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Addressing Personal Protective Equipment (PPE) Decontamination: Methylene Blue and Light Inactivates SARS-CoV-2 on N95 Respirators and Medical Masks with Maintenance of Integrity and Fit

--Manuscript Draft--

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Abstract:	Objective: The coronavirus disease 2019 (COVID-19) pandemic has resulted in shortages of personal protective equipment (PPE) underscoring the urgent need for simple, efficient, and inexpensive methods to decontaminate SARS-CoV-2-exposed masks and respirators. We hypothesized that methylene blue (MB) photochemical treatment, which has various clinical applications, could decontaminate PPE contaminated with coronavirus. Design: The two arms of the study included: 1) PPE inoculation with coronaviruses followed by MB with light (MBL) decontamination treatment, and 2) PPE treatment with MBL for 5 cycles of decontamination (5CD) to determine maintenance of PPE performance. Methods: MBL treatment was used to inactivate coronaviruses on three N95 filtering facepiece respirator and two medical mask models. We inoculated FFR and MM

	materials with three coronaviruses, including SARS-CoV-2, and treated with 10 μM MB and exposed to 50,000 lux of white light or 12,500 lux of red light for 30 minutes. In parallel, integrity was assessed after 5CD using multiple US and international test methods and compared to the FDA-authorized vaporized hydrogen peroxide plus ozone (VHP+O3) decontamination method. Results: Overall, MBL robustly and consistently inactivated all three coronaviruses with 99.8 - to >99.9% virus inactivation across all FFRs and MMs tested. FFR and MM integrity was maintained after 5 cycles of MBL treatment, whereas one FFR model failed after 5 cycles of VHP+O3. Conclusions: MBL treatment decontaminated respirators and masks by inactivating three tested coronaviruses without compromising integrity through 5CD. MBL decontamination is effective, low-cost and does not require specialized equipment, making it applicable in all-resource settings.

Dear Editors of Infection Control and Hospital Epidemiology,

We cannot continue to be oppressed by the COVID19 pandemic.

Whether it be from global underestimation of the far-reaching impact of a pandemic or whether due to political ambivalence, our healthcare workers and global citizens do not have the adequate supply of personal protective equipment needed to mitigate the spread of SARS-CoV-2. This supply shortage requires a decontamination solution that is simple, safe, inexpensive, resource-light and globally accessible. In our study, the first of its kind, we have shown that methylene blue (MB, a common dye) when activated with bright white light, can inactivate human SARS-CoV-2 on commonly used N95 respirators, medical masks, and community masks without degrading the PPE materials.

We represent a consortium of virology labs and mask and respirator integrity testing sites around the globe (including the National Institute of Occupational Safety and Health) testing the hypotheses that methylene blue with light can inactivate coronaviruses on PPE. Our team is comprised of members of the WHO Infection Prevention and Control (IPC) Group, the WHO COVID 19 Task Force, and the Centers for Disease Control and Prevention in the US. Three different coronaviruses were tested – Human SARS-Co-V-2, Murine Hepatitis Virus (MHV), and a porcine coronavirus to understand the inactivation potential of methylene blue.

We leveraged existing clinical evidence of the safety and efficacy of methylene blue and its ability to generate singlet oxygen to develop a potential recipe for PPE decontamination. Furthermore, we are adding to the literature about the stable integrity of PPE when subjected to repeated (X5) cycles of MB decontamination. These data provide guidance and reassurance to the PPE wearer that reuse of PPE is not providing additional risk in high exposure environments and situations.

In the course of this study, one of our senior researchers lost her husband to COVID19 making the need to get our results and message to the globe that more pressing.

All authors have approved this manuscript, contributed significantly to this work, and this manuscript has not been previously published in a peer reviewed journal, nor is being considered by another journal for publication. We have pre-published the methylene blue details in MedRxiv doi: https://doi.org/10.1101/2020.12.11.20236919

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We thank you for the opportunity to submit this research to a wide and diverse community.

Sincerely,

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Addressing Personal Protective Equipment (PPE) Decontamination: Methylene Blue and Light Inactivates SARS-CoV-2 on N95 Respirators and Medical Masks with Maintenance of Integrity and Fit

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Using MB to decontaminate PPE for reuse and wear

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ABSTRACT

Objective: The coronavirus disease 2019 (COVID-19) pandemic has resulted in shortages of personal protective equipment (PPE) underscoring the urgent need for simple, efficient, and inexpensive methods to decontaminate SARS-CoV-2-exposed masks and respirators. We hypothesized that methylene blue (MB) photochemical treatment, which has various clinical applications, could decontaminate PPE contaminated with coronavirus.

Design: The two arms of the study included: 1) PPE inoculation with coronaviruses followed by MB with light (MBL) decontamination treatment, and 2) PPE treatment with MBL for 5 cycles of decontamination (5CD) to determine maintenance of PPE performance.

Methods: MBL treatment was used to inactivate coronaviruses on three N95 filtering facepiece respirator and two medical mask models. We inoculated FFR and MM materials with three coronaviruses, including SARS-CoV-2, and treated with 10 µM MB and exposed to 50,000 lux of white light or 12,500 lux of red light for 30 minutes. In parallel, integrity was assessed after 5CD using multiple US and international test methods and compared to the FDA-authorized vaporized hydrogen peroxide plus ozone ($VHP+O_3$) decontamination method.

Results: Overall, MBL robustly and consistently inactivated all three coronaviruses with 99.8 - to >99.9% virus inactivation across all FFRs and MMs tested. FFR and MM integrity was maintained after 5 cycles of MBL treatment, whereas one FFR model failed after 5 cycles of $VHP+O_3$.

Conclusions: MBL treatment decontaminated respirators and masks by inactivating three tested coronaviruses without compromising integrity through 5CD. MBL decontamination is effective, low-cost and does not require specialized equipment, making it applicable in all-resource settings.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 has resulted in critical personal protective equipment (PPE) shortages, especially filtering facepiece respirators (FFRs, also known as N95 respirators). Although designed for single use, healthcare personnel (HCP) are reusing potentially contaminated FFRs and medical masks (MMs) on an emergency basis due to supply shortages. These shortages have necessitated the rapid development and deployment of disinfection processes, leading to the World Health Organization (WHO) issuing interim guidance on rational PPE use (1,2). The US Food and Drug Administration (FDA) granted Emergency Use Authorization of hydrogen peroxide and steam sterilization systems to decontaminate FFRs for reuse (3). These technologies remain less available in low-resource settings, where frontline HCP have inadequate protection (4,5), thus we need novel methods for PPE decontamination.

Photochemical disinfection uses a photosensitive chemical, which combined with visible light generates singlet oxygen (Supplemental Section). Singlet oxygen inactivates viruses by damaging viral nucleic acids and membranes (6). One such photosensitizer is methylene blue (MB), which is FDA-approved to treat methemoglobinemia, and used to sterilize human plasma transfusions in Europe (7). MB inactivates SARS-CoV-2 and many other viruses (8-10) [Supplemental TABLE S3].

This Development and Methods for N95 Respirators and Mask Decontamination (DeMaND) study aimed to evaluate methods that inactivate SARS-CoV-2 on respirators and masks, which can be applied anywhere at low-cost. We examined if MB with light (MBL) could effectively decontaminate commonly-used FFRs and MMs while maintaining mask integrity (filtration, breathability, fluid resistance and fit) after multiple decontamination cycles. We leveraged four virology laboratories and six PPE integrity testing sites to examine: (a) MBL

virucidal effect on two SARS-CoV-2 isolates (*Betacoronavirus*) and two coronaviruses requiring a lower level of biocontainment (the *Betacoronavirus* murine hepatitis virus (MHV) and the *Alphacoronavirus* porcine respiratory coronavirus (PRCV)), and (b) the impact of five cycles of decontamination (5CD) on PPE integrity [FIGURE 1].

We selected the number of decontamination cycles based on the Centers for Disease Control and Prevention (CDC)'s recommended maximum number of donnings as part of crisis capacity strategies (11). We chose FFRs and MMs based on availability during recent outbreak responses and variations in shape, material and structure. For integrity testing, we compared MBL to the FDA-authorized vaporized hydrogen peroxide plus ozone system (VHP+O_3) (2).

METHODS

Respirators and Masks

We tested three FFRs and MM models, both fluid resistant and non-fluid resistant, [Supplemental Figure S9]. These FFRs are surgical FFRs: National Institute of Occupational Safety and Health (NIOSH)-approved particulate respirators FDA-cleared as medical devices. Supplemental TABLE S1 testing matrix.

Viruses

We obtained SARS-CoV-2 isolates from a patient at the George Washington University Hospital (Lab 1) and from Dr. Darryl Falzarano (VIDO) (GISAID accession ID: EPI_ISL_425177) (Lab 2). We used a SARS-CoV-2 clinical sputum specimen with University of Calgary Conjoint Health Research Ethics Board approval (ID#REB20-0444). Recombinant MHV strain rA59-E-FL-M and PRCV strain 91V44 have been described previously (12-15).

Virus Inoculation and Elution

We cut FFRs and MMs into 7x10 mm coupons and inoculated them with the maximum available virus dose of SARS-CoV-2 or MHV on the outer layer (or inner layer where specified) with a pipette and dried for 20 mins before treatment. Virus was eluted in media by vortex or orbital rocker. Alternatively, we injected PRCV under the outer layer, and then we excised 34x34 mm coupons and vortexed in a media-containing tube. We quantified the remaining infectious virus by median tissue culture infectious dose ($TCID_{50}$) or plaque assays.

Methylene Blue Treatment

We added 10µM MB to the inoculated coupons or sprayed on intact inoculated masks, dried for 30 minutes protected from light, and exposed to 50,000 lux of white light or 12,500 lux of red light for 30 min. We used red light at a lower intensity because red light contains a higher percentage of wavelengths that activate MB. Dark controls were left in the biosafety cabinet with the light off or covered by aluminum foil (<100 lux).

For pre-treatment testing, we soaked R3 coupons with MB for >1 hour and dried 2 days protected from light. We then spotted SARS-CoV-2 on outer or inner mask layers and dried for 20 minutes before exposure to 50,000 lux of white light for 30 min. Intact RM and FW were sprayed with 7-8mL MB and dried overnight. We added MHV to three points on the outer surface, dried for 20 minutes, exposed to light (50,000 lux) for 30 min, excised the inoculated area, eluted, and quantified by $TCID_{50}$ assay.

Light Sources

The Seattle Children's Research Institute, George Washington University, University of Calgary, and Nelson Laboratories used lightboxes developed at Colorado State University and included 4000K Husky LED lights. The University of Alberta used 3500K Husky LEDs. The University of Liège and Centexbel used a custom lightbox containing horticultural lamps. All laboratories verified light intensity using light meters [Supplemental Methods].

Integrity Testing

We assessed respirator and mask integrity by determining filtration efficiency, breathability, fluid resistance and fit. We tested FFRs/MMs untreated and after 5CD with VHP+O_3 or 10μM MB plus white light (50,000 lux) or red light (12,500 lux) for 60 minutes. For the VHP+O_3 treatment, we used the pre-set cycle (Cycle 1) of Stryker's STERIZONE VP4 Sterilizer at 41°C.

Filtration Efficiency Testing

We assessed filtration efficiency using NaCl sub-micron charged-neutralized particles ranging in size from 0.022–0.259μm with a median count diameter of 0.075 ± 0.020μm and a geometric standard deviation of less than 1.86 to give a mass median aerodynamic diameter of 0.3μm, with air flow at 85 L/min (simulating inhalation at heavy workload) (16). We measured bacterial filtration efficiency (BFE) of MMs using aerosolized droplets containing *Staphylococcus aureus* at a 28.3 L/min air flow rate (17,18) [Supplemental Methods and Results].

Breathability Testing

We assessed breathability by measuring inhalation and exhalation breathing resistances using standard test methods, and pressure drop measurements for MMs. Additionally, we determined Sheffield Dummy airflow differences for both FFRs and MMs [Supplemental Methods and Results].

Fluid Resistance Testing

Testing of resistance to splash and spray by synthetic blood is required for surgical masks in the US and fluid-resistant MMs in Europe. We tested fluid resistance for MMs [Supplemental Methods and Results].

Fit Testing

We conducted human fit testing with the PortaCount® Pro+ 8038 (TSI). Fit testing was exempted from ethics board review by both the Research Compliance Office, Stanford University and the Conjoint Health Research Ethics Board, University of Calgary. We tested multiple dynamic tasks: regular breathing, heavy breathing, turning head side-to-side, moving head up-and-down, talking, and bending over breathing. We performed each set of tests twice and calculated Fit Factor (FF) for each mask. We conducted Manikin fit testing using an advanced, realistic manikin head and according to the NIOSH/National Personal Protective Technology Laboratory (NPPTL) Decontaminated Respirator Assessment Plan (16). We examined the changes in the elastic recovery of the FFR straps and MM ear loops to determine changes in strap/ear loop integrity after 5CD [Supplemental Methods and Results].

Statistical Analysis

We calculated means and standard deviations or percent pass of each integrity test method separately by FFR or MM model. We combined data for integrity test methods conducted at more than one test site to create overall means and standard deviations or percent pass. We tested normality of the data distribution using the Shapiro-Wilk test. We calculated significant differences between untreated and treated FFRs and MMs with Student's t-tests, Mann-Whitney U tests, or Fisher's exact tests (SAS v9.4) (19).

RESULTS

Methylene blue and light (MBL) tissue culture plate inactivation

To confirm that MBL can inactivate a coronavirus, varying concentrations of MB were mixed with PRCV and exposed to red light (12,500 lux). Treatment with 0.1µM MB plus light resulted in complete inactivation. In the absence of additional light, complete inactivation required a dose 1µM MB [FIGURE 2A].

To confirm the ability of MBL to specifically reduce SARS-CoV-2 infectivity, varying concentrations of MB were mixed with SARS-CoV-2 and exposed to 50,000 lux of white light. MB inhibited SARS-CoV-2 infectivity with a dose-dependent effect, with or without exposure to light. This virucidal effect was enhanced in the presence of light [FIGURE 2B].

MBL FFR and MM material inactivation

To examine the MBL dose required to decontaminate FFR and MM material inoculated with SARS-CoV-2 or MHV, we cut coupons from a representative FFR (R3) or MM (FW) and inoculated and treated with MBL for the indicated time periods. Both viruses displayed sensitivity to MBL treatment. Using 10µM MB and light resulted in complete inactivation of SARS-CoV-2 and MHV on both FFR and MM materials after 5 minutes [FIGURE 3A]. Using 1µM MB, we observed complete inactivation after 30 minutes of light exposure, though we observed a 2-4 log viral titer reduction after 5 minutes. MB treatment in the absence of additional light also resulted in substantial reduction of viral titers.

To ensure that MBL can efficiently decontaminate a variety of masks, we tested three more masks, including two additional FFRs (RH) and (RM), and one additional MM (FH). We inoculated coupons or intact masks, treated with 10µM MB and exposed to light for 30 minutes [FIGURE 3B-E], conditions which demonstrated robust inactivation in the previous experiment. We observed complete inactivation (up to 4 logs) of SARS-CoV-2 for all respirators and masks tested. Treatment with MB without exposure to white light resulted in substantial virus reduction [FIGURE 3D]. We observed complete inactivation (4-5 log reduction) of MHV for FH, R3, RH, and RM masks. A low level of virus was detectable in one replicate for FW [FIGURE 3B]. For PRCV, which was injected under the outer mask layer, we observed a >5-log virus reduction after treatment of FH, FW, R3 and RH masks. In contrast, we observed a 3-log reduction in RM [FIGURE 3E]. The overall percent reduction in virus titer after treatment across all FFRs/MMs

and viruses ranged from 99.8->99.9%[TABLE 1]. In addition, we tested the effect of MBL inactivation on FFR and MM straps inoculated with PRCV and noted 2-4 log reduction in titers [Supplemental FIGURE S4].

Evaluation of Potential Applications of MBL in a Clinical Setting

We examined three potential applications of MBL in a clinical environment. First, since some clinical settings may not have access to bright light, we investigated whether 10µM MB and ambient light would be sufficient to inactivate SARS-CoV-2. MB treatment and exposure to 700 lux (ambient light generated by light in a biosafety cabinet) for 60 minutes inactivated nearly 5-logs of SARS-CoV-2. MB and <100 lux of light inactivated almost 3-logs of virus [FIGURE 4A].

Second, we investigated the possibility of pre-treating respirators or masks with MB. We treated coupons with 10µM MB, dried overnight, and inoculated them with SARS-CoV-2 on either the hydrophobic outer layer or the hydrophilic inner layer before exposure to 50,000 lux of white light for 30 min. We could not recover infectious virus from either side of the light-exposed coupons, signifying inactivation of >4 logs of virus [FIGURE 4B]. We sprayed intact RM respirators and FH masks with 10µM MB, dried overnight, inoculated with MHV, and exposed to 50,000 lux of white light for 30 min. No viable virus was recovered. [FIGURE 4D].

Lastly, we added 10µl of a clinical specimen (saliva) with a titer of 1.15×10^5 PFU/ml obtained from a COVID-19 patient onto respirator coupons, and treated with 10µM MB and white light (50,000 lux) for 30 min. No viable virus was detected post-treatment, thus indicating the potential for this inactivation method in clinical settings in which viable virus may be protected by proteinaceous matrixes [FIGURE 4C].

Integrity Testing

We employed standard test methods to examine if MBL decontamination affected integrity and compared results to the FDA-authorized VHP+O_3 decontamination method. The following sections describe each of the integrity test methods and results [Supplemental TABLES S2A-B for complete results]. Additional testing for MMs included bacterial filtration efficiency, differential pressure, Sheffield dummy, fluid resistance, and strap/ear loop integrity testing [Supplemental FIGURES S5-S8].

Filtration Efficiency

FIGURE 5A depicts the filtration efficiency before (untreated) and after 5CD. We expected high filtration efficiencies for the FFRs as they are all NIOSH-approved N95 FFRs, which requires ≥95% sub-micron filtration efficiency. All FFR models surpassed the minimum 95% filtration efficiency requirement before and after 5CD. Untreated FW and FH masks achieved 76% and 86% filtration efficiency, respectively. Overall, MBL and VHP+O_3 treatment of FFRs and MMs did not cause any significant differences in the filtration efficiency of the studied models ($p>0.01$). MM models continued to meet requirements of differential pressure after 5CD [Supplemental FIGURE S5 and TABLE S2A].

Breathability

The resistance to airflow via inhalation and exhalation (breathability), is an indication of the difficulty in breathing through the respirators or masks. The FFR models achieved inhalation/exhalation resistances >60% below respective NIOSH 42 CFR Part 84 and EN 149 requirements after 5CD [FIGURE 5B, C]. These resistance changes would not make it harder to breathe through the mask. MMs demonstrated similar inhalation resistances, and lower exhalation resistance values compared to FFRs after 5CDs. Both MM models were below their respective allowable maximum differential pressure limit after 5CD [Supplemental FIGURE S6 and TABLE S2A]. Furthermore, we determined air flow differences using the Sheffield Dummy

[Supplemental FIGURE S7 and TABLE S2A-B]. The MBL treatment did not affect the breathability of FFRs or MMs, in terms of either inhalation and exhalation resistances or pressure drop, respectively.

Fluid Resistance

We evaluated MMs for their fluid resistance and found them to be resistant when challenged from the outside (conventional challenge direction) but less so from the inside of the mask [Supplemental FIGURE S8 and TABLE S2A].

Fit Testing

Fit testing measures how well a respirator or mask seals around the contours of the face. A good fit ensures exchanged air is filtered through the respirator. Human fit testing demonstrated that respirators maintained quantitative fit values, or fit factors (FF), above 100 after 5CD. In contrast, VHP+O_3 decontamination decreased RH and RM fit to the point of failure [FIGURE 6A]. Note that two VHP+O_3 decontamination cycles is the maximum authorized by FDA for N95 FFRs (20). We also performed human fit testing for the MMs to demonstrate that these types of masks are not designed to ensure a tight fit [FIGURE 6A]. On some of the VHP+O_3-treated FFRs and MMs, human participants noted a "strong acrid odor" and some observed partial elasticity loss on treated straps and ear loops, and discoloration of the nosepiece foams (RM only). In contrast, some participants wearing the MBL-treated FFRs noted a "not unpleasant slight odor" at one of the fit testing sites. In addition to the discoloration, the nose bridge was more rigid for the three MBL-treated RMs.

We determined Manikin FF using an advanced, realistic manikin headform which resulted in similar overall passing of OSHA criterion of 100 FF for all three FFRs [FIGURE 6B](16,21). Observed differences between human and manikin fit can be attributed to testing procedure variability and structural facial variations. FFRs do not provide a universal fit for all

wearers. Overall, MBL decontamination after 5CD did not negatively impact integrity performance and fit while the VHP+O_3 decontamination yielded some detrimental performance issues.

DISCUSSION

We demonstrate that MB activated by white or red light effectively inactivates SARS-CoV-2 on FFR and MM surfaces, and with a clinical specimen, without affecting integrity or fit. MBL can be applied as a decontamination method for single-use FFRs and MMs. Residual MB on the mask surface could potentially provide a novel means of continual inactivation of viral particles to decontaminate a mask while donned, since even under ambient light conditions, MB inactivated SARS-CoV-2 on mask surfaces. .

For decades, MB has been recognized to have disinfection capabilities against a range of viral and bacterial pathogens (7-9,22), and MB is currently used to sterilize plasma for transfusion (23)and to sterilize convalescent serum for COVID-19 treatment (10).

MBL is suitable for high- and low-resource settings since MB is inexpensive, globally available, and does not require specialized equipment. Light sources can vary from white high-intensity lamps to ambient lighting to generate singlet oxygen [Supplemental TABLE S6]. Future studies are warranted to investigate whether MBL could be used to inactivate additional pathogens and decontaminate other forms of PPE such as gowns, gloves, and boots.

One limitation was that we only tested a minority of FFR and MM models, yet there are approximately 500 NIOSH-approved FFR models, and many others used globally. MBL worked well on all FFRs and MMs tested, except the “RM” FFR possibly owing to a distinct design characteristic which resulted in the variable outcome. CDC recommends that a decontamination

method's effectiveness be evaluated for specific FFR models in collaboration with the manufacturer, and if needed, a third-party laboratory (25).

During our integrity testing, we did not simulate extended wear or multiple donnings and doffings, which could affect FFR fit and performance (26,27). In addition, off-gassing of MB or VHP+O_3 was not evaluated. The biocompatibility of MB wearer inhalation was not tested, however, MB concentrations used were below those administered clinically (intravenously, orally or intranasally) (7,28,29). If the entire dose of 10μM MB sprayed onto a mask was inhaled, which is unlikely, the total inhaled dosage would be 0.02 mg. The quantity of MB inhalation overtime while wearing a MB-pretreated mask is under investigation.

We generalized our findings by demonstrating complete MBL inactivation employing the same methodology across multiple virology laboratories using three coronavirus species and a SARS-CoV-2 clinical sample. This signifies that emergent variants of SARS-CoV-2 would also be inactivated by MBL and that viruses requiring lower levels of biocontainment can be used for similar inactivation studies. Integrity tests in multiple testing centers using heterogeneous light administration methods reaffirms the reproducibility of our findings and we replicated practical light scenarios expected in the real-world settings.

In conclusion, MBL treatment inactivates SARS-CoV-2 on FFRs and MMs without decreasing integrity and fit. Our findings provide a method for inexpensive, accessible, effective decontamination of PPE for reuse, applicable in high- and low-resource settings during supply shortages. Pretreatment of masks with MB could provide a novel means of continual disinfection reducing exposure to SARS-CoV-2.

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Conflict of Interest

Potential conflicts of interest. TSL, JC and TC are co-founders of Singletto, Inc. DE has stock investment in Singletto, Inc. YC, SC are co-founders of 4C Air, Inc. MZ and LL are employees of 4C Air, Inc. JMC has received research funding from Pfizer. MC received funding from BMGF.

No conflict. BHH, FEMS, FSK, YLL, MML, LFC, AP, YCL, CNM, JS, KBK, EH, ET, BH, JL, SS, JMD, TW, TC, SJS, RP, TG, ET, LLB, MN, CM, KH, KP, SR, PF, OJ, AP, JLL, V<S. KH, SRT, CW, MM, RM, JFW, HN, LD, SdeJ.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Table 1: Virus Reduction by 10 µM Methylene Blue with Light

	MHV		[b]PRCV		Lab 1 SARS-CoV-2		Lab 2 SARS-CoV-2		Avg. SARS-CoV-2		Overall avg.	
Mask	Log reduction	% Inactivation	Log reduction	% Inactivation	Log reduction	% Inactivation	Log reduction	% Inactivation	Log reduction	% Inactivation	Log reduction	% Inactivation
RH	4.4	>99.9%	5.5	>99.9%	2.2	>99.4%	4.3	>99.9%	3.2	99.6%	4.1	99.8%
RM	3.5	>99.9%	2.7	99.8%	2.8	>99.8%	4.0	>99.9%	3.4	99.9%	3.2	99.9%
R3	5.1	>99.9%	5.0	>99.9%	[a]3.3	>99.9%	3.1	>99.9%	3.2	>99.9%	4.1	>99.9%
FW	5.8	>99.9%	5.5	>99.9%	[a]3.6	>99.9%	3.1	>99.9%	3.4	>99.9%	4.5	>99.9%
FH	3.8	>99.9%	5.1	>99.9%	3.1	>99.9%	4.2	>99.9%	3.6	>99.9%	4.0	>99.9%

[a]Data extracted from inactivation curve.

[b]Light source for testing MB on PRCV = 12500 lux red light; Light source for testing MB on all other virus typ

50000 lux white light.

Due to the limit of detection of the assays used to titer the virus, the highest % inactivation is indicated as >99.9

Abbreviations: RH, Halyard duckbill respirator (Fluidshield-46727); RM, 3M half-sphere respirator (1860); R3

Panel respirator (1870+);

FW, Type II EN14 683 generic face mask; FH, Type II RASTM F2100 Level 2 Halyard face mask.

Figure legends

FIGURE 1. Graphical representation of the DeMaND study methodology. (A) *Overview of the coronaviruses, respirators, masks and decontamination methods used.* ***(B)*** *FFRs and MMs were inoculated with virus and treated with MB and light (MBL). Remaining infectious virus was quantified using $TCID_{50}$ or plaque assay.* ***(C)*** *In parallel with the virucidal testing of MBL, intact FFRs and MMs were subjected to 5 cycles of decontamination before mask integrity was tested using the indicated methods. PRCV = porcine respiratory coronavirus, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, MHV = murine hepatitis virus, MBL = methylene blue + light, VHP+O_3 = vaporized hydrogen peroxide plus ozone. See Supplemental Table S1 for the respirator and mask decontamination and testing matrix.*

FIGURE 2. Inactivation of PRCV and SARS-CoV-2 using methylene blue and light (MBL). (A) *To determine the efficacy of different MB concentrations, we added serial dilutions of MB to wells of a 48-well plate containing 10 µL PRCV ($2x10^7$ $TCID_{50}$/ml). Plates were either exposed to red light (12,500 lux) for 30 minutes or were protected from light (<100 lux). Dotted line indicates limit of detection.* ***(B)*** *We added serial dilutions of MB to wells of a 12-well plate containing ~50 PFU of SARS-CoV-2 in MEM plus 15% FCS. Plates were either exposed to white light (50,000 lux) for 45 min or protected from light (<100 lux). We determined viral titers using 2-3 replicate samples. ND= not detected.*

FIGURE 3. MBL inactivates MHV, SARS-CoV-2, and PRCV on FFR and MM material. (A) *Effect of methylene blue and light treatment on MHV and SARS-CoV-2 titers. We applied a 10 µl aliquot of MHV or SARS-CoV-2 to coupons derived from a FFR (R3) or MM (FW) and they were left to dry for 20 min. Subsequently, we added 10 or 30 µl of MB to each coupon at the indicated concentrations. We exposed the samples to white light (50,000 lux) for the indicated time periods or left them in the biosafety cabinet with the lights off. We measured each virus titer using 2-6*

replicate samples by $TCID_{50}$ or plaque assay. . ***(B-D)*** *We applied a 10 µl aliquot of SARS-CoV-2 or MHV to coupons of the indicated masks and dried for 20 min. Depending on coupon size, we added 10-30 µl of 10 µM MB to each coupon, and treated the samples with light (50,000 lux) or protected them from light.* ***(E)*** *We injected 100 µl of PRCV under the outer layer of intact MMs or FFRs and allowed them to dry for 30 minutes. Subsequently, we sprayed the FFRs and MMs with 10 µM MB and dried them for 30 minutes in the dark before exposure to red light (12,500 lux). We determined each virus titer using 2-6 replicate samples by $TCID_{50}$ or plaque assay. Data is represented as mean +/- SD. ND = Not detected. RH= Halyard duckbill respirator (Fluidshield-46727). RM= 3M half-sphere respirator (1860). R3= 3M panel respirator (1870+). FW= Type II EN 14683 generic face mask. FH= Type IIR ASTM F2100 Level 2 Halyard face mask. Dotted line indicates limit of detection.*

FIGURE 4. Potential applications of MBL in a clinical setting. (A) *Effect of low light levels on SARS-CoV-2 inactivation using MB. We applied a 10 µl aliquot of SARS-CoV-2 to R3 coupons and dried for 20 min. We added 10 µL of 10 µM MB to each coupon before treatment with 700 lux or <100 lux of light (the light level produced by the biosafety hood lights).* ***(B)*** *Effect of MB pre-treatment on SARS-CoV-2 inactivation. We cut coupons from R3 masks and soaked for 1 hr in 10 µM MB. We then dried the coupons in the dark for 2 days before adding 10 µl of virus to either the inner or outer layers. We exposed the samples to white light (50,000 lux) for 30 min and determined the virus titer by plaque assay.* ***(C)*** *Inactivation of a SARS-CoV-2 clinical specimen by MBL. We obtained a saliva specimen from a COVID-19 patient with a titer of 1.1 x 10^5 PFU/ml for SARS-CoV-2. We applied 10 µl aliquots to coupons cut from a R3 mask and treated with 10 µM MB and exposed to white light (50,000 lux) for 30 min. We determined virus titer by plaque assay.* ***(D)*** *Effect of MB pre-treatment on MHV inactivation using intact masks. We pretreated intact R3 and FW masks with 10 µM MB by spraying the front and back with a*

total of 7-8 ml of MB and allowing to dry overnight in the dark. We inoculated the dried masks with MHV and exposed to white light (50,000 lux) for 30 minutes. We then excised inoculated areas before elution and titration. We determined virus titer by $TCID_{50}$ assay. ND = Not detected. RM= 3M 1860 half-sphere respirator. FH= Type IIR Halyard face mask. Dotted line indicates limit of detection.

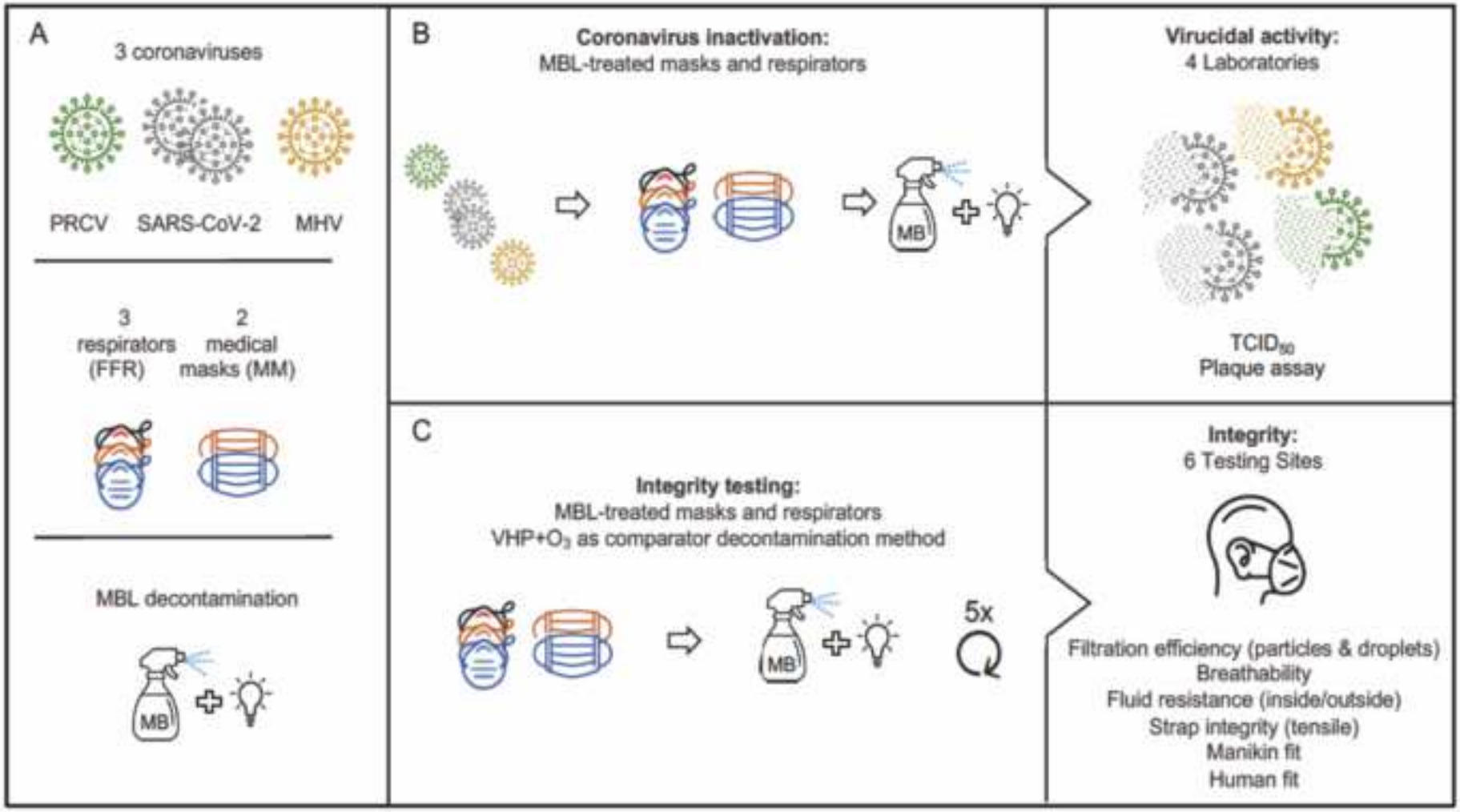
FIGURE 5. Effect of MBL and VHP+O_3 treatments on NaCl sub-micron filtration efficiencies and breathability before and after 5CD. (A) *NaCl sub-micron filtration efficiency is a measure of the ability of an FFR or MM to capture aerosolized particles smaller than one micron, expressed as a percentage of particles that do not pass the material at a given velocity or flow rate.* ***(B)*** *Inhalation and* ***(C)*** *exhalation breathing resistances before and after 5CD. The resistance to airflow during inhalation and exhalation is an indication of the difficulty in breathing through the respirators/masks. *Results from decontaminated FFRs and MMs are significantly different from untreated masks (Student's t test or Mann-Whitney U test $p<0.01$).RH= Halyard duckbill respirator (Fluidshield-46727). RM= 3M half-sphere respirator (1860). *Horizontal solid line represents the N95 filtration efficiency requirement of ≥95% particle filtration efficiency according to 42 CFR Part 84. **Horizontal lines represent the following breathing resistance standards: Inhalation: ≤35 mmH2O; exhalation: ≤25 mmH_2O for respirators according to 42 CFR Part 84. Note: EN 149 maximum inhalation resistance at 95 L/min is 2.4 mbar, or approximately 24 mmH_2O. At higher flow rate according to EN 149, the equivalent breathing resistance may increase slightly but can be similar to the 42 CFR Part 84 maximum inhalation resistance at 85 L/min.*

RH= Halyard duckbill respirator (Fluidshield-46727). RM= 3M half-sphere respirator (1860). R3= 3M panel respirator (1870+). FW= EN 14683 Type II generic face mask. FH= ASTM F2100 Level 2 Halyard face mask.

FIGURE 6. Effect of MBL and VHP+O_3 treatments on human and manikin fit factor of FFR/MMs. (A) *We performed human fit testing with volunteer participants who adjusted the FFR and MM to achieve the highest fit factor or seal and subsequently performed head movements and remeasured fit or seal.* ***(B)*** *Manikin fit factors using advanced, realistic manikin headforms is a reproducible method to test fit without volunteer participants. We used the PortaCount® PRO+ 8038 machine to determine overall fit for both human participants and manikins headforms. *Indicates significantly different values between treated and untreated FFR or MM at $p<0.05$, Student's t- test or Mann-Whitney U test, as appropriate. **Horizontal line represents the following standard: ≥100; OSHA 29 CFR 1910.134(f)(7). Percentages on or above each bar represents % of respirators or masks tested that surpassed this standard. While the standard does not apply to face masks, we present the % to note the strong difference between respirator and face mask test results. †Horizontal solid line represents the following standard: Per OSHA 1910.134(f)(7), if the Overall Fit Factor as determined through an OSHA-accepted quantitative fit testing protocol is equal to or greater than 100 for tight-fitting half facepieces, then the fit test has been passed for that respirator. RH=Halyard duckbill respirator (Fluidshield-46727). RM=3M half-sphere respirator (1860). R3=3M panel respirator (1870+). FW=EN 14683 Type II generic face mask. FH=ASTM F2100 Level 2 Halyard face mask.*

Figure 1_Graphical Abstract

DeMaND Graphical Abstract – Decontamination of masks and filtering facepiece respirators using methylene blue photochemical treatment



Panel A: Viruses, decontamination methods and personal protective equipment used in the DeMaND study. Panel B: Virus inactivation testing process. Panel C: Integrity testing process. Abbreviations: PRCV = porcine respiratory coronavirus, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, MHV = murine hepatitis virus, MBL = methylene blue + light, VHP+O_3 = vaporized hydrogen peroxide plus ozone

Figure 2_Methylene blue and light (MBL) tissue culture plate inactivation

Click here to access/download;Figure;Lendvay_Figure_2.tiff

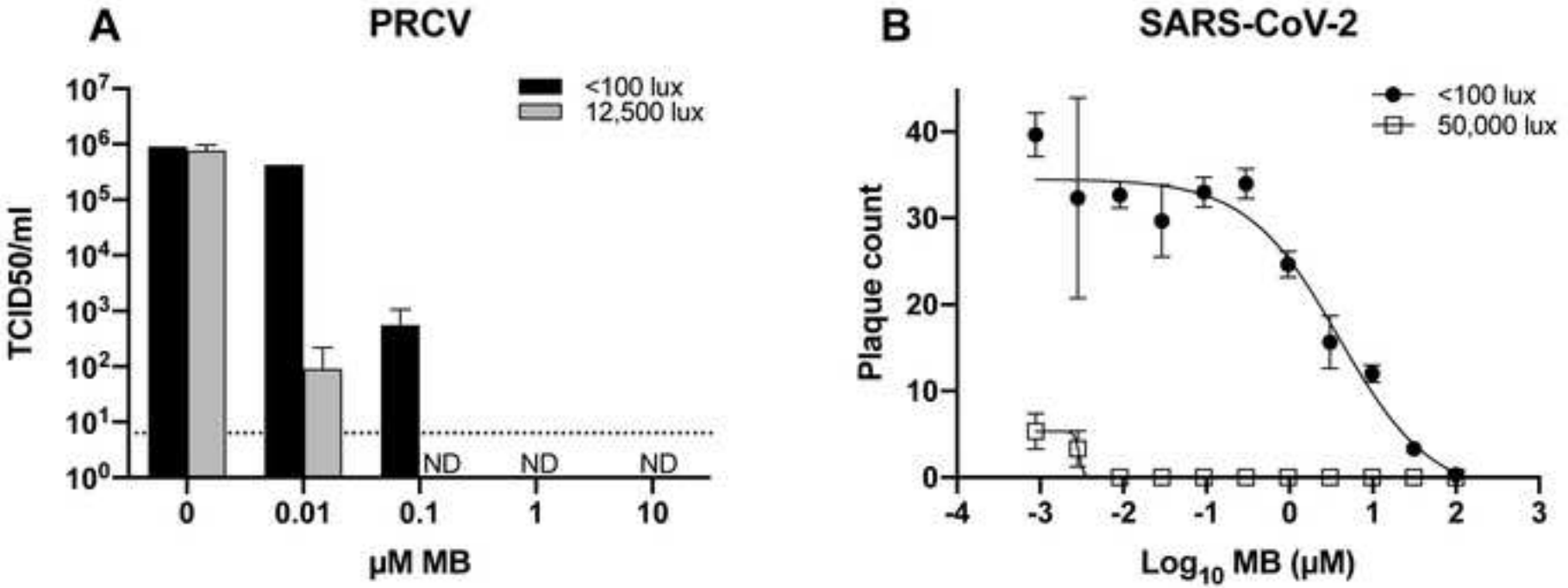


Figure 3_MBL FFR and MM material inactivation

Click here to access/download;Figure;Lendvay_Figure_3.tiff

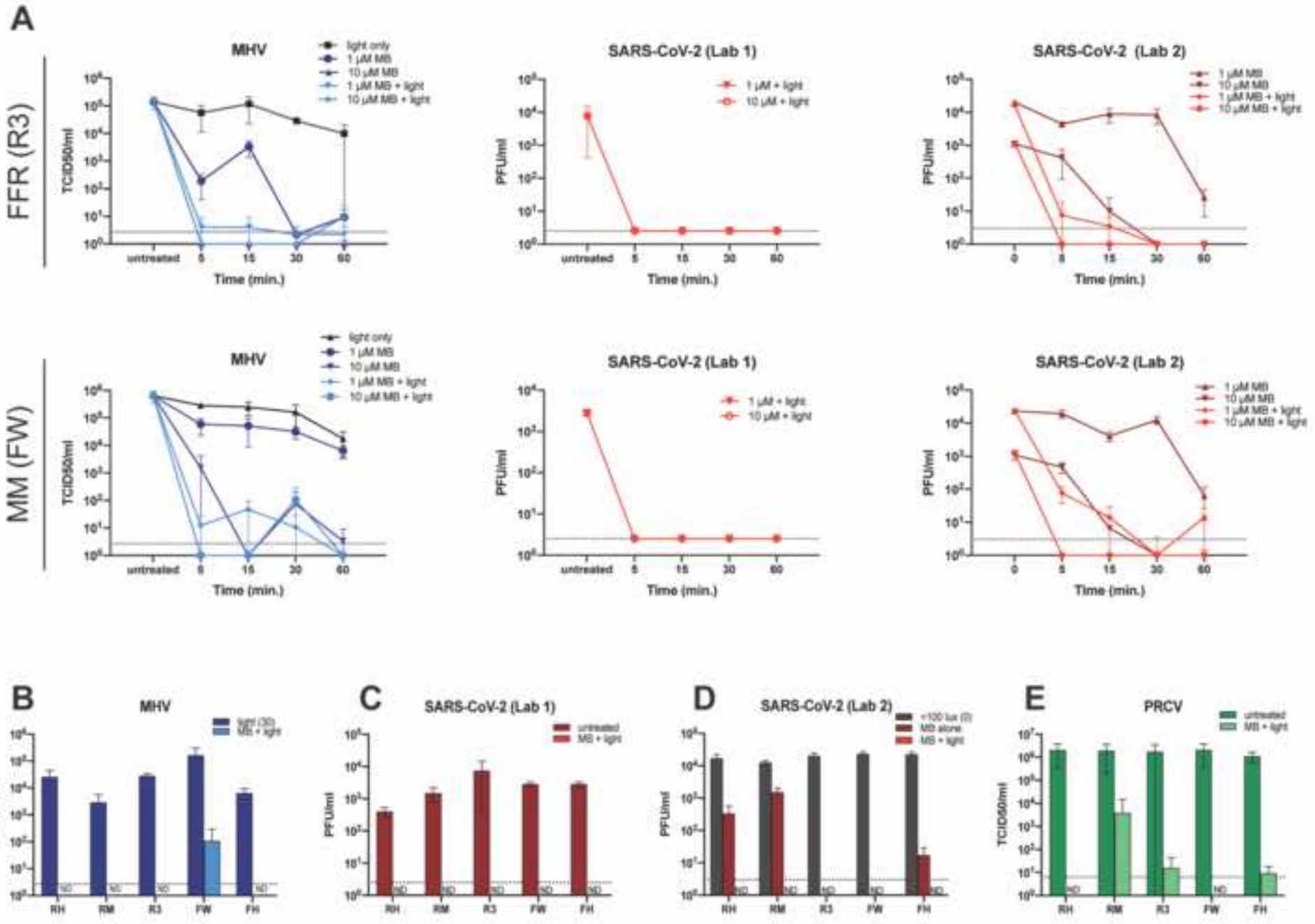


Figure 4_Clinical specimen and pretreatment with MB

Click here to access/download;Figure;Lendvay_Figure_4.tiff

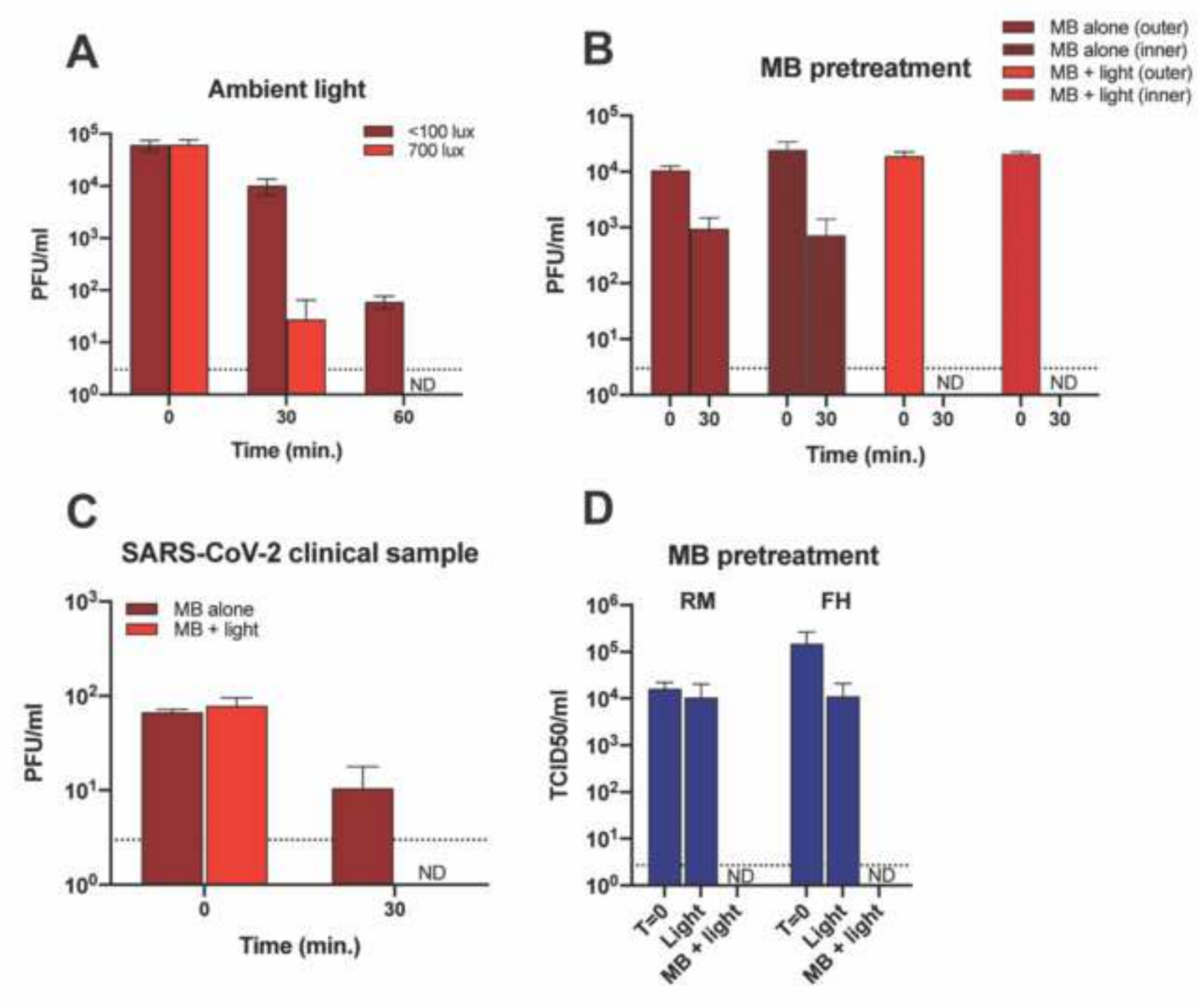
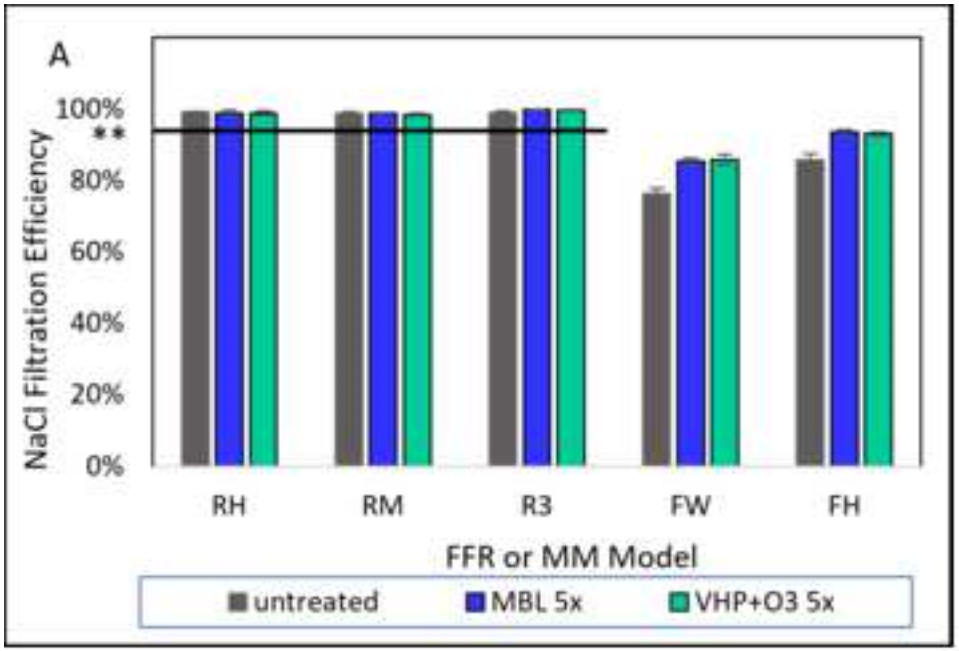
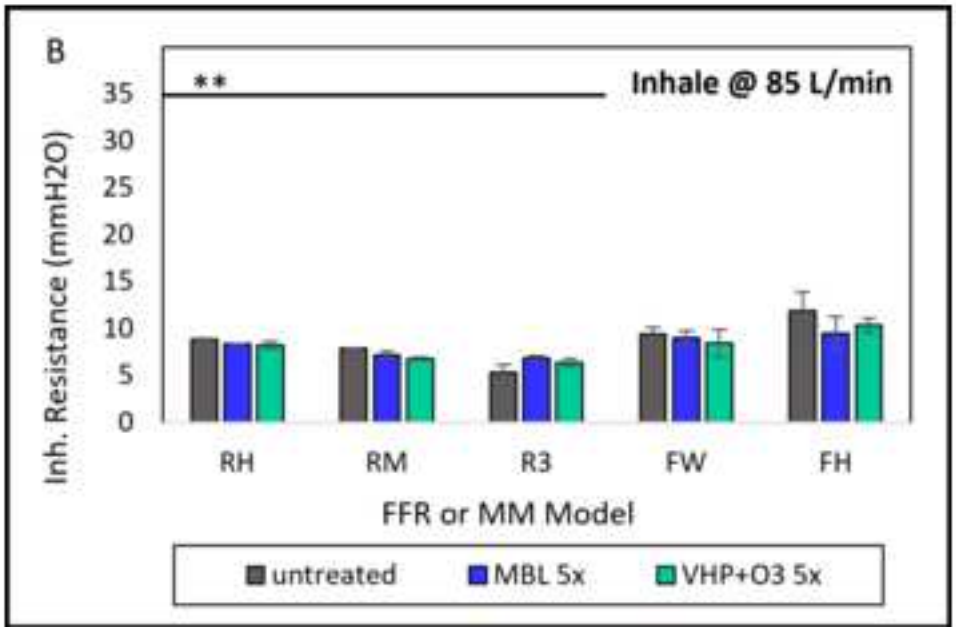


Figure 5_Integrity testing_Filtration and Breathability

Click here to access/download;Figure;Lendvay_Figure_5.tiff





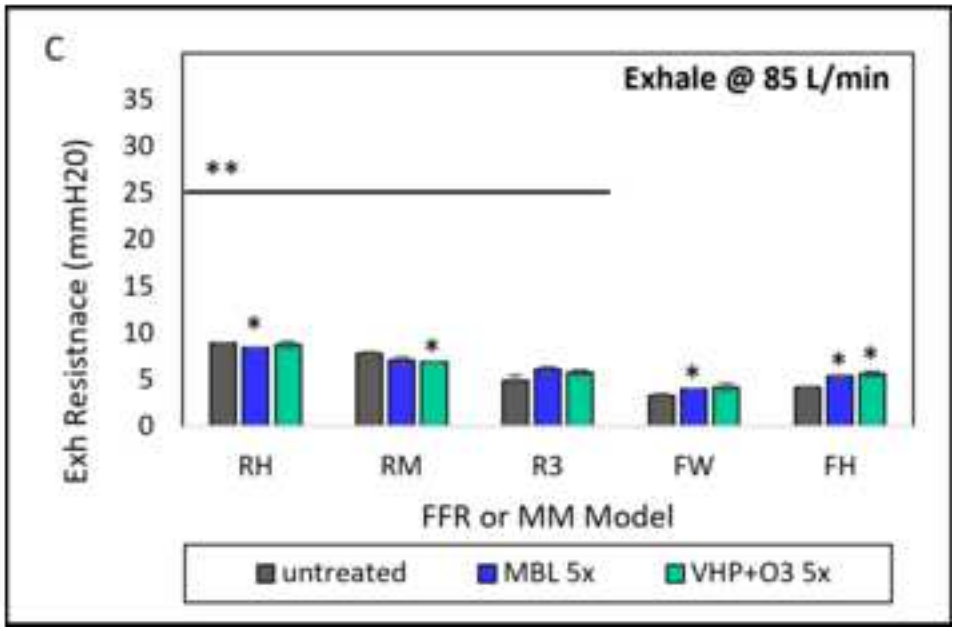


Figure 6_Fit Testing

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