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MAJOR ARTICLE

Validation of N95 Filtering Facepiece Respirator Decontamination Methods Available at a Large University Hospital

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Background. Due to unprecedented shortages in N95 filtering facepiece respirators, healthcare systems have explored N95 reprocessing. No single, full-scale reprocessing publication has reported an evaluation including multiple viruses, bacteria, and fungi along with respirator filtration and fit.

Methods. We explored reprocessing methods using new 3M 1860 N95 respirators, including moist (50%–75% relative humidity [RH]) heat (80–82°C for 30 minutes), ethylene oxide (EtO), pulsed xenon UV-C (UV-PX), hydrogen peroxide gas plasma (HPGP), and hydrogen peroxide vapor (HPV). Respirator samples were analyzed using 4 viruses (MS2, phi6, influenza A virus [IAV], murine hepatitis virus [MHV)]), 3 bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Geobacillus stearothermophilus* spores, and vegetative bacteria), and *Aspergillus niger*. Different application media were tested. Decontaminated respirators were evaluated for filtration integrity and fit.

Results. Heat with moderate RH most effectively inactivated virus, resulting in reductions of >6.6-$\log_{10}$ MS2, >6.7-$\log_{10}$ Phi6, >2.7-$\log_{10}$ MHV, and >3.9-$\log_{10}$ IAV and prokaryotes, except for *G stearothermohphilus*. Hydrogen peroxide vapor was moderately effective at inactivating tested viruses, resulting in 1.5- to >4-$\log_{10}$ observable inactivation. *Staphylococcus aureus* inactivation by HPV was limited. Filtration efficiency and proper fit were maintained after 5 cycles of heat with moderate RH and HPV. Although it was effective at decontamination, HPGP resulted in decreased filtration efficiency, and EtO treatment raised toxicity concerns. Observed virus inactivation varied depending upon the application media used.

Conclusions. Both moist heat and HPV are scalable N95 reprocessing options because they achieve high levels of biological indicator inactivation while maintaining respirator fit and integrity.

Keywords. decontamination; inactivation; N95; reprocessing; virus.

The N95 respirator is the most commonly used filtering facepiece respirator (FFR) and removes at least 95% of airborne particles [1]. When caring for patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the Centers for Disease Control and Prevention (CDC) recommends the use of an N95 or better respirator [2]. Due to unprecedented N95 respirator shortages, many healthcare systems have implemented reprocessing of N95 respirators [3]. Until recently, few studies addressed effectiveness and feasibility of large-scale N95 decontamination and reuse [4–9].

In March 2020, the CDC issued guidance stating that hydrogen peroxide vapor (HPV), ultraviolet germicidal irradiation, and moist heat were "the most promising FFR decontamination methods" [10]. The US Food and Drug Administration (FDA) issued Emergency Use Authorizations (EUA) for N95 respirators, but important data are lacking with regards to virucidal and bactericidal efficacy of different modalities. The FDA recommendations for decontamination [11] offer a hierarchy of decontamination EUAs, ranging from ≥6-$\log_{10}$ inactivation of resistant spores or *Mycobacterium* (Tier 1) to ≥3-$\log_{10}$ inactivation of a nonenveloped virus or 2 vegetative bacteria (Tier 3). The recommendations do not specify an experimental medium for depositing microorganisms. Most FFR decontamination studies have used single biological indicators [4–6, 8, 12, 13].

Maintaining respirator fit [14] and N95 filtration performance are also critical safety components to consider. The

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National Institute for Occupational Safety and Health (NIOSH) standard for N95 respirators requires that 95% of particles sized between ~10 and 300 nm are prevented from passing through during respirator challenge tests [1].

We evaluated 5 different FFR decontamination strategies that were deemed practical for the University of Michigan Health System (UMHS) with regards to inactivation of several biological indicators, respirator fit, and filtration efficiency. We also addressed issues of practicality, scalability, and experimental approach.

METHOD AND MATERIALS

We analyzed several FFR treatment options, including moist heat, EtO, UV-PX, HPGP, and HPV for biological inactivation, filtration integrity, and fit. Biological inactivation was tested only on 3M 1860 respirators, whereas integrity and fit tests were conducted for other respirator types as well (Supplement Table S1).

Filtering Facepiece Respirator Integrity Testing

Particle filtration efficiency tests were conducted using a custom-built apparatus (Supplement Figure S1; Supplement Table S1). Assessments were based on differences in the number of NaCl aerosol particles that penetrated FFRs compared with the number used in the challenge test for each particle size and resulted in a calculated filtration efficiency (Supplement Figure S2). Filtration testing was conducted at higher flow rates than what is specified by NIOSH standard TEB-APR-STP-0059. This resulted in lower calculated filtration (Supplement Figure S3) and more conservative evaluation of FFR integrity. Breathability was assessed using differential pressure measured across FFRs at the test aerosol flow rate. Fit testing was performed by the Occupational Health Services [14] on the same individual pre- and postdecontamination.

Decontamination

Heat

Two approaches were tested to achieve moderate RH. First, we used Steris Vision Reliance Washers with controlled local humidity by placing FFR in Ziploc containers [15]. In brief, ~300 µL deionized water was added to a 7.5 × 7.5-cm^2 paper towel in sealed Ziploc medium square polypropylene containers. When run in the dry cycle of the instrument washer at 82°C for 30 minutes (after a 15- to 30-minute warm up), the inside of the container achieved RH between 65% and 75% and 80°C (Supplement Figure S4). The second method involved humidity controlled ovens (Supplement Figure S5). A laboratory-scale oven was initially used (123H; TestEquity, Moorpark, CA). Later, a hospital-scale humidity controlled oven was used for decontamination (Memmert model HCP240). Ovens were equilibrated at the desired RH (40%–90%) and heat (180–190°F) for >30 minutes and then run for a cycle time of 30 minutes.

Hydrogen Peroxide Vapor

We used a Bioquell Q10 whole room decontamination system. N95 respirators or respirator coupons were placed on racks with clips strung on wire. Treatment consisted of 3 phases: a Gassing phase in which hydrogen peroxide was vaporized and emitted into the room; a Dwell phase in which hydrogen peroxide levels were maintained; and an Aeration phase in which hydrogen peroxide was filtered from air. This process was tested under 2 conditions, with the second test mimicking the FDA EUA certified method (Supplement) [16].

Pulsed Xenon UV-C, Ethylene Oxide, and Hydrogen Peroxide Gas Plasma Treatments

A LightStrike Pulsed Xenon UV lamp (model PXUV4D; Xenex) was used to deliver polychromatic (200–315 nm) wavelengths of UV light across the UV-C and UV-B range (Supplement Table S2). Coupons were placed with the outer facing layers toward the light source, 1.8 m from the lamp. For EtO decontamination, we used a 3M Steri-Vac 5XL Sterilizer/Aerator. A low temperature Sterrad 100NX system was used for HPGP treatment (Supplement).

Inactivation Experiments

Biological Indicators

We used 4 model viruses (Supplement Table S3): 3 bacteria and 1 fungus. The viruses included +ssRNA bacteriophage MS2 and dsRNA bacteriophage Phi6 because they (1) are common human virus indicators [17, 18], (2) can be produced at high concentrations, (3) have rapid turnaround times for culture-based enumeration, and (4) do not require biosafety level (BSL)2 or BSL3 facilities. We also used a recombinant influenza A H3N2 strain (IAV) that produces firefly luciferase in infected cells because, like SARS-CoV-2, it is an enveloped ssRNA virus that is transmitted via droplets and perhaps aerosols [19]. We used the mouse coronavirus, murine hepatitis virus (MHV), which is in the same genus as SARS-CoV-2. For bacterial inactivation experiments, we used *Escherichia coli* obtained from the American Type Culture Collection (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Geobacillus stereothermophilus* (ATCC 12980; both vegetative bacteria and spore tablets [Pharmaceutical Biological Indicator For 6-Log Reduction: HPV-BI; Bioquell Inc., United Kingdom] were used), and *Aspergillus niger* (a patient isolate) (Supplement).

Deposition on N95 Filtering Facepiece Respirator Coupons

Circular coupons with 1-inch diameter were prepared from 3M 1860 N95 FFRs. Each coupon was weighed and placed in a petri dish. The MHV stock in its culture Dulbecco's modified Eagle's medium ([DMEM]; MHV medium) (Supplement Table S4) was deposited directly on coupons. The IAV was deposited in its culture DMEM (IAV medium) (Supplement Table S4).

The MS2/Phi6 deposition solutions consisted of the combined stock diluted to ~10^{10} plaque-forming units (pfu)/mL in either 1× phosphate-buffered saline (PBS) (Supplement Table S5) or IAV medium (Supplement Table S4). Fifty microliters of virus solutions were deposited on each coupon in 2-µL droplets resulting in a total of ~10^6 $TCID_{50}$ influenza, 5×10^8 pfu/mL MS2 and phi6, and ~10^4 pfu/mL MHV deposited on each coupon. For the EtO experiment, MS2 was sprayed onto the coupon. Coupons were dried in biosafety cabinets for 1.25 hours. For bacteria and fungus, overnight grown cultures were diluted in saline to a final concentration of 10^6–10^7 colony-forming units (CFU)/mL. For bacteria and fungus, overnight grown cultures were diluted in saline to a final concentration of 109 CFU/mL. For each prokaryote, 50 µL of a 109 suspension was applied as multiple drops to 1.25 × 0.25-inch rectangular coupons from 3M 1860 N95 FFRs. Coupons were dried before treatment. Each coupon with a specific indicator microorganism had a corresponding no-treatment control.

Extraction From N95 Filtering Facepiece Respirator Coupons

After treatments, biological indicators were extracted and enumerated with infectivity assays. For viruses, coupons were cut into 5–6 small pieces and then deposited in 1.3 mL of extraction solutions, consisting of (1) PBS with 1% bovine serum albumin (BSA) for MS2 and Phi6, (2) IAV medium supplemented with 1% BSA, HEPES, and antibiotics for IAV, and (3) MHV medium for MHV. The tubes were vortexed for 1 minute at half-speed. Viruses in the extract solutions were titered with their respective assays [20]. For bacteria and fungus, coupons were placed into 8 mL trypticase soy broth and vortexed for 10 minutes. Liquid aliquots (1, 10, or ~60 µL) were plated in duplicate in trypticase soy sheep blood agar for all strains except *A niger*, which was plated on Sabouraud Dextrose Agar. *Staphylococcus aureus* and *E coli* were incubated at 35°C, *G stereothermophilus* was incubated at 56°C, and *A niger* was incubated at 30°C. Plates were enumerated after 72 hours of incubation. The recoveries of the deposited viruses after drying and extraction ranged from 12% to 113%.

For all culture-based measurements, plates with at least 1 infectious unit (CFU) were included in calculations. For plates with no detectable colonies, we assume <1 infection unit for the least diluted sample when calculating concentrations. No-treatment controls were used for log reduction value (LRV) calculations.

RESULTS

Filtering Facepiece Respirator Integrity Under Different Decontamination Treatments

Filtering facepiece respirators met the NIOSH-approved N95 respirator standard through several cycles of decontamination by moderate RH heat, HPV, EtO, and UV-PX, but not HPGP treatment (Table 1 and Supplement Table S6). Although particle size-dependent filtration efficiency varied across cycles, it consistently remained above 95% (Supplement Figure S6). Breathability resistance was maintained between 16.5 and 17.5 mm H_2O, which are the same as new FFRs and higher than other reports [9, 21]. Fit testing was conducted only for decontamination methods that were considered promising, and it passed in all cases across multiple cycles (Table 1 and Supplement Table S6).

Table 1. Filtration Performance and Fit Test Results for 3M 1860 FFRs After Treatment for Decontamination

Decontamination Treatment	Minimum Filtration Efficiency[a,b] (No. of Treatment Cycles[c])	Fit Test Outcome No. Passed/No. Tested After (No. Cycles)
Low RH heat	>95% (10)	NT
Moderate RH heat	>98% (10)	3/3 (5)
Bioquell HPV	>99% (5)	5/5 (5)
HPGP	>74% (5)[d]	NT
EtO	>99% (1)	NT

Abbreviations: EtO, ethylene oxide; FFR, filtering facepiece respirators; HPGP, hydrogen peroxide gas plasma; HPV, hydrogen peroxide vapor; NT, not tested; RH, relative humidity.

[a]According to *National Institute for Occupational Safety and Health* guidelines, an N95 respirator is acceptable if filtration efficiency is larger than 95%.

[b]In all cases, pressure differential across FFR was maintained at 17.0 ± 0.5 mm H_2O.

[c]Single-cycle duration values, by treatment method using the method designated under Materials and Methods.

[d]Minimum filtration efficiency was <95% after 3 treatments.

Inactivation of Biological Indicators Under Different Decontamination Treatments

Pulsed Xenon UV-C

Treatment resulted in 0.5- to 1.3-log_{10} MS2, 0.0- to 2.0-log_{10} phi6, 0.8- to 1.7-log_{10} IAV, and >1.3- to 1.7-log_{10} MHV inactivation (Figure 1). The deposition solution impacted the extent of inactivation, with viruses deposited in IAV medium exhibiting less inactivation than viruses deposited in PBS. This effect was more pronounced for Phi6 than MS2.

Prokaryotic results showed that UV-PX yielded zero to 0.3-log_{10} inactivation for *G stereothermophilus*, and the *Geobacillus* spore tablet tested positive after treatment (Table 2). *Aspergillus niger* experienced 2.3-log_{10} inactivation. *Staphylococcus aureus* inactivation was 0.5 log_{10} for one test and >0.3 log_{10} for another, although the maximum detectable LRV was <1 log_{10}. *Escherichia coli* experienced 0.85- to >1.0-log_{10} inactivation.

Heat Treatment

Our industrial instrument washers had low RHs, even when wet towels were added (Figure 2). Moderate RH heat was initially achieved using Ziploc containers inside the washers as recently reported [15]. In these containers, the temperature stabilized at 80°C, and RH stabilized between 65% and 75% after ~13 minutes, and these conditions were maintained for ~15 minutes (Supplement Figure S4). The conditions resulted in high levels of virus inactivation for all tested viruses (Figure 2A). MS2, Phi6, IAV, and MHV inactivation was >6.9 log_{10}, >7.2 log_{10}, >3.4 log_{10}, and >.4 log_{10},

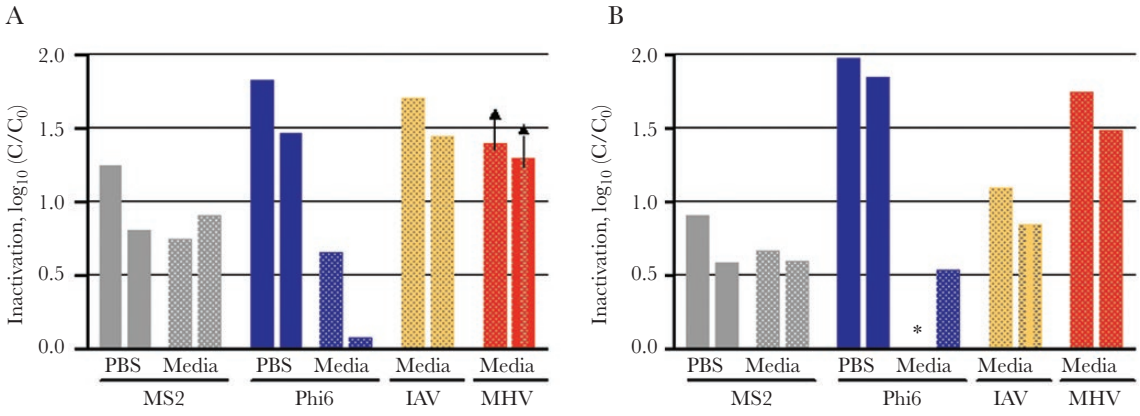

Figure 1. Virus inactivation on N95 respirators with pulsed xenon ultraviolet (UV) treatment. Studies A and B conducted on 2 different days. Arrows identify samples that exceeded assay detection limits after treatment. Asterisk indicates negligible inactivation. Replicates (n = 2) for each treatment condition are shown. Viruses were deposited on the coupons in either phosphate-buffered saline (PBS) or influenza A virus (IAV) media (Supplement Table S4 and Supplement Table S5). MHV, murine hepatitis virus.

respectively. Subsequently, UMHS purchased an industrial-scale, humidity-controlled oven. A pilot experiment with the humidity-controlled oven at 82°C and 45%–55% RH for 30 minutes resulted in inactivation levels exceeding the dynamic range of each virus (Figure 2B); >6.6-$\log_{10}$ MS2, >6.7-$\log_{10}$ Phi6, >3.9-$\log_{10}$ IAV, and >2.7-$\log_{10}$ MHV inactivation.

Moderate RH heat decontamination was not effective at inactivating the bacterial thermophile *G stereothermophilus* (Table 2). *Geobacillus* spore tablets were positive after treatment. Inactivation of *A niger* was >2.8 $\log_{10}$. *Staphylococcus aureus* inactivation was >2.4 $\log_{10}$ in the humidity-controlled containers and >3.2 $\log_{10}$ in the humidity-controlled hospital oven. *Escherichia coli* colonies were not detected on treated coupons for either method.

Hydrogen Peroxide Treatment

The Sterrad HPGP system resulted in high levels of virus inactivation (Supplement Figure S7). Phi6 and IAV inactivation exceeded the assay dynamic range after treatment, corresponding to >8.2- and >4.0-$\log_{10}$ inactivation, respectively. MS2 was inactivated by an average of 5.6 $\log_{10}$. Respirator integrity declined after 1 cycle and dropped below 95% after 3 cycles (Table 1).

The Bioquell HPV decontamination system was tested under 2 conditions. Condition 2 was consistent with the FDA EUA-approved protocol [16], and Condition 1 generated ~40% less vaporous hydrogen peroxide. Virus inactivation was inconsistent after both treatments, ranging from 1.5 $\log_{10}$ for Phi6 to >4.5 $\log_{10}$ for MS2 (Figure 3). More inactivation was observed when MS2 and Phi6 were deposited in IAV medium than in PBS.

Geobacillus spore tablets were negative after treatment with both Conditions 1 and 2 (Table 2). The *G stereothermophilus* spore culture was inactivated to >1.4 $\log_{10}$, >3.0 $\log_{10}$, and 2.8 $\log_{10}$ under conditions 1, 2, and 2, respectively. *Escherichia coli* exhibited >3.8 $\log_{10}$ inactivation. *Staphylococcus aureus* was inactivated only 1.0 $\log_{10}$ under Condition 1 and >1.6 and >2.3 $\log_{10}$ under Condition 2. *Staphylococcus aureus* colonies were detected on all treated coupons.

Ethylene Oxide Treatment

We conducted a single experiment with EtO and achieved > 4.8-log reduction of MS2 after treatment (Supplement Figure S8). Although effective for inactivating the MS2 virus, we did not expand testing due to concerns about residual EtO and toxicity to FFR wearers.

DISCUSSION

This work provides comprehensive information about multiple N95 FFR decontamination methods evaluated by assessing the impact of treatment method on respirator fit and integrity, the ability to inactivate multiple biological indicators, and the ability to scale each treatment approach to reprocess hundreds of FFRs daily. Three treatment approaches (UV, heat, and HPV) were selected for more intensive assessment under a variety of media matrix conditions.

Integrity testing did not reveal significant changes in filtration efficiency, breathability resistance, or fit for most decontamination methods after at least 5 cycles. The exception was HPGP, which reduced filtration integrity beyond standard criteria after 3 cycles. Our results are consistent with prior studies, which showed that moist heat [15], UVC [22], and HPV [23] are not harmful to the integrity of 3M 1860 FFRs after at least 5 cycles. Although there are no data on filtration integrity after UV-PX treatment, 3M 1860 FFRs maintained filtration efficiency after 4 cycles when treated with both UV-PX and moderate RH heat (Supplement Table S6). All respirators passed fit testing for moderate RH heat and HPV after 5 cycles (Supplement Table S6).

Using the FDA's EUA recommendation of 3-$\log_{10}$ virus inactivation as a baseline for decontamination of viruses, UV-PX

Table 2. Inactivation Data for Bacterial and Fungus Indicators

Treatment[a]	*Staphylococcus aureus*		*Escherichia coli*		*Geobacillus stereothermophilus*			*Aspergillus niger*	
	Measured LRV[b]	CFUs on Coupons[c]	Measured LRV[b]	CFUs on Coupons[c]	Measured LRV[b]	CFUs on Coupons[c]	Tablet[e]	Measured LRV[b]	CFUs on Coupons[c]
HPV Condition 1[d]	1.0	$3.3 \times 10^3/3.2 \times 10^4$	>3.8	$<1.3 \times 10^{2f}/>8.0 \times 10^5$	>1.4	$<1.3 \times 10^{2f}/3.3 \times 10^3$	Negative	>1.2	$<1.3 \times 10^{2f}/3.3 \times 10^3$
HPV Condition 2[d]	>2.3	$3.6 \times 10^3/>8.0 \times 10^5$	>3.8	$<1.3 \times 10^{2f}/>8.0 \times 10^5$	>3.0	$<1.3 \times 10^{2f}/1.4 \times 10^5$	Negative	>3.8	$6.7 \times 10^1/>8.0 \times 10^5$
HPV Condition 2[d]	>1.6	$1.8 \times 10^4/>8.0 \times 10^5$	NT		2.8	$6.7 \times 10^1/4.0 \times 10^4$	Negative	NT	
UV-PX, round 1	>0.3	$1.4 \times 10^6/>2.4 \times 10^6$	>1.0	$<1.7 \times 10^{2f}/1.3 \times 10^3$	0	$1.4 \times 10^5/1.4 \times 10^5$	Positive	NT	
UV-PX, round 2	0.5	$4.2 \times 10^5/1.3 \times 10^6$	0.85	$4.0 \times 10^2/2.8 \times 10^3$	0.33	$1.0 \times 10^4/2.1 \times 10^4$	NT	2.3	$2.8 \times 10^3/5.6 \times 10^5$
Moderate RH heat, round 1	>2.4	$8.8 \times 10^3/>2.4 \times 10^6$	>1.0	$<1.7 \times 10^{2f}/1.3 \times 10^3$	0.10	$1.1 \times 10^5/1.4 \times 10^5$	Positive	NT	
Moderate RH heat, round 2	>3.2	$<8.0 \times 10^{2f}/1.3 \times 10^6$	>0.5	$<8.0 \times 10^{2f}/2.8 \times 10^3$	0.60	$5.6 \times 10^3/2.1 \times 10^4$	NT	>2.8	$<8.0 \times 10^2/5.6 \times 10^5$
UV-PX + moderate RH heat, round 1	>2.7	$4.3 \times 10^3/>2.4 \times 10^6$	>1.0	$<1.7 \times 10^{2f}/1.3 \times 10^3$	0.17	$9.2 \times 10^4/1.4 \times 10^5$	Positive	NT	
UV-PX + moderate RH heat, round 2	>3.2	$<8.0 \times 10^{2f}/1.3 \times 10^6$	>0.5	$<8.0 \times 10^{2f}/2.8 \times 10^3$	1.4	$1.6 \times 10^3/2.1 \times 10^4$	NT	>2.8	$<8.0 \times 10^2/5.6 \times 10^5$

Abbreviations: CFUs, colony-forming units; HPV, hydrogen peroxide vapor; LRV, log reduction value; NT, not tested; RH, relative humidity; UV-PX, pulsed xenon UV-C.

[a]Treatments include condition 1 (short run) and condition 2 (long run that simulates the Battelle protocol); UV-PX treatment condition is the same for all rounds at fluence rate = 24 mJ/cm^2; low RH heat is in hospital instrument washer dry cycle; moderate RH heat round 1 is with sealed containers in the hospital instrument washer with drying cycle; moderate RH heat round 2 is with the hospital humidity controlled oven.

[b]LRV = (−1) × $\log_{10}$(CFU remaining after treatment/CFU applied).

[c]The average viable, extractable CFU remaining after treatment/viable, extractable CFU per untreated control, for coupons.

[d]Colonies were applied on the backside of respirators because of difficulty in applying to hydrophobic front side; consequently, recoveries were higher for *E coli*.

[e]Results shown are for treated *Geobacillus* tablets. Untreated controls are not shown but were always positive.

[f] No colonies detected so <1 colony assumed for calculations.

alone was insufficient. Others have reported higher MS2 and IAV $\log_{10}$ reductions on N95 respirators after UVC treatment [4, 5, 8, 24], although the lamps used in these studies generated primarily UV_{254} and provided a larger fluence rate (>1000 mJ/cm^2) than the Xenex UV-PX unit, which provided 200–280 nm at a distance of 1.8 m (~24 mJ/cm^2). No studies have directly compared inactivation of microorganisms with UVC_{254} to UV-PX on N95 respirators. Overall, UV-PX did not effectively inactivate prokaryotic indicators.

At moderate RH (50%–70%), heat treatment was effective, inactivating all viruses beyond the dynamic assay ranges. Previous work has demonstrated that IAV and MS2 are susceptible to moist heat treatment [4, 5, 25]. In contrast, the conventional sterilization bacterial spore indicator *Geobacillus* showed poor inactivation; notably, our heat temperature was well below the condition in autoclaves where this indicator is typically used. Poor inactivation of spore formers with moist heat is a notable limitation, and it has important infection control implications with regards to *Clostridium difficile*.

When the Bioquell HPV system was operated according to the FDA EUA-approved conditions, virus inactivation was less robust then with moist heat. Not all virus indicators exhibited greater than 3-$\log_{10}$ inactivation. This result is different from that of Kenney et al [26] who report ≥6-$\log_{10}$ inactivation of Phi6; however, they treated at 19.4 g/m^3 (our study used 12.8 g/m^3) with a much shorter dwell time but with more than twice the aeration time. The current study suggests that HPV results may be sensitive to methods used, and it is necessary to provide detailed protocols for comparisons to be made between studies. Although *Geobacillus*, *E coli*, and *A niger* inactivation levels were ~3 $\log_{10}$, we could not affirm >3-$\log_{10}$ inactivation for *S aureus*, and *S aureus* colonies were always detected on coupons. There is no standard for *S aureus* inactivation with HPV treatment, and it is known to be resistant to HPV treatment [27, 28]. The infection control implications of this are important, because *S aureus* is a nosocomial threat. In addition, *S aureus* commonly colonizes the nares of healthcare workers [29], and there is risk that the inner surfaces of respirators can become colonized with *S aureus*.

Experimental protocols vary, and we find that they can significantly influence results. The FDA EUA recommends that treatments achieve >3-$\log_{10}$ removal of viruses, but it does not give guidance on the application medium used to deposit viruses. The outcomes of this study suggest that achieving >3 $\log_{10}$ for a specific virus depends not only on the virus tested but also on the deposition solution used. With HPV, MS2 exhibited >3-$\log_{10}$ removal when it had been dried in IAV medium but <2 $\log_{10}$ when it had been dried in PBS. Thus, utilization of a cell culture medium for depositing SARS-CoV-2 may lead to overestimating the inactivation that takes place in respiratory droplets. Our complimentary study [20]

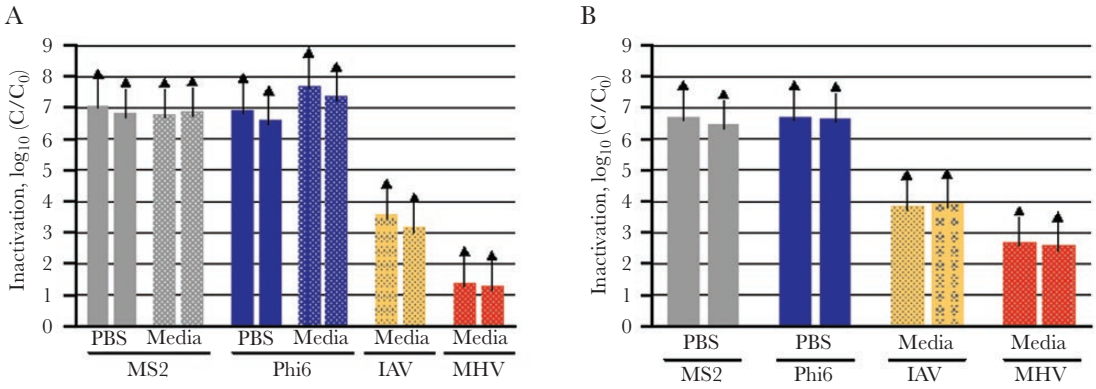

Figure 2. Virus removal with moderate relative humidity (RH) heat. Virus removal with moderate RH heat using (A) Ziploc container with ~70% RH and 80°C and (B) an industrial-scale temperature and humidity-controlled oven with ~50% RH and 82°C. Replicates (n = 2) for each treatment condition are shown. Arrows identify samples that exceeded assay detection limits after treatment. Viruses were deposited on the coupons in either phosphate-buffered saline (PBS) or influenza A virus (IAV) media (Supplement Table S4 and Supplement Table S5). MHV, murine hepatitis virus.

further explores the role of deposition matrix on virus inactivation by heat. Future studies on decontamination should clearly specify the microorganism deposition media that is used and, if possible, use the appropriate human material (eg, saliva).

The methodology in this study, where a diverse set of viruses were analyzed, can be used for evaluation of a wide range of viruses and for potential future pandemic scenarios. Murine hepatitis virus most closely resembles SARS-CoV-2; however, demonstrating 3-$\log_{10}$ reduction with this virus was technically challenging. The IAV experiments had larger dynamic range, up to 4 $\log_{10}$. The bacteriophage surrogates added additional experimental value because they had much higher dynamic ranges, usually >6-$\log_{10}$ inactivation, and experiments could be carried out quickly, without BSL2 facilities.

The results from this study present important issues that should be addressed by future research studies. Based on our results and the results of others, there seems to be a complex relationship between drying conditions, matrix, heat, and humidity, but a mechanistic understanding of how the organisms are inactivated does not yet exist. Mechanistic descriptions of virus inactivation in solution have been published, but they are lacking for viruses on fomites. For example, we observed an enhancement of inactivation when viruses were deposited in their culture medium. Future work should seek to describe the drivers for this enhanced inactivation. Mechanistic understandings of inactivation would help guide more accurate design and evaluation of N95 reprocessing.

When evaluating FFR decontamination modalities, hospitals should consider practical and logistical issues, including costs and aspects of implementation and maintenance (Table 3). The UV-PX had the shortest treatment cycle duration and HPV had the longest. The smallest dedicated space was required for moist heat, and the most space was required for HPV. Capital costs will vary by product and purchaser, and personnel effort required for each approach will also vary by site; consequently,

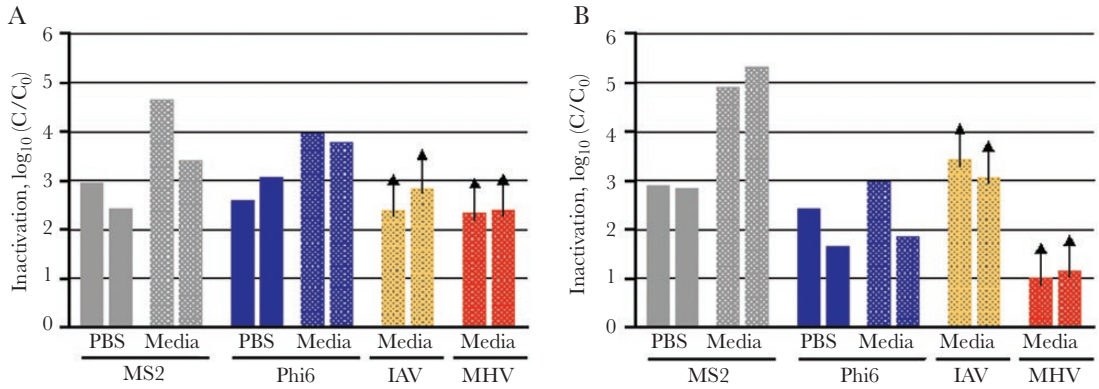

Figure 3. Virus removal with Bioquell hydrogen peroxide vapor (HPV) system. Virus removal with Bioquell hydrogen peroxide vapor (HPV) system with either (A) Condition 1 or (B) Condition 2. Replicates of treated coupons (n = 2) are shown. Arrows illustrate samples that exceeded assay detection limits after treatment. Viruses were deposited on the coupons in either phosphate-buffered saline (PBS) or influenza A virus (IAV) media (Supplement Table S4 and Supplement Table S5). MHV, murine hepatitis virus.

Table 3. A Comparison of Different Treatment Options Relative to Practical Considerations

	Microbiologic Activity		Practical and Operational Considerations for Treatment[a]		
Treatment Modality	Viruses	Bacteria	Time Needed/Cycle	Space Needed	Maintenance Costs[b]
UV-PX	+	+	<1 hour	Moderate	$
Moist heat	++++	+++[c]	<1 hour	Least	$
HPV	+++	+++[d]	~8 hours	Most	$$$

Abbreviations: HPV, hydrogen peroxide vapor; UV-PX, pulsed xenon UV-C.

[a]Estimates based on the filtering facepiece respirator (FFR) capacity per treatment cycle: hydrogen peroxide vapor (HPV) – 800 (~ 100/hour); moist heat – 75 (~ 75/hour); pulsed xenon UV-C (UV-PX) – 200 (~ 200/hour).

[b]Cost does not including initial purchase.

[c]Minimal activity against spore-forming bacteria (via *Geobacillus* experiments).

[d]Did not completely eradicate *Staphylococcus aureus*.

these costs were not considered in our analysis. With regards to operation and maintenance costs, heat with moderate RH and UV-PX were least expensive, with minimal ongoing costs after purchase. Hydrogen peroxide vapor was the costliest. Ease of implementation was similar for the 3 processes.

CONCLUSIONS

This research will help hospitals understand the capabilities and limitations of several N95 FFR decontamination approaches. Heat with moderate RH performed the best with regards to pathogen inactivation and was the reprocessing method implemented by our hospital; however, no approach was without limitation (Table 3). Ultimately, the needs of hospitals are likely to vary with regards to reprocessing volume, available capacity, and infrastructure needed to address N95 decontamination demands. Consequently, we conclude that no single FFR decontamination solution will work across all hospitals. Our study also highlights the importance of methodologic issues (eg, type of deposition media used) in evaluating FFR decontamination modalities. Future studies to validate FFR decontamination should include several viruses and deposit viruses in a range of solutions.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Extraction From N95 Filtering Facepiece Respirator Coupons

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