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Decontamination of SARS-CoV-2 on Filtering Face Piece Respirators (Ffrs) With Vapor Phase Hydrogen Peroxide and Post Decontamination Performance

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Abstract

Background: During the early months of the COVID-19 pandemic, N95 filtering face piece respirators (FFRs) were in short supply or not available. As a result, decontamination for reuse was authorized to deal with the temporary shortages under Emergency Use Authorizations (EUAs) by the U.S. Food and Drug Administration (FDA). The use of vapor phase hydrogen peroxide (VPHP) is the method used in 13 of the EUAs issued. While the efficacy of the VPHP method has been demonstrated for a variety of organisms, it previously had not been shown for an EUA authorized process with SARS-CoV-2 in biologically relevant matrices. This study demonstrated the efficacy of VPHP decontamination on FFRs contaminated with SARS-CoV-2 as well as the impact of decontamination on FFR performance.

Methods: SARS-CoV-2 in culture media, simulated saliva, or simulated lung fluid was spiked onto coupons cut from the filter material of eight different FFR models, allowed to dry and then subjected to VPHP decontamination using an FDA-authorized process in an active EUA. In addition, whole FFRs were subjected to 20 decontamination cycles and then assessed for performance by filtration efficiency, breathing resistance, hydrogen peroxide off-gassing, visual inspection and strap elasticity.

Results: Levels of SARS-CoV-2 on FFRs treated with VPHP were reduced to levels below detection for all conditions tested. Performance was also maintained after 20 decontamination cycles with the filtration efficiency and breathing resistance still meeting criteria, hydrogen peroxide off-gassing being below the acceptable level, and no visual damage upon inspection. Some changes in strap elasticity were observed for some FFR models though these were relatively small.

Conclusions: Decontamination of FFRs using VPHP in the system tested proved effective at reducing levels of SARS-CoV-2 to levels below detection limits while not impacting the performance criteria examined. The relationship between respirator fit and changes in strap elasticity is not well-established. Thus, individuals should perform user seal checks when donning a decontaminated mask. For FFRs that have been donned more than five times, the U.S. Centers for Disease Control and Prevention (CDC) recommends considering implementing a qualitative fit performance evaluation.

Keywords

SARS-CoV-2, N95 Filtering face piece respirator, Decontamination, Vapor phase hydrogen peroxide

Introduction

The COVID-19 pandemic created a high demand for N95 filtering face piece respirators (FFRs) which are used by medical personnel to reduce the risk of infection when providing care to persons with a contagious disease. These demands have been greater than the available supply causing shortages around the country. The CDC and the National Institute of Occupational Safety and Health (NIOSH) issued guidance for how to deal with FFR shortages [1]. Among these recommendations, decontamination for reuse was identified as an option for crisis shortages with treatment of FFRs using vapor phase hydrogen peroxide (VPHP) identified as a promising method.

The U.S. Food and Drug Administration (FDA) issued 13 Emergency Use Authorization (EUA) requests for systems utilizing VPHP in their decontamination process versus just one using steam and another using ultraviolet

germicidal irradiation (UVGI) [2]. A variety of studies have shown that VPHP treatment is able to inactivate microbes on the surface of FFRs without damaging FFR performance. One study examined the efficacy of VPHP to decontaminate SARS-CoV-2 on N95 FFRs using the VHP® ARD System (Steris, Mentor, OH) and found it to be effective [3]. Others examined the efficacy of VPHP to inactivate a variety of viruses including Phi-6, Influenza A, Mouse Hepatitis Virus (MHV) and bacteria including *Escherichia coli*, *Staphylococcus aureus*, and *Geobacillus stearothermophilus* spores [4-7]. In addition, it has been shown that VPHP treatment does not impact key performance parameters such as filtration efficiency and air flow resistance [8-11]. However, issues for fit testing have been observed for some VPHP decontamination methods [6,12,13]. Thus, it is prudent to perform tests with the specific decontamination system to ensure performance is maintained. To this end, this study examines the inactivation of SARS-CoV-2 on eight different FFR models, some covered by the EUA and others included simply to assess feasibility, as well as the performance after 20 decontamination cycles through the Battelle Critical Care Decontamination System™ (CCDS™). Note, the EUA was amended after this work was performed to limit the number of uses to five in total, which is only four decontamination cycles.

Materials and methods

Cells

Vero (African green monkey kidney) clone E6 cells (ATCC Cat. No. NR-596, Manassas, VA, USA) were used to propagate stock SARS-CoV-2 and perform virus infectivity assays. Cells were incubated at 37 °C with 5% carbon dioxide (CO_2) in complete cell culture media (Dulbecco's Modified Eagle Medium, Corning Cat. No. 10-010-CV, Corning, NY, USA) supplemented with 2% fetal bovine serum (FBS) (Gibco Cat. No. 10082147, Carlsbad, CA, USA) and penicillin-streptomycin (Gibco Cat. No. 15140122).

Virus

SARS-CoV-2 strain USA-WA1/2020 was obtained from BEI Resources (Manassas, VA, USA) and propagated in Vero E6 cells. Following the one-hour infection period in Vero E6 cells with the virus seed stock, the virus lysate supernatant was harvested after a 48- to 72-hour incubation time. The supernatant was centrifuged at 800 x g to remove cellular debris. The resulting suspension was a liquoted and frozen in single-use vials for testing.

The concentration of infectious virus was determined by a cell-based assay in 96-well plates to determine the median tissue culture infectious dose ($TCID_{50}$). This assay was performed by serially diluting the virus stock suspension and transferring each of the dilutions to corresponding wells that contained Vero E6 cells. Determination for cytopathic effects (CPE) on the Vero E6 cell mono layer was performed after 72 hours of incubation. Quantitation of CPE was determined by using the Spearman-Karber method. The limit for detection of this assay was 13.1 $TCID_{50}$ which is 1.12 $\log_{10}$ $TCID_{50}$.

Filtering face piece respirators

Eight FFRs were selected for this study. These FFRs were selected based upon their common use in the field, presence of unique features, and availability given that FFRs were in extremely short supply during the conducting of this work. The FFRs used were 3M Models 1860, 1870, 8210, 8233 and 8511 (St. Paul, MN, USA), Northern Safety (NS) Model 7210 (Utica, NY, USA) and Moldex Models 1512 and 2200 (Culver City, CA). The 3M 1860, 1870 and 3M 8210 as well as the Moldex models are commonly used by healthcare professionals, first responders and security professionals who interact with the public. Three models tested are not covered by the EUA; these are the NS 7210, which is not on the NIOSH list of certified N95s, the 3M 8511, which has an exhalation valve, and the 3M 8233, which is an N100 and has a higher filtration efficiency (99.97%) than the N95 FFRs. Their inclusion was for scientific purposes only.

Simulated saliva and simulated lung fluid

Simulated saliva was prepared as described elsewhere [14]. Simulated saliva was stored at 4 °C until use and any unused portion was discarded after one week and a new preparation was made. Simulated lung fluid was prepared based upon the work of Hassoun, et al. [15] and Kumar, et al. [16] and modified to use Hanks' Balanced Salt Solution (HBSS) as the diluent for the protein and antioxidant components [17].

Virus was concentrated and resuspended in simulated saliva or simulated lung fluid by using a centrifugal concentrator (Spin-X UF Concentrator, Corning Cat. No.CLS431491, Corning, NY, USA) with a 100 kiloDalton (kDa) molecular weight cutoff that retained the SARS-CoV-2 but allowed the complete cell culture media components to be removed. The retentate virus was resuspended (i.e., *quantum satis*; Q.S.) with either the simulated saliva or simulated lung fluid prior to spiking representative FFR coupons or whole FFRs.

Virus inactivation assays

Inactivation was performed with representative coupons (2 cm by 2 cm) cut from the FFRs. Coupons were inoculated with 100 µL of SARS-CoV-2 stock by placing nine droplets of 11.1 µL each onto the coupon. Droplets were allowed to dry on the coupons by evaporation (1 to 2 hours). Coupons were treated directly using VPHP

in a 498 L acrylic glove box (Plaslabs Lansing, MI) at ambient temperature. Fixed humidity salts were used to adjust relative humidity (RH) to a target of 75% for each experiment. Temperature and RH inside the test chamber were measured using a NIST-traceable data logger (Onset, Bourne, MA; MX1101). VPHP was generated using a commercial generator (Bioquell Clarus C, Horsham, PA) and VPHP concentration in the chamber was measured using a calibrated ATI B12 2-wire gas transmitter (Analytical Technology, Inc., Collegeville, PA) connected to a CNI-822 process controller (Omega Engineering, Norwalk, CT). VPHP concentrations were recorded using the iLOG software provided with the controller and readings were used to help control VPHP concentration in the chamber.

After placing the coupons in the chamber, FFRs coupons contaminated with SARS-CoV-2 were conditioned for 10 min. Then, VPHP was injected at a rate of 2 g/min into the chamber until a saturated atmosphere was achieved as indicated by micro-condensation. A saturated atmosphere was maintained by injecting VPHP at a rate of 0.5 g/min until a total exposure time of 150 minutes was reached. The chamber was then aerated for 300 min. Once complete, virus was extracted from each coupon by placing it in 10 mL of complete cell culture media that included 5% FBS and then agitating for 15 minutes at 200 rotations per minute (RPM) on a platform orbital shaker. The solution was then removed from the tube and concentrated down to 2 mL using a centrifugal concentrator (Spin-X UF Concentrator, Corning Cat. No. CLS431491) with a 100 kDa cutoff. The 2-mL concentrated samples were then filter-sterilized (Thermo-Fisher Cat. No. 720-1320, Waltham, MA, USA) through a low-binding, 0.2-µm filter before being assayed for infectious virus by $TCID_{50}$ assay.

Performance testing

VPHP exposure was performed exactly as described for the virus inactivation tests except that uncontaminated, whole FFRs were used. Assessment of performance included characterization of the initial aerosol collection efficiency, inhalation resistance, strap tensile properties and visual inspection of the FFR. Collection efficiency and airflow resistance were measured using a Model 8130A automated filter tester (TSI Inc., Shoreview, MN). The challenge aerosol and test flow rate were consistent with those used in NIOSH certification testing {National Institute for Occupational Safety and Health (NIOSH), 2019 #122}. Only the initial collection efficiency was measured, and aerosol loading was not performed. This test was a check of performance and not for recertification, and FFRs were not subjected to pre-conditioning prior to testing. Any changes in performance could be attributed to the VPHP decontamination exposure. FFRs were glued onto the test plate using hot glue to ensure a seal along the edge and then placed into the tester. FFRs were challenged with a salt aerosol and the tester reported the collection efficiency and inhalation resistance at 85 L/min. Strap elasticity was tested as described elsewhere [18]. In brief, this was a three-step process where a 10-centimeter section of the strap was stretched three times to 200%, 150% and then 200% of its initial length at a rate of 1 cm/s. Stress and load are reported for the straps at 200% strain. Visual inspection was performed to evaluate the integrity of the FFR.

Results

Virus inactivation

Coupons of all eight FFR models examined in the study were subjected to VPHP decontamination and then infectious SARS-CoV-2 virus was quantified by $TCID_{50}$ assay. In all cases, virus in cell culture media was inactivated below detection with a single exposure. These results are summarized in Table 1. Titers reported for the untreated samples differ according to the recovery of virus for the FFR type in that experiment.

In addition, coupons for two of the FFRs were also tested with virus in simulated saliva and simulated lung fluid. In both cases, virus was inactivated to levels below detection for our assay (Table 2). Note, the recoveries for the test with simulated saliva were less than 3 log_{10} of virus which is lower than the desired target, but as FFRs were in short supply at the time of this work, it was not possible to repeat these experiments to obtain results with a higher recovery.

FFR performance after VPHP treatment

Performance assessments were done after 20 cycles and included measuring aerosol collection efficiency, inhalation resistance, off-gassing of hydrogen peroxide and strap elasticity (Table 3). Visual inspection was also performed, and no visual damage was observed.

Table 1: Inactivation of SARS-CoV-2 in culture media on coupons from eight FFR models.

	FFR Model and Titer ($log_{10}TCID_{50}$)							
Condition	3M 1860	3M 1870	3M 8210	3M 8233	3M 8511	Moldex 1512	Moldex 2200	NS 7210
Untreated	4.1	4.0	3.8	4.7	3.2	3.8	3.9	4.4
Treated	BD*	BD	BD	BD	BD	BD	BD	BD

*Below Detection

Table 2: Inactivation of SARS-CoV-2 in simulated saliva and simulated lung fluid on coupons from two FFR models.

	Simulated Saliva Titer ($log_{10}TCID_{50}$)		Simulated Lung Fluid Titer ($log_{10}TCID_{50}$)	
FFR Model	Untreated	Treated	Untreated	Treated
3M 1860	2.1	BD*	4.8	BD
3M 8511	1.9	BD	5.2	BD

*BD: Below Detection

Table 3: Performance testing of eight FFR models after 20 VPHP decontamination cycles.

FFR Model [a]	Cycles	Collection Efficiency (%)[b]	Inhalation Resistance (mm H_2O)[c]	VPHP Off-Gassing (ppm)[d]	Strap Stress at 200% Strain (MPa)	Strap Load at 200% Strain (N)
3M 1860	0	99.7 ± 0.1	8.4 ± 0.6	< 0.1	0.75 ± 0.07	3.71 ± 0.34
	20	99.6 ± 0.1	9.0 ± 0.4	0.2 ± 0.1	1.14 ± 0.08	5.74 ± 0.38
3M 1870	0	99.7 ± 0.3	7.1 ± 0.4	0.1 ± 0.0	1.23 ± 0.14	2.39 ± 0.22
	20	99.8 ± 0.1	7.0 ± 0.1	0.2 ± 0.1	1.12 ± 0.06[e]	2.13 ± 0.11[e]
3M 8210	0	99.7 ± 0.4	7.0 ± 0.5	< 0.1	1.44 ± 0.09	5.77 ± 0.14
	20	99.8 ± 0.1	6.8 ± 0.2	0.2 ± 0.1	1.21 ± 0.30	4.65 ± 0.30
3M 8233	0	99.994 ± 0.002	15.1 ± 0.6	< 0.1	2.01 ± 0.50	22.9 ± 5.51
	20	99.995 ± 0.004	15.3 ± 0.2	0.1 ± 0.0	2.38 ± 0.42[e]	26.6 ± 4.7[e]
3M 8511	0	98.9 ± 0.8	6.2 ± 0.4	0.1 ± 0.0	1.06 ± 0.10	5.49 ± 0.53
	20	99.2 ± 0.3	6.5 ± 0.2	0.4 ± 0.1	1.16 ± 0.03	6.29 ± 0.18
Mx 1512	0	98.8 ± 0.5	8.9 ± 0.5	< 0.1	1.63 ± 0.05	5.83 ± 0.21
	20	98.9 ± 0.5	9.2 ± 0.4	< 0.1	1.28 ± 0.13	4.42 ± 0.51
Mx 2200	0	98.2 ± 0.5	9.8 ± 0.4	< 0.1	2.24 ± 0.16	7.08 ± 0.41
	20	98.5 ± 0.5	9.6 ± 0.8	< 0.1	1.98 ± 0.20	6.11 ± 0.58
NS 7210	0	99.5 ± 0.2	9.1 ± 0.3	< 0.1	1.86 ± 0.07	4.94 ± 0.18
	20	99.2 ± 0.3	8.2 ± 0.3	< 0.1	1.66 ± 0.11	4.52 ± 0.29

[a]Mx: Moldex; [b]Requirement is > 95% for N95 models and > 99.97% for N100 models (i.e., 3M 8233); [c]Requirement is < 32 mm H_2O for N95 and N100 models; [d]Compared to OSHA PEL of 1 ppm; [e]Strap elasticity not statistically different from untreated samples by one-way ANOVA with $p < 0.05$.

Specific requirements exist for collection efficiency and inhalation resistance. In all cases, the FFRs subjected to 20 cycles of VPHP decontamination still met the requirements for these performance measures. Off-gassing of hydrogen peroxide vapor was compared against the OSHA PEL which is 1 ppm [19]. For strap elasticity, statistically significant differences between the untreated and treated FFRs were observed for most FFRs for both stress and load at 200% strain. Two FFRs, the 3M 1870 and 3M 8233, did not have statistically different changes in strap stress and load. Increases in strain/load were observed for the 3M 1860, 3M 8511, and Northern Safety 7210. Reductions in strain/load were observed for the 3M 8210, Moldex 1512, and Moldex 2200. In all cases, visual inspection did not reveal any potential issues with the FFRs.

Discussion

Treatment of FFRs contaminated with SARS-CoV-2 with VPHP using the Battelle CCDS™ reduced infectious virus titers to levels below detection. Reduction of virus was seen in selected models even when virus was placed in simulated saliva or simulated lung fluid. This is similar to the results reported by Kumar, et al. for a different VPHP treatment process for SARS-CoV-2 [3] and consistent with the many reports showing inactivation of viruses and bacteria by this method [4-7]. Performance testing for filtration efficiency and air flow resistance demonstrated no meaningful difference in performance after 20 VPHP decontamination cycles as the initial filtration efficiency was > 95% and the inhalation resistance was < 35 mm H_2O. Visual inspection of the FFRs did not reveal any apparent physical changes or damage. None of the straps broke, but there were some slight changes in elasticity (both increasing and decreasing depending on the make/model and associated strap material). However, fit testing was not performed in this study because FFR

quantities were extremely limited at the time of this study. Future studies that include fit testing are needed to fully understand the impact of decontamination on these masks. Other considerations worthy of additional assessment include the impact of contaminating the FFRs with aerosols containing virus, more similar to what would be experienced during actual use, and inoculation with different concentrations of virus. In addition, this work was done with new FFRs without normal wear and multiple uses which will introduce other soil to the mask that could impact performance and decontamination efficacy. Though, the extent of soil on the mask should limited because the recommendation to discard soiled FFRs remained even when decontamination for reuse efforts were underway.

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Competing Interests Statement

It is acknowledged that Battelle manufactured and operated the Battelle CCDS™ on contracts for the U.S. Government that was tested here in. Persons involved in the performance of this work were assigned to test and evaluation functions and hence were not involved in the performance of work for the CCDS™ contracts.

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