tracheal humidity (RH). Increases in tracheal RH level beyond the ambient value was indicative of ultra-fine water vapor permeating beyond the different masks and filters. A pediatric Monaghan AeroChamber Plus Z STAT valved mask (Monaghan Medical; Plattsburgh, NY) incorporates a series of low resistance one-way valves that allows decoupling of inspiratory

Figure 2. Experimental set up. (A) The experimental set-up of the Harvard Apparatus use to emulate spontaneous breathing via 3D- printed realistic adult airway model. (B) Assessment of fine particle efficiency was characterized by measuring time to reach 95% humidity levels acquired from within the simulated trachea during spontaneous breathing. A valved, aerosol mask allowed egress of exhaled water vapor particles to atmosphere to prevent saturation of masks/filters and limit measurement to incoming water vapor particles. (C) Direct application of aerosol applied to the face with a cloth mask. Aerosol was eluted from upper airways, and lung model filter placed in filter housing following nebulization of 10 mg albuterol and quantified using spectrophotometry.
and expiratory flow paths. This prevented saturation of the hygrometer but also limited changes in RH and penetration of incoming fine particles to inhalation without rebreathing exhaled particles. Following preconditioning of the mask and filter as applicable, the valved, aerosol mask was secured on the 3D model and masks using rubber bands. A Fisher and Paykel (MR 850, Fisher & Paykel; Panmure, Auckland) heated humidifier was preset at 37°C in the invasive mode and applied to 20 L/min air via a heated-wire circuit (see Figure 2B). RH value of 99–100% was confirmed at the AeroChamber mask opening using a hygrometer. Following ambient RH measurement, humidified air was administered directly to the airway with each mask/filter. The masks efficiency in filtering fine airborne particles was determined based on time (seconds) to reach 95% relative humidity within the trachea. A new mask/filter was used for each of the 5 runs with the exception of the 3 N95 respirators. Three of the respirators were pre-conditioned again in the dryer and re-tested for a total of 5 runs (n=5).

**Course particle deposition**

The first step for the aerosol testing was to characterize the course particle size emitted from a nebulizer. Coarse aerosol particles were generated with a vibrating mesh (VM) nebulizer (Aerogen Solo, Aerogen Ltd., Galway, Ireland), with a residual drug volume < 0.1 mL. (Li et al., 2019) A high-performance, multistage, next generation impactor (Model #NGI-0670, NGI, TSI Incorporated, Shoreview, MN) was used to quantify the distribution of coarse aerosol droplet size produced by the VM nebulizer and classify into respirable size fractions. The NGI uses eight different particle trays to collect aerosol droplets. The vacuum flow was adjusted with a frit resistor (S/N 511197-9, Cole Palmer, Vernon Hills, IL) at 15 L/min and confirmed with a TSI 5200 Flow and Pressure Analyzer (TSI Industries, Shoreview, MN). A standard leak test was performed per manufacturer’s specifications before each test. 10 mg of aqueous albuterol was aerosolized in continuous output mode. Following nebulization, aerosol droplets were eluted by instilling 10mL of hydrochloric acid in each plate and then withdrawing the solution from each individual stage. The mass was quantified using ultraviolet-visible spectrometry (UV-Vis) (Molecular Devices SpectraMax M3; San Jose, CA). The albuterol sulfate was measured by UV-Vis spectrophotometry at its local spectral maximum of approximately 276 nm. Using the Spectra Max M3 (Molecular Devices; San Jose, CA). The albuterol sulfate was measured using UV-Vis spectrophotometry at its local spectral maximum of approximately 276 nm. Using the Spectra Max M3 (Molecular Devices; San Jose, CA). Samples were be pipetted into 96 well plates with UV transparent flat bottom, (Thermo Scientific, or equivalent). Albuterol sulfate powder (USP Reference Standard, Cat #1012633) was used to make standard concentrations for comparison of unknown samples. A calibration curve was created using 500 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, and 12.5 µg/mL known concentrations of albuterol. Three readings from each of these known concentration samples were plotted and analyzed by the Spectra Max M3 software. The standard curve was then be used to determine the concentrations of test samples from the absorbance measurements. This was repeated for a total of 5 runs (n=5). We then calculated median mass aerodynamic diameter (MMAD), geometric standard deviation (GSD) using a log-probit equation, fine particle fraction (FPF, %) based on proportional mass of respirable particles (MMAD <5.4 µm) and total drug mass delivered to the impactor stages.

Following the characterization of aerosols emitted from the nebulizers, we evaluated filtration efficiency of coarse aerosols between the different mask and no mask conditions using a similar experimental set-up as the previous study and is shown in Figure 2A. A low dead-space filter housing containing a low resistance medication filter (PARI Filter; Starnberg, Germany), was placed between the lung model and simulated trachea to collect inspired aerosol just prior to entering the lung model. 10 mg of aqueous albuterol sulfate (5mg/mL) was administered through the VM nebulizer with an operational flow of compressed at 3 L/min. The nebulizer was attached directly to the 3D printed face mask or overprotective masks using a vented aerosol mask (see Figure 2C).

Following nebulization, the lung filter was removed and the 3D printed face was wiped with a PARI filter. The 3D printed model airway was eluted with 20mL of hydrochloric acid (0.1 N). PARI filters, mask and mask filters (when applicable) were placed in a sealing plastic bag and eluted with 20 mL of HCL. They were manually agitated for 3 minutes. All eluents were filtered through a 5-micron filter needle to remove debris. Recovered samples were then quantified for absolute albuterol mass (µg) using UV-Vis spectrophotometry (Molecular Devices SpectraMax M3; San Jose, CA). The albuterol sulfate was measured by UV-Vis spectrophotometry at its local spectral maximum of approximately 276 nm. Using the Spectra Max M3 (Molecular Devices; San Jose, CA). Samples were be pipetted into 96 well plates with UV transparent flat bottom, (Thermo Scientific, or equivalent). Albuterol sulfate powder (USP Reference Standard, Cat #1012633) was used to make standard concentrations for comparison of unknown samples. A calibration curve was created using 500 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, and 12.5 µg/mL known concentrations of albuterol. Three readings from each of these known concentration samples were plotted and analyzed by the Spectra Max M3 software. The standard curve was then be used to determine the concentrations of test samples from the absorbance measurements. A new mask (with or without filter) was used for a total of 5 runs (n=5). Each of the 3 N95 respirators were tested once for a total of 3 runs (n=3).

For these testing purposes, we reported coarse aerosol as the cumulative deposited aerosol dose (%) because patients are most likely to become infected by touching the face or when infectious droplets are inhaled directly into the naso-oral airway airways, bronchial tubes or lungs. As such, cumulative deposited aerosol dose (%) included the sum of albuterol mass deposited on face, airway, and lung as referenced to the nominal dose placed into the nebulizer. We also included descriptive measurements for mask and filter deposition.
Analysis

Fine particle permeability based on time to 95% humidity was calculated in seconds and assessed for normality using histograms and QQ-plots and summarized using means and standard deviations for each mask/filter type. ANOVAs were used to compare the cumulative deposited dose (%) as well as the mean amount of time, in seconds, until the mask/filter reached 95% humidity between each mask/filter. The sum of cumulative albuterol particles deposited and recovered from face, airway, and lung was calculated and assessed for normality using histograms and QQ-plots. The cumulative mean value was referenced to the nominal dose placed into the nebulizer (10 mg) and at each individual location (face, airway, lung, filter, and mask) and expressed as means and standard deviations for cumulative deposited aerosol dose (%) for each condition. A Tukey’s post-hoc analysis was used to assess specific differences between each mask/filter type. A p-value < 0.05 was considered statistically significant. SAS 9.4 (Cary, NC) was used for all analyses. R is an open access alternative that could be used for the analysis. The deposited dose (%) at each individual location (face, airway, lung, filter, and mask) was not tested for differences but were included as descriptive data.

Sample size

Estimates from previous pilot runs were used to estimate a standard deviation of 92 for total drug deposition. With 7 conditions of 5 runs each, this estimates a standard deviation of means of 64. An overall difference of 183 between mask/filter types was able to be detected using an alpha of 0.05 and 80% power. Post-hoc analyses could detect a difference of 232 between each mask/filter type with 80% power using a Bonferroni correction and an alpha of 0.007.

Results

Fine particle permeability

The results from the fine particle testing can be found in Figure 3. Based on time to 95% RH, permeability with the PM2.5, MERV 15, Swiffer™, and N95 was lower than no mask (Ringer, 2021). The cloth mask and surgical mask alone did not demonstrate significant (P<0.05) filtration of water vapor when compared to no mask condition. The cloth mask with Swiffer™ filter performed as well as the N95 respirator with filtration of the fine water vapor particles.

Course particle size characterization

Nebulized coarse aerosol albuterol particle sizes ranged from 0.54 to 11.72 µm with MMAD of 4.87 ± 2.18 µm (Figure 4). The particle size fractions (%) were based on the total mass (µg) of albuterol recovered from the individual impactor stages with 43, 27, and 30% for particles >5.4, 3.2- 5.4 and <3.2 µm, respectively (n=5).

Course particle deposition

Data shown in Figure 5 are the sum of total cumulative mass of albuterol particles recovered from the face, airway, and lung.
Figure 4. Particle size characterization of nebulized coarse aerosols. The particles generated by the Aerogen. Aeroneb (n=5) were characterized using an aerosol impactor prior to testing with masks. Particle sizes ranged from 0.54 to 11.72 µm with median mass aerodynamic diameter (MMAD) 4.87±2.18 µm. The particle size fractions (%) were based on the total mass (µg) of albuterol recovered from the individual impactor stages with 43, 27, and 30% particle for particles >5.4, 3.2- 5.4 and <3.2 µm, respectively (n=5).

Discussion

The major finding of this study was that a cloth mask, cloth mask/filter combination, surgical mask, and N95 respirator provide filtration of coarse aerosol particles capable of containing viral pathogens from reaching the face, airway and lungs when compared to no face covering. For the fine water vapor particles, also capable of transmitting infectious pathogens, the addition of a filter to a cloth mask or wearing an N95 respirator showed better protection than a cloth with no filter or surgical mask.

This study is one of the first to use realistic 3D printed airway models and a spontaneously breathing test lung to measure mask efficiency with aerosols. Although these findings are not generalizable to all face coverings currently recommended by the CDC, they do show benefit of wearing a cloth mask, cloth mask with filter, surgical mask and N95 respirator in protecting...
Figure 5. Cumulative deposited dose of coarse aerosol particles. Data shown are the sum of total cumulative mass of albuterol particles recovered from the face, airway, and lung with and without different masks. These data were then referenced to the nominal albuterol dose (10 mg) placed into the nebulizer and shown as delivered dose %. Data are mean ± SD (standard deviation) with n=5 runs with each configuration and n=3 for N95. Data were analyzed with one-way ANOVA (analysis of variance), with Tukey test post hoc comparisons between testing conditions. Means with the same letter are not significantly different, P<0.05.

Since the emergence of COVID-19, much of the public health messaging has promoted face coverings, when social distancing is challenging, to lower the spread of the virus. Previous studies have demonstrated advantages of mask wearing for source control. (Patel et al., 2016) In this in vitro study, we demonstrated that there may be some benefits of wearing facemasks as they filtered large particles and some effectively filtered tiny water vapor particles. Makison Booth et al. (2013) measured the permeability of surgical masks and their ability to protect the wearer from influenza bioaerosols. Their study, in vivo, suggested that the influenza virus can survive in particles that penetrated a surgical mask. Davies et al. (2013) demonstrated that both surgical mask and cloth masks reduce the total number of microorganisms expelled when coughing, however the surgical mask had superior performance especially with smaller particles. Our water vapor testing results align with a Li et al. (2006) study that showed surgical masks have significantly higher water vapor permeability than N95 respirators. Our study added to the body of literature, by testing cloth masks and filters in comparison with both the surgical mask and N95 respirator.

A limitation of this study is that the efficacy of filtration was measured with non-biologic particles. Despite this the effectiveness of the masks is best estimated on the measurement of blocking aerosols as essentially any aerosolized particles will behave similarly despite having different biologic properties. (Davies et al., 2013) While we considered fine and coarse inhaled particle transmission, not all particles penetrating masks in the community may be infective or be indicative of viral viability or exposure to the airway, lungs or of the virus. In addition, we were also unable to test the wide range of respirable aerosols that may be emitted by infectious individuals. Additionally, the study methods exposed the masks, airway and face directly to large amounts of inhaled particles. Findings from this comparative study should be approached with trepidation as this simulates a ‘worst case scenario’; wherein a potentially unmasked infected individual is speaking or coughing directly into direct proximity of an unmasked/masked individual. In most situations in the community, a mask...
Figure 6. Location of course particle deposition. Data shown represent the mass of albuterol particles recovered from the face, airway, and lung with and without different masks. These data were then referenced to the nominal albuterol dose (5 mg) placed into the nebulizer as delivered dose %x. Data are mean ± SD (standard deviation) with n=5 runs with each configuration and n=3 for N95. These descriptive data were not analyzed for statistical differences.

This study does not suggest that a cloth mask alone or any mask will prevent the airborne transmission and spread of this virus. However, the addition of a filter to the cloth mask may improve the performance. The possible contribution of infective aerosols to the current pandemic suggests the advisability of wearing a suitable mask in combination with other measures such as social distancing and handwashing.

Data availability

Underlying data


This project contains the following underlying data:

- Course Particle Deposition.xlsx
- Water Vapor Permeability.xlsx

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).


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We would strongly suggest that the methods/study design for testing fine particle filtration using relative humidity should be removed until it is verified as a valid method of determining filter efficiency of mask material for ultra-fines. They also need to cite many of the studies in the literature that have already tested mask efficiency using more traditional/validated methodologies for quantifying particle concentrations penetrating mask material or the masks as worn by human subjects (e.g. Sickbert-Bennett et al. and Clapp et al. papers).

General comments:

You have attempted to measure efficacy of masks for filtering (not preventing transmission) fine and coarse particles. The use of a 3D printed face seems novel but the experimental setup did not allow for the assessment of leaks (i.e. mask fit) so it was unclear what the purpose was for having a “realistic face” as part of your study design. In effect you measured material efficiency by only exposing a small portion of the mask surface to the emitted aerosol. The methods for assessing filtration efficiency were novel but cumbersome and not well validated compared to others who have tested mask efficacy. This was especially true for the measure of relative humidity (RH) as an index of ultrafine water particles. RH is a measure of water vapor not water particles/aerosol. You need to validate that the RH measure is indeed a surrogate for particle concentration. More specific comments are given below.

Specific comments:

Major:

1. Title is overstated. You did not “Assess mask efficiency for preventing transmission of
airborne illness ...”. You assessed mask efficiency for preventing penetration of particles to the wearer.

2. Title and throughout. Water vapor is a gas – not fine particles. You tested penetration of ultrafine particles.

3. Abstract. Conclusion. “The findings from this study suggest that a mask can filter these potentially infectious airborne particles.” Is this really a unique finding? What is new about your findings?

4. The Introduction could be reduced in description of SARS-CoV-2 but greater emphasis given to what is already known about mask efficiency testing methods and results. There are a number of recent in-vitro and in-vivo studies that have tested a variety of masks using a variety of methods, e.g. material efficiency, fitted efficiency on a mannekin, and fitted efficiency on human subjects (e.g. O’Kelly et al., Rengasamy et al., Asadi et al., Mueller et al., Pan et al., Sickbert-Bennett et al., Clapp et al.). What are the advantages/limitations of your method compared to these other approaches and findings? For example, your use of an adult 3D printed airway model ignores the effect of mask fit for preventing penetration of particles via leaks around the mask fit to the face.

5. Study Design. What was the humidifier? How was the humidifier particle size (0.03-0.05 um) determined? MMAD? CMAD? Were they sized at the outlet of the humidifier or at the surface of the mask?

6. Methods. “...of fine water vapor particles..”. Not fine nor water vapor. Ultrafine water particles. Masks are designed to remove particles not gases (i.e. water vapor).

7. Fine Particle Permeability. How were the printed plastic face/nasal/lung airways checked/accounted for electrostatic surface charges? Since these are ultra-fine particles with high diffusion constant, charge plays a large factor in their motion. This may be a limitation to the use of a plastic face.

8. Masks and Filters. The masks and filters were pre-conditioned in a vacuum warmer prior to testing. Why was this done and what effect could it have that is not normally seen outside the lab? Does it increase efficiency or decrease it, or have no effect?

9. Methods. “Increases in tracheal RH level beyond the ambient value was indicative of ultrafine water vapor permeating beyond the different masks and filters. The masks efficiency in filtering fine airborne particles was determined based on time (seconds) to reach 95% relative humidity within the trachea. A traceable hygrometer(Model #4185, Fisher Scientific, Pittsburg, Penn) was placed within the simulated trachea in series between the 3D printed airway and the lung model to measure relative tracheal humidity (RH).” RH is a measure of water vapor not particles. Masks are not designed to filter water vapor. What was the response time of the hygrometer? Was the model kept warm at body temperature (37deg)? How did you measure the size of ultrafine water particles introduced to the mask? Your method may be more sensitive to hydrophilic/hydrophobic retentive nature of the filter medium to water vapor than actual particle filtering efficiency. In sum can you validate that the measure of RH is an index of ultrafine particles penetrating the mask?
10. Figure 2 is very confusing – a schematic of the setup instead of, or in addition to, would be helpful.

11. Methods. Figure 2 and “...aerosol mask was secured on the 3D model and masks using rubber bands.” This seems unrealistic to how a mask would be fit to a face. What was the purpose of having a realistic 3D model if you were going to perfect the fit onto the face with rubber bands? Essentially you tested the filtration of the mask material (no consideration of mask fit) which does not require a 3D model. Figure 2B and C seem to confirm this – you are only applying aerosol from the humidifier or nebulizer to the small portion of the mask surface area directly over the mouth/nose. If the fit is somehow held constant from one arm of the study to another, then, in essence, this study simply measures the filtering efficiency of the different materials. The face, lung, and aerosol egress mask are immaterial and may be confounding additions.

12. Was breathability assessed for the different mask/filter combinations, e.g. pressure drop across the mask? For example, the Swiffer/cloth mask seemed by your methods to be almost as effective as an N95 for filtering the humidifier particles but is it breathable?

13. Results for surgical masks using humidification. Your finding of poorer performance for surgical vs. cloth masks seems at odds from fitted filtration data (Sickbert-Bennett et al. and Clapp et al.) tested with 0.05 um particles on an actual human head. In fact, procedure masks available to the public are constructed with nonwoven polypropylene, the same highly efficient filtering medium used in respirators. You need to discuss why your data are at odds with other published results.

14. Figure 5 data and calculations of “Cumulative deposited aerosol dose” These data are difficult to interpret and compare to filtration efficiencies. Can you recalculate as a % relative/normalized to the no mask condition?

15. Discussion. “The major finding of this study ...” Finding that masks filter particles from being inhaled is not a novel finding (e.g. Sickbert-Bennett et al. and Clapp et al.).

16. Discussion. “It would be beneficial to test the permeability of mask on research subjects with the ability to be fit-tested.” This has been done with realistic masks as fitted on human subjects. See:
   ○ Sickbert-Bennett et al. (2020).
   ○ Clapp et al. (2020).
   ○ Sickbert-Bennett et al. (2021).

Minor comments:
1. Method. “…inhaled ‘fine’ particles (0.03–0.05 μm)” Technically these are ultrafine particles (< 0.1 um).

2. The spelling of “Coarse” and “Course” are intermixed throughout.

3. Method. “…spontaneously breathing 3D printed airway.” How is this possible in-vitro? I think
you mean a “controlled or simulated breathing 3D printed airway”.

4. Fine Particle Permeability. The TSI 5200 is a mass flow meter, not an analyzer.

References

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aerosol Scientist and Pulmonary Physiologist

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.