Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Journal of Infectious Diseases 100 (2020) 224–229

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

The effect of ultraviolet C radiation against different N95 respirators inoculated with SARS-CoV-2

David M. Ozog[a,*], Jonathan Z. Sexton[b,c,d], Shanthi Narla[a], Carla D. Pretto-Kernahan[e], Carmen Mirabelli[e], Henry W. Lim[a], Iltefat H. Hamzavi[a], Robert J. Tibbetts[f], Qing-Sheng Mi[g,h,i]

[a] Photomedicine and Photobiology Unit, Department of Dermatology, Henry Ford Health System, Detroit, MI 48202, USA
[b] Department of Internal Medicine, Gastroenterology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
[c] Department of Medicinal Chemistry, College of Pharmacy, Ann Arbor, MI 48109, USA
[d] University of Michigan Center for Drug Repurposing, USA
[e] Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
[f] Department of Microbiology, Department of Pathology and Laboratory Medicine, Henry Ford Health System, Detroit, MI 48202, USA
[g] Center for Cutaneous Biology and Immunology, Department of Dermatology, Henry Ford Health System, Detroit, MI 48202, USA
[h] Immunology Program/Henry Ford Cancer Institute, Henry Ford Health System, Detroit, MI 48202, USA
[i] Department of Internal Medicine, Henry Ford Health System, Detroit, MI 48202, USA

ARTICLE INFO

Article history:
Received 19 July 2020
Received in revised form 24 August 2020
Accepted 28 August 2020

Keywords:
Ultraviolet C
N95
SARS-Co-V2
Healthcare personnel
Reuse
COVID-19

ABSTRACT

Objectives: There are currently no studies that have examined whether one dosage can be uniformly applied to different respirator types to effectively decontaminate SARS-CoV-2 on N95 filtering facepiece respirators (FFRs). Health care workers have been using this disinfection method during the pandemic. Our objective was to determine the effect of UVC on SARS-CoV-2 inoculated N95 respirators and whether this was respirator material/model type dependent.

Methods: Four different locations (facepiece and strap) on five different N95 FFR models (3M 1860, 8210, 8511, 9211; Moldex 1511) were inoculated with a 10 μL drop of SARS-CoV-2 viral stock (8×10^7 $TCID_{50}$/mL). The outside-facing and wearer-facing surfaces of the respirators were each irradiated with a dose of 1.5 J/cm^2 UVC (254 nm). Viable SARS-CoV-2 was quantified by a median tissue culture infectious dose assay ($TCID_{50}$).

Results: UVC delivered using a dose of 1.5 J/cm^2, to each side, was an effective method of decontamination for the facepieces of 3M 1860 and Moldex 1511, and for the straps of 3M 8210 and the Moldex 1511.

Conclusion: This dose is an appropriate decontamination method to facilitate the reuse of respirators for healthcare personnel when applied to specific models/materials. Also, some straps may require additional disinfection to maximize the safety of frontline workers. Implementation of widespread UVC decontamination methods requires careful consideration of model, material type, design, and fit-testing following irradiation.

Introduction

The shortage of personal protective equipment (PPE) affects healthcare workers worldwide during the coronavirus disease 2019 (COVID-19) pandemic. The ability to decontaminate and reuse N95 filtering facepiece respirators (FFRs) is a partial solution to the current shortage (Prevention CfDCa, 2020). We previously proposed decontaminating respirators with repurposed dermatology office phototherapy devices, which serve as a platform for ultraviolet C (UVC) (254 nm) germicidal disinfection (Hamzavi et al., 2020). On March 31, 2020, during the height of the pandemic, Henry Ford Health System (HFHS) began decontaminating 3M 1860 respirators with UVC and returning them to their original

Abbreviations: FFRs, filtering facepiece respirators; UVC, ultraviolet C; SARS-CoV-2, Severe Acute Respiratory Syndrome coronavirus 2; COVID-19, coronavirus disease 2019; PPE, personal protective equipment; BSL3, Biosafety Level 3; DMEM, Dulbecco's Modified Eagle Medium; FBS, Fetal Bovine Serum; HEPES, hydroxyethyl piperazineethanesulfonic acid; CPE, cytopathic effect; HCP, healthcare personnel; WHO, World Health Organization; PBS, phosphate-buffered-saline; HFHS, Henry Ford Health System; UVGI, ultraviolet germicidal irradiation; $TCID_{50}$, 50% tissue culture infectious dose.

* Corresponding author at: Photomedicine and Photobiology Unit, Department of Dermatology, Henry Ford Medical Center New Center One, 3031 W. Grand Blvd Suite 800, Detroit, MI 48202, USA.
E-mail address: DOZOG1@hfhs.org (D.M. Ozog).

https://doi.org/10.1016/j.ijid.2020.08.077

users. UVC dosing was extrapolated from earlier virology work. Since then, several thousand respirators have been decontaminated; one HFHS hospital alone decontaminated 5797 respirators from April 7, 2020 to July 2, 2020, with a single device.

Recently, Fischer et al. demonstrated that UVC (260−285 nm) effectively decontaminated N95 respirators inoculated with SARS-CoV-2 (Fischer et al., 2020). However, previous studies have demonstrated that UVC (254 nm) decontamination is dependent on the material/model type of N95 respirators (Mills et al., 2018; Heimbuch and Harnish, 2019 Heimbuch and Harnish, 2019). Furthermore, there are currently no studies that have examined whether one dosage can be uniformly applied to different respirator types to effectively decontaminate SARS-CoV-2 on N95 filtering facepiece respirators (FFRs). Therefore, this study's objective was to determine the effect of UVC on decontamination of SARS-CoV-2-inoculated N95 respirators using a variety of FFRs that are available to healthcare employees at Henry Ford Health System in Detroit, MI.

Methods

The study was performed as a collaboration between HFHS and the University of Michigan. All study procedures were approved and conducted according to the University of Michigan Institutional Biosafety Committee BSL3 (Biosafety Level 3). The appropriate training and medical surveillance for experimental procedures and manipulations performed in the BSL3 facility were satisfied by all individuals directly involved in laboratory testing at the University of Michigan.

Virus and preparation of viral stocks

The SARS-CoV-2 strain used was USA-WA1/2020 NR-52281. Viral stocks of SARS-COV-2 were obtained from the Biodefense and Emerging Infections Research Resources Repository. They were propagated in Vero-E6 cells grown in Dulbecco's Modified Eagle Medium (DMEM) without phenol red, with 2% Fetal Bovine Serum (FBS), L-glutamine, penicillin/streptomycin, non-essential amino acids, and hydroxyethyl piperazineethanesulfonic acid (HEPES). The virus stock was purposely produced in a phenol red-free medium to avoid photodegradation or photooxidation that could affect the results. For stock virus titration, aliquots of viral stock were applied on confluent Vero-E6 cells in 96-well plates for a 50% tissue culture infectious dose ($TCID_{50}$) assay. Viral stocks were determined to be 8×10^7 $TCID_{50}$/mL.

Test respirators and UVGI device

Respirators were tested 100% intact and included the following models: 3M 1860 (St. Paul, MN); 3M 8210 (St. Paul, MN); Moldex 1511 (Culver City, CA); 3M 8511 (St. Paul, MN); and 3M 9211 (St. Paul, MN). The low-pressure mercury lamp ultraviolet germicidal irradiation device (UVGI) (254 nm, 1 series) was manufactured by Daavlin (Byron, OH), with custom dimensions (22 in. × 10 in. × 8 in.) to fit under the BSL3 biosafety hood. The device's irradiance was approximately 16.5 mW/cm^2 at a distance of 11.5 cm from the lamps (approximately at the apex of the N95 respirator). The UVGI device used four lamps, spaced 4.5 cm apart. In comparison, the devices used by HFHS to decontaminate respirators for healthcare personnel had an irradiance of approximately 10 mW/cm^2 at a distance of 11.5 cm from the lamps. This UVGI device had ten lamps, spaced 11 cm apart. Despite the differences, the units are similar in performance. Before initiating the experiment, the irradiance of the device was measured using a calibrated UVC meter UV512C (General Tools and Instrument, Secaucus, NJ, USA), as we have described previously (Kohli et al., 2020).

Decontamination studies

Intact FFRs in a donned position were inoculated on the outside-facing surface with a single 10 μL drop of viral stock 8×10^7 $TCID_{50}$/mL) on four areas to account for differing received doses on complex surfaces: nosepiece, apex, chin-piece, and strap (Figure 1A). Inoculated respirators were dried in a biosafety cabinet at room temperature for 40 min. For each N95 respirator model, FFRs were UVC-irradiated or left untreated as positive controls for viral load recovery. The respirators were then placed under the UVGI device, in the center, and were individually treated with a dose of 1.5 J/cm^2. Then, they were rotated, and the wearer-facing side of the N95 was again irradiated with 1.5 J/cm^2. The irradiation time for each side was approximately 60–70 s. The device does not generate any heat; thus, all FFRs were exposed to UVC at room temperature. Immediately after the completion of the irradiation, 4 mm circles containing the inoculated surface were obtained with a leather belt eyelet hole punch tool and were placed in 300 μL (microliters) of PBS for one hour at room temperature.

Recovered viral loads were determined by $TCID_{50}$ assay of the absorbed samples. Briefly, 25 μL aliquots of serially 10-fold diluted samples were inoculated into 96-well plates with a Vero-E6 cell monolayer in sextuplicate and cultured in DMEM with 2% FBS, L-glutamine, penicillin/streptomycin, non-essential amino acids, and HEPES. The plates were observed for cytopathic effects for four days. Viral titer was calculated with the Reed and Müench endpoint method (Reed and Muench, 1938). Viral yields were expressed as total $TCID_{50}$ recovered in 300 μL or $TCID_{50}$/4 mm punch. $TCID_{50}$ negative controls were cells with media only and were included on each plate assayed. All negative controls had no cytopathic effect (CPE). The limit of detection (LOD) for the $TCID_{50}$ assay was determined to be $10^{1.3}$ $TCID_{50}$/4 mm punch. If the number of viral particles was below the LOD, then a theoretical, yet low content of viruses may be present. However, an absence of CPE in the Vero E6 cells at four days post-inoculation indicates a loss of infectivity and is evidence of inactivation of the SARS-CoV-2 samples (Figure 1B). We considered effective decontamination to be the elimination of all infectious SARS-CoV-2 defined as results below the LOD with no CPE.

Results

Following preliminary testing (Appendix Figure 1, Appendix Table 1), virus inoculation was performed on all five types of respirators. For each type, three were irradiated with UVC, and one was not irradiated to serve as a positive control. Similar results were seen for the 3M 1860 respirators, as in the preliminary study. All facepiece locations were below the LOD with absent CPE. Two straps were above the LOD, and one strap was below the LOD with absent CPE. Sufficient virus ($\leq$1 log reduction) was recovered from the untreated positive controls on all facepiece locations; however, a lower yield was recovered from the untreated control strap (Figure 2, Appendix Table 2).

On the 3M 8210 respirators, location 1 had two respirators above the LOD and one respirator below the LOD with absent CPE. Location 2 had one FFR at the LOD and two FFRs below the LOD with absent CPE. Location 3 and all the straps were below the LOD with absent CPE. Lower virus yields were recovered from the untreated positive control on all facepiece locations. In contrast, the strap did not absorb the droplet, and a sufficient yield was obtained. (Figure 2, Appendix Table 2). Of note, the amount of virus recovered from the strap on the untreated positive control was higher than the untreated control virus stock (10 μL in PBS control). This could have been due to a loss of viral titer of the stock as the control sat in PBS solution for an hour during the experiment.

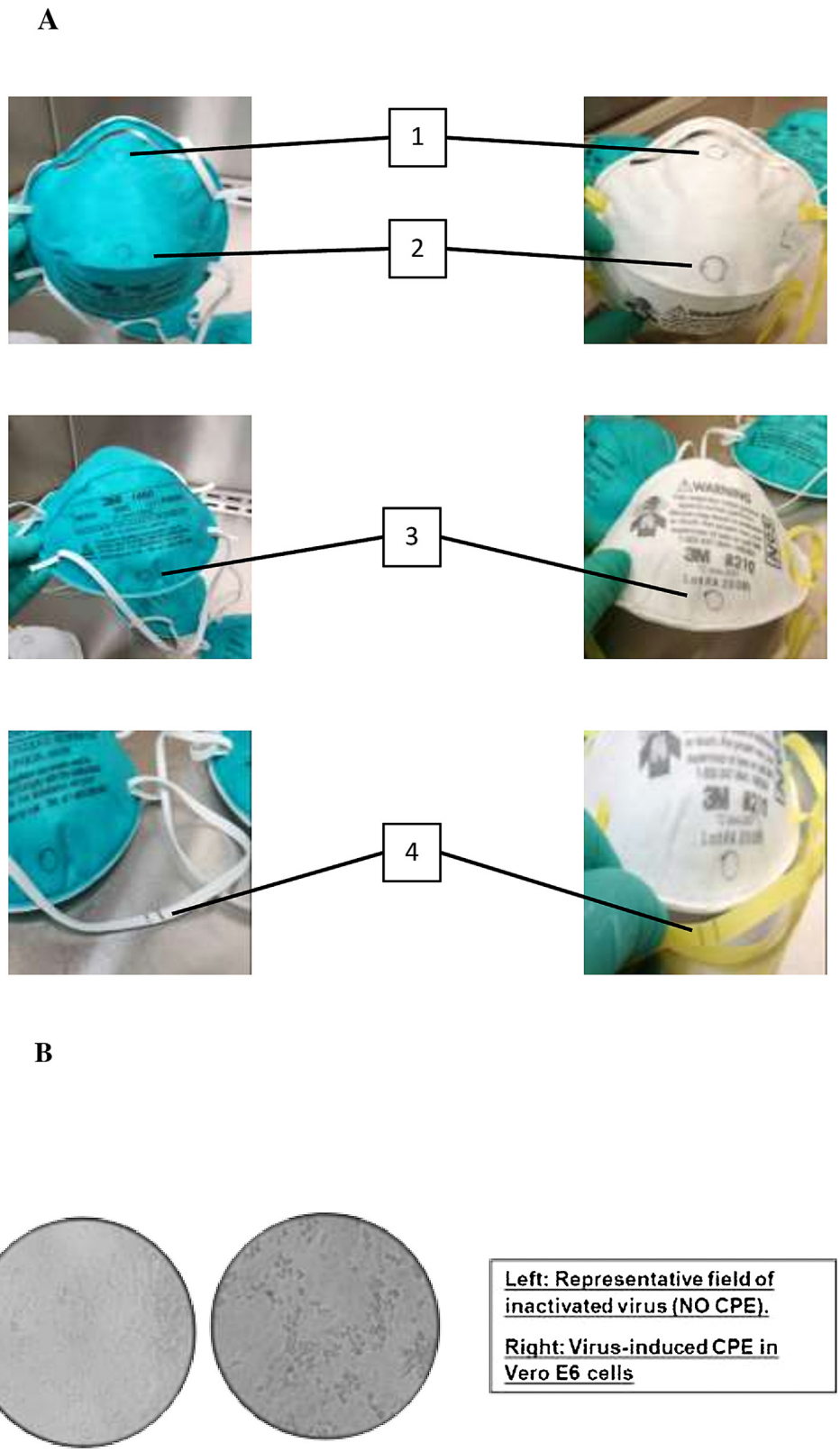

Figure 1. (A) Locations 1-4 (Nosepiece, Apex, Chin, Strap) on models 1860 and 8210.
[a]Similar locations were sampled on each of the five N95 respirators.
(B) Bright-field microscopy of wells with Vero-E6 cells and SARS-CoV-2.
[a]CPE = cytopathic effect.

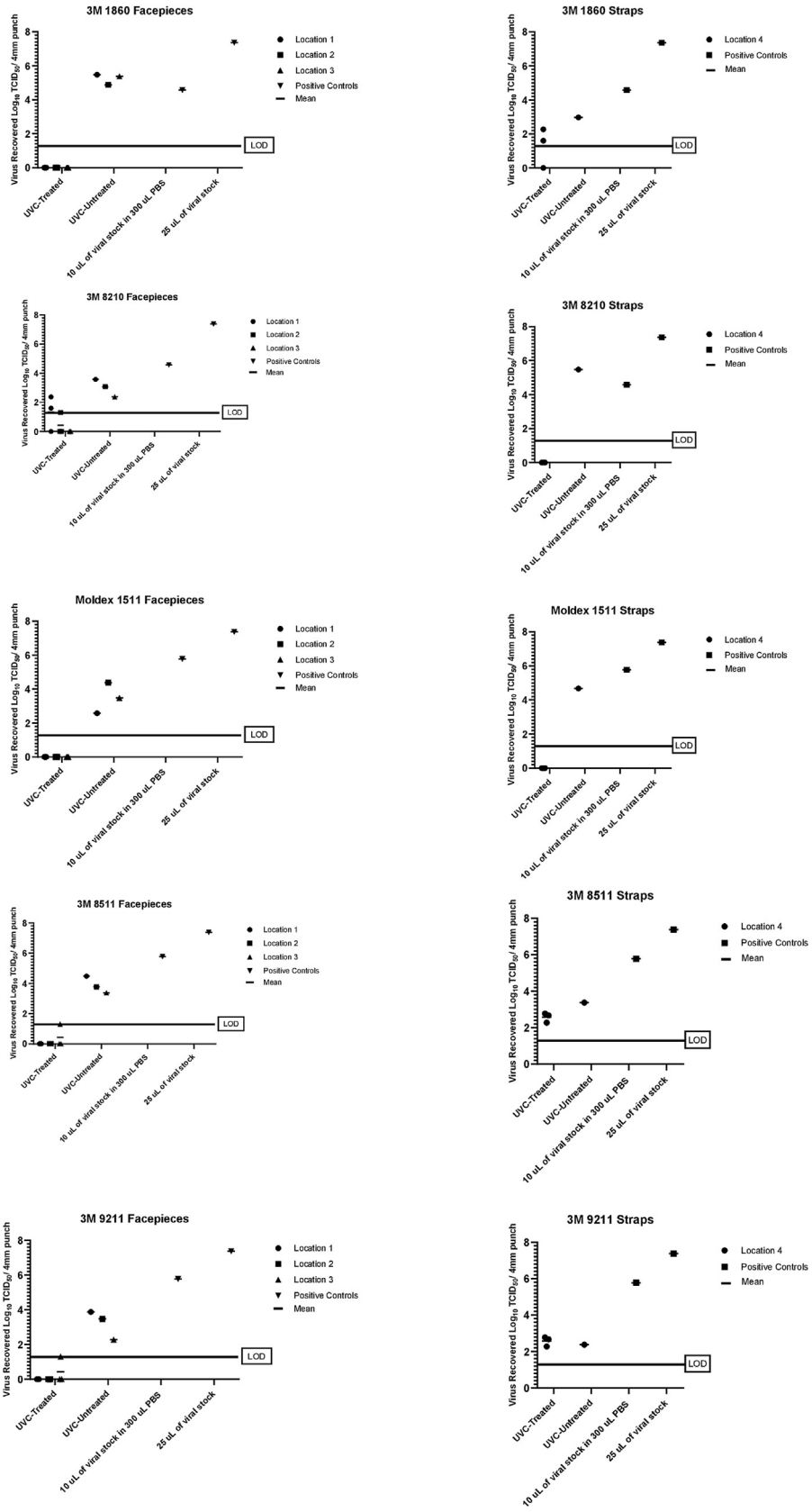

Figure 2. Recovered SARS-CoV-2 TCID50/4 mm punch.
[a]Wells that were below the limit of detection (LOD) and had no cytopathic effect were arbitrarily assigned the value of zero to represent this phenomenon in the above graphs.

On the Moldex 1511, all facepiece locations and straps were below the LOD with absent CPE. However, there was a lower virus recovery from specific facepiece locations (1 and 3) on the untreated positive respirator (Figure 2, Appendix Table 2).

For the 3M 8511 and 3M 9211, locations 1 and 2 had FFRs all below the LOD with absent CPE. Location 3 had one FFR at the LOD and two respirators below the LOD with absent CPE. All the straps were above the LOD. All facepiece locations and the strap on the untreated control had lower virus recovery as compared to the 10 μL in PBS control (Figure 2, Appendix Table 2).

Discussion

Five N95 respirator models were inoculated with SARS-CoV-2 and tested. UVC, delivered using a dose of 1.5 J/cm^2 to each side, was an effective method of decontamination for the facepieces of 3M 1860 and Moldex 1511, and for the straps of 3M 8210 and the Moldex 1511. This is consistent with previous results with H1N1 influenza, demonstrating that UVC decontamination is dependent on model and material type (Mills et al., 2018; Heimbuch et al., 2019). Mills et al. and Heimbuch et al. reported 1 J/cm^2 dose may not be adequate to kill H1N1 influenza depending on the N95 respirator used. Mills et al. found that only facepieces on twelve of 15 models and straps on seven of 15 models showed a significant (≥3 log) reduction of H1N1 influenza viability. Similarly, Heimbuch et al. found that only facepieces on eleven of 15 models and straps on four of 15 models showed a significant (≥3 log) reduction of H1N1 influenza viability (Narla et al., 2020).

Some respirator models have materials, such as the straps of the 3M 1860, that demonstrate hydrophilic characteristics when inoculated. Moreover, these seemingly hydrophilic surfaces showed consistently lower mean log reduction <3 $\log_{10}$ $TCID_{50}$ (Heimbuch and Harnish, 2019). In contrast, seemingly hydrophobic materials, such as the 3M 1860 facepiece, were found to demonstrate a >3 $\log_{10}$ $TCID_{50}$ reduction (Heimbuch and Harnish, 2019). In our study using SARS-CoV-2, we observed similar results with the 3M 1860 facepiece and strap. Further, the facepieces of the 3M 8210 have hydrophilic properties, which were reflected in the reduced decontamination results, while the straps did not readily absorb the droplets, and hence were adequately disinfected. The Moldex 1511 facepiece and straps also appeared to be hydrophobic and did not absorb the droplets.

Some straps are prone to twisting. Consequently, when the respirator is flipped during the irradiation process, care must be taken to ensure the appropriate surface of the strap is exposed to UVC. Also, straps should not inadvertently lay on top of the respirator, hence creating a shadowing effect. Reduced decontamination seen amongst the straps may not only be a result of material but may also be secondary to receiving a reduced dosage. UVC devices that provide 360 degrees of irradiation may obviate this issue. Possible respirator-based solutions include a secondary disinfection step (e.g., Environmental Protection Agency recommended cleansers) applied only to the straps. Further, ancillary disinfection testing was performed on the 3M 1860 straps using over-the-counter 70% isopropyl alcohol prep pads (TopCare, Elk Grove Village, IL). The straps were inoculated with SARS-CoV-2 and wiped three times with the alcohol pad. Results showed that regardless of UVC irradiation, alcohol alone was sufficient to decontaminate the 3M 1860 straps (Appendix Figure 2, Appendix Table 3). Additionally, manufacturers may consider using, for example, the same material as the straps of the 3M 8210 for all the other models of FFRs to improve UVC decontamination.

Our dosage for this study was partially based on previous work with Influenza A (H1N1), Avian influenza A virus (H5N1), Influenza A (H7N9) A/Anhui/1/2013, Influenza A (H7N9) A/Shanghai/1/2013, MERS-CoV, and SARS-CoV (Mills et al., 2018; Heimbuch and Harnish, 2019; Narla et al., 2020; N95DECON, 2020), where it was determined that all areas of a respirator should receive at least 1 J/cm^2. Data from the Photomedicine and Photobiology Unit at HFHS demonstrated through theoretical and measured models that the curvature and the distance of the 3M 1860 N95 respirator from the light source affected the dosage delivered, in a predictable way. Moreover, extrapolating from this model, after irradiating one side of the respirator with 1.5 J/cm^2, some of the lateral aspects may only receive 900 mJ/cm^2 while the apex of the respirator may receive almost 3 J/cm^2. Further, it was also observed that a certain percentage of the dosage received in an area (~10%) permeates to the other side (Kohli et al., 2020). Therefore, 1.5 J/cm^2 was chosen as the lowest radiant exposure (i.e., fluence) to ensure that all areas received at least 1 J/cm^2.

Increasing the dosage delivered may improve decontamination, but UVC radiation can degrade certain polymers in a dose-dependent manner, and the effects may vary significantly between different models (Torres et al., 2020). Lindsley et al. exposed circular coupons from different N95 respirators to UVC doses ranging from 120 to 950 J/cm^2. The exposure led to a small increase in particle penetration (up to 1.25%). At dosing >950 J/cm^2, a more significant effect on the strength of the layers of the respirator materials (in some cases, >90% decrease) was observed. Less effect was seen on the respirator straps tested, requiring a dose of 2360 J/cm^2 to reduce the breaking strength by 20–51% (Lindsley et al., 2015). The limitation of this study was that fit-testing was not performed, which limits applicability. Heimbuch et al. tested 15 different types of respirators for up to 20 cycles of UVC treatment (approximately 1 J/cm^2 per cycle). Their results demonstrated that 20 cycles did not have any significant effect, including fit-testing, airflow resistance, or particle penetration. However, while 10 cycles did not have any significant effect on the straps, 20 cycles may have a significant effect on a few models, including the 3M 1860. Further, while ten donning/doffing cycles did not demonstrate any meaningful effect, 20 donning/doffing cycles may affect respirator fit, affecting the performance of certain models (Heimbuch and Harnish, 2019). Therefore, fit-testing of UVC decontaminated respirators must be performed each time a new model and/or dose is introduced into the healthcare system (Ozog et al., 2020). Through our previous work, we have demonstrated outstanding fit-testing performance with 3M model 1860 after 20 cycles totaling 60 J/cm^2 (Ozog et al., 2020). For this reason, HFHS treated only the 3M model 1860 respirators with a maximum of five cycles.

Our study sampled different areas of each respirator to ensure that all ranges of dosages were accounted for in a real-world setting against SARS-CoV-2. Other strengths included the testing of different model types. Of note, the hydrophilic surfaces (e.g., 3M 8210 facepiece and 3M 1860 strap) of untreated positive controls demonstrated a lower virus recovery than control. Additional testing was performed to determine if the droplet was drying larger than the 4 mm area tested. The results showed that there was limited to no virus in the periphery of the 4 mm area tested and that no virus could be detected on the wearer-facing surface. Moreover, the lower yield reflects a diminished ability to re-suspend the virus after drying. Limitations of the study include that no soiling agents were used. However, at Henry Ford Health System, as in other healthcare facilities, personnel are instructed not to reuse respirators that are visibly soiled.

Further, it is still unclear what the infectious dose is for SARS-CoV-2; therefore, it is unknown if a significant reduction in viral load eliminates contagious risk. Based upon clinical observation at the HFHS sites, since beginning UVC decontamination of N95 respirators, it does not appear that there was a significant increase in healthcare personnel-related COVID-19 infection that could be potentially attributed to wearing N95 respirators that were not

decontaminated of infectious SARS-CoV-2. However, we were unable to trace whether COVID-19 positive employees had used UVC treated respirators, which is a major limitation to this assumption.

In conclusion, UVC at a dose of 1.5 J/cm^2 applied to both sides effectively decontaminates SARS-CoV-2 on some N95 respirators. This dose may only be an appropriate decontamination method to facilitate the reuse of PPE for healthcare personnel, when applied to specific models/materials. Further, this UVC dosage may not provide adequate decontamination against other more robust hospital-acquired respiratory infections; however, treatable respiratory infections have not been a significant cause of morbidity during the COVID-19 pandemic. Also, some straps may require additional disinfection to maximize the safety of the frontline workers. Implementation of widespread UVC decontamination methods requires careful consideration of model, material type, design, and fit-testing following irradiation. It should also be emphasized that similar cautions should be practiced for all other methods of respirator decontamination.

Conflict of interests

HWL is an investigator for the LITE study funded by PCORI in which the home phototherapy machines are provided by Daavlin. HWL has participated as a speaker in a general educational session for Ra Medical System. IHH is an investigator for the LITE study funded by PCORI in which the home phototherapy machines are provided by Daavlin.

Funding sources

Partial funding was provided by the William Clay Ford, Jr. and Lisa V. Ford Foundation.

Ethical approval

All study procedures were approved and conducted according to the University of Michigan Institutional Biosafety Committee BSL3 (Biosafety Level 3). The appropriate training and medical surveillance for experimental procedures and manipulations performed in the BSL3 facility were satisfied by all individuals directly involved in laboratory testing at the University of Michigan.

This article does not contain any studies with human or animal subjects. There are no human subjects in this article, and informed consent is not applicable.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2020.08.077.

References

Prevention CfDCa. Decontamination and Reuse of Filtering Facepiece Respirators. 2020. https://www.cdc.gov/coronavirus/2019-ncov/hcp/ppe-strategy/decontamination-reuse-respirators.html.

Hamzavi Iltefat H, Lyons Alexis B, Kohli Indermeet, Narla Shanthi, Parks-Miller Angela, CCRP, et al. Ultraviolet germicidal irradiation: possible method for respirator disinfection to facilitate reuse during COVID-19 pandemic. J Am Acad Dermatol 2020;82(6):1511–2, doi:http://dx.doi.org/10.1016/j.jaad.2020.03.085.

Fischer Robert J, Morris Dylan H, van Doremalen Neeltje, Sarchette Shanda, Jeremiah Matson M, Bushmaker Trenton, et al. Effectiveness of N95 respirator decontamination and reuse against SARS-CoV-2 virus. Emerg Infect Dis 2020;26(9).

Mills Devin, Harnish Delbert A, Lawrence Caryn, Sandoval-Powers Megan, Heimbuch Brian K. Ultraviolet germicidal irradiation of influenza-contaminated N95 filtering facepiece respirators. Am J Infect Control 2018;46(7):e49–55.

Heimbuch BK, Harnish D. Research to Mitigate a Shortage of Respiratory Protection Devices During Public Health Emergencies. 2019. https://www.ara.com/news/ara-research-mitigate-shortage-respiratory-protection-devices-during-public-health-emergencies.

Kohli Indermeet, Lyons Alexis B, Golding Bob, Narla Shanthi, Torres Angeli E, Parks-Miller Angela, et al. UVC germicidal units: determination of dose received and parameters to be considered for N95 respirator decontamination and reuse. Photochem Photobiol 2020;, doi:http://dx.doi.org/10.1111/php.13322 [published online ahead of print, 2020 Aug 7].

Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Epidemiol 1938;27(3):493–7.

Narla Shanthi, Lyons Alexis B, Kohli Indermeet, Torres Angeli E, Parks-Miller Angela, Ozog David M, et al. The importance of the minimum dosage necessary for UVC decontamination of N95 respirators during the COVID-19 pandemic. Photodermatol Photoimmunol Photomed 2020;.

Torres Angeli Eloise, Lyons Alexis B, Narla Shanthi, Kohli Indermeet, Parks-Miller Angela, Ozog David, et al. Ultraviolet-C and other methods of decontamination of filtering facepiece N-95 respirators during the COVID-19 pandemic. Photochem Photobiol Sci 2020;, doi:http://dx.doi.org/10.1039/d0pp00131g [published online ahead of print, 2020 May 15].

Lindsley William G, Martin Jr. Stephen B, Thewlis Robert E, Sarkisian Khachatur, Nwoko Julian O, Mead Kenneth R, et al. Effects of ultraviolet germicidal irradiation (UVGI) on N95 respirator filtration performance and structural integrity. J Occup Environ Hyg 2015;12(8):509–17.

Ozog David, Parks-Miller Angela, Kohli Indermeet, Lyons Alexis B, Narla Shanthi, Torres Angeli E, et al. The importance of fit-testing in decontamination of N95 respirators: a cautionary note. J Am Acad Dermatol 2020;83(2):672–4.