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## Major Article

## N95 reprocessing by low temperature sterilization with 59% vaporized hydrogen peroxide during the 2020 COVID-19 pandemic

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## Key Words:

Decontamination  
N95 filtering facemask

**Background:** Response to the COVID-19 pandemic by hospital systems has been strained by severe shortages in personal protective equipment (PPE), particularly N95 respirators. Recently, the Centers for Disease Control and Prevention endorsed decontamination strategies to prolong the lifespan of single use respirators. Battelle and Duke University have validated hospital protocols to decontaminate respirators using vaporized hydrogen peroxide (VHP) at 30%-35% concentrations. To prolong our supply of respirators, we evaluated and implemented VHP decontamination at 59% hydrogen peroxide concentration while detailing the effects of this process on the filtration efficiency and quantitative fit of single-use respirators. This study may help other health systems develop local solutions to their N95 mask shortage during this COVID-19 pandemic.

**Methods:** N95 respirators (3M 8211 FF and 9210 FF) that were treated with 5 and 10 cycles of VHP by the V-PRO maX Low Temperature Sterilization System were evaluated quantitatively for filtration efficiency as well as with quantitative fit testing per Occupational Safety and Health Administration standards. A decontamination protocol was concurrently implemented at our institution. This process involved depositing used masks, reprocessing, and re-distributing treated masks efficiently back to frontline providers. Furthermore, we implemented patient safety officers on COVID-19/person under investigation units to ensure optimized donning/doffing of respirators through frontline provider education.

**Results:** There were no statistically significant changes in mean filtration efficiency between the control and VHP-treated respirators. Furthermore, both treated and untreated respirators demonstrated fit factors above the minimum pass requirement.

**Conclusions:** We have successfully demonstrated that N95 respirator decontamination with VHP at 59% hydrogen peroxide can be safely utilized to decontaminate single-use N95 respirators without significant effects on filtration efficiency or quantitative fit testing. With the COVID-19 pandemic and N95 respirator shortage, health systems without access to commercial decontamination processes should investigate the viability of such a process in their facilities.

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## INTRODUCTION

The current COVID-19 pandemic has considerably disrupted the global supply chain of personal protective equipment (PPE), thus straining the capacities of healthcare systems around the world.<sup>1</sup> First isolated from the lower respiratory tract of a cluster of patients who

developed viral pneumonia after visiting the Huanan Seafood Market, SARS-CoV-2 is an enveloped single-stranded RNA virus within the family of betacoronavirus that causes coronavirus disease. (COVID-19).<sup>2,3</sup>

As numerous hospitals in the United States care for COVID-19 patients as well as patients under investigation, national, state, and local stockpiles of PPE have reached critical levels. As cases rise, a shortage of PPE, particularly N95 respirators will be prevalent.<sup>4</sup> The National Institute for Occupational Safety and Health (NIOSH) and Centers for Disease Control and Prevention (CDC) have recommended extended use and reuse of respirators and, most recently,

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recommended multiple decontamination methodologies for reuse when systems are faced with supply shortages. One of the decontamination methodologies supported by the CDC included the utilization of vaporized hydrogen peroxide (VHP).<sup>5</sup> The decontamination or sterilization of single-use N95 respirators is not recommended by 3M.<sup>6</sup> VHP has been previously evaluated for its virucidal properties for the purposes of surface disinfection.<sup>7</sup> VHP has also been evaluated as a promising decontamination modality for N95 respirators, combining the reliable inactivation of respiratory viruses with the maintenance of structural integrity of treated masks even after multiple cycles of decontamination.<sup>8–11</sup>

Bergman et al demonstrated that 3 cycles of VHP treatment with the Clarus R HPV generator from Bioquell (which utilizes 30% hydrogen peroxide) resulted in no significant changes in penetration levels of respirator filter material nor noticeable physical changes to respirators.<sup>12</sup> VHP treatment of the 3M 1860 FFR models for 50 treatment cycles was also evaluated and scaled up by the Battelle Memorial Institute with the Clarus R HPV generator from Bioquell (utilizing 30% hydrogen peroxide). Their pilot study demonstrated 6 log inactivation of heat- and hydrogen peroxide-resistant *Geobacillus stearothermophilus* spores inoculated using both liquids and aerosols on respirators.<sup>13</sup> Kenney et al successfully demonstrated the virucidal activity of a single cycle of VHP with the Bioquell BQ-50 system against 3 aerosolized bacteriophage strains. At a vapor concentration of 35% hydrogen peroxide, they were able to demonstrate complete eradication of inoculated viral strains at a limit of detection of 10 plate forming units, which was lower than the infectious dose for the majority of the respiratory viral pathogens studied.<sup>14</sup> Recently, Smith et al demonstrated that the treatment of SARS-CoV-2 inoculated on single-use N95 respirators with VHP at a concentration of 35% hydrogen peroxide lowered viral infectivity in 2 of 3 N95 masks studied.<sup>15</sup> Successful inactivation of SARS-CoV-2 inoculated on cut-outs of single-use respirators has been demonstrated with VHP from Steris generators.<sup>16,17</sup> Other studies have shown that VHP can inactivate numerous pathogens such as *Clostridium difficile*, Middle East Respiratory Syndrome Virus, herpes simplex virus 1, and influenza inoculated on single-use respirators to greater than 6-log clearance.<sup>18,19</sup> Furthermore, the US Food and Drug Administration authorized an Emergency Use Authorization for the utilization of the V-PRO maX Low Temperature Sterilization System by Steris for the decontamination of single-use N95 respirators. However, to our knowledge no data regarding the effects of 59% vaporized hydrogen peroxide utilized by these sterilizers on the filtration efficiency of single-use N95 respirators has been published.<sup>20–22</sup>

VHP respirator decontamination has been further scaled up and codified into a protocol for implementation by researchers at Duke University Health System during the SARS-CoV-2 pandemic as a measure to mitigate N95 shortages.<sup>23</sup> In another protocol, researchers were able to validate room-based VHP decontamination of N95 respirators over 50 cycles using qualitative fit testing and demonstrate that, even after multiple cycles of VHP decontamination, respirator performance was more a function of fit in the setting of elastic fiber degradation.<sup>13</sup>

Hydrogen peroxide gas plasma is a decontamination modality that differs from VHP and utilizes hydrogen peroxide in its plasma phase.<sup>20</sup> This methodology has been associated with degradation with mask components and has been demonstrated to reduce filtration efficiency of single-use N95 respirator masks.<sup>16,24,19</sup> Viscusi et al evaluated gas plasma decontamination of 9 respirator models in a single 55-minute STERRAD 100S H<sub>2</sub>O<sub>2</sub> gas plasma sterilizer at 59% concentrations. Though their results demonstrated initially that a single cycle did not significantly impact filter aerosol penetration or air-flow resistance for any of the models as compared to the controls, a follow-up study did demonstrate a reduction in filtration efficiency by >5% in some of the respirators tested with hydrogen peroxide gas

plasma.<sup>12,24</sup> Ionized hydrogen vapor has also been evaluated for its virucidal activity and effectiveness in decontaminating single-use N95 respirators.<sup>25,19</sup>

With this study, we aimed to use a readily available local resource to prolong our institutional supply of N95 respirators during a crisis capacity while maintaining the safety of frontline providers. We thus evaluated the effect of multiple decontamination cycles using 59% vaporized hydrogen peroxide (VHP) on single-use N95 masks using the V-PRO maX Low Temperature Sterilization System by Steris. In reporting the continued fitness and filtration efficiency of the respirators using a higher concentration of hydrogen peroxide than previously reported, we also demonstrate the collaborative actions of our infection prevention, sterilization cycle and civil engineer teams.

## METHODS

### Mask decontamination protocol

In our presented workflow, each respirator is assigned to a single healthcare provider (HCP) and must be labeled with a permanent marker. HCPs are tasked with tracking mask reprocessing cycles on the log that is provided to each worker and are instructed to utilize respirators per the CDC PPE Optimization Strategy regarding extended use.<sup>26</sup> Per our institutional use policy, HCPs must appropriately doff the respirator and place the mask within a breathable paper bag after each extended use. These bags containing used respirators are placed in designated dirty plastic bins in each unit's soiled utility closet. HCPs are instructed to not stack paper bags containing respirator to mitigate distortion of the respirator. We have implemented unit-based patient safety officers to ensure that HCPs are appropriately donning/doffing respirators and other PPE. As we were concurrently implementing ultraviolet germicidal irradiation decontamination at our institution, respirators that undergo either decontamination modality are kept separate. Any N95 respirators that are visibly soiled, including with makeup, are discarded. Due to incompatibility of the elastic material of the straps, 3M 8210 models are not sent for VHP sterilization in our process.

Designated HCPs from each unit are tasked with transporting plastic bins containing dirty respirators from the soiled utility closet to the VHP decontamination area (and subsequently referred to as "transporters"). When the transporter arrives at the soiled utility closet to retrieve dirty masks, the plastic bin is cleaned with a hospital-grade disinfectant effective against coronavirus. The transporter must log requested information onto the "Used Respirator Log Sheet" and place this sheet on top of the bin (Fig 1). The transporter then performs guideline-directed hand hygiene and dons gloves only. Gloves are the only PPE required for transport. The transporter trolleys the plastic bin containing dirty respirators to the VHP decontamination area. Upon arriving to the VHP decontamination area, the transporter leaves the bin on a designated table in the decontamination area. After moving the plastic bin, the transporter doffs gloves and performs hand hygiene.

In the decontamination area, central sterile personnel wear standard personnel protection of hair and shoe coverings, gowns, gloves, face shield, and surgical mask. Masks are removed from the transport bin and packaged in peel packs with external process indicators, approved for use in the device, allowing ample room in the pack as to not crush the mask. A chemical indicator strip is placed into each peel pack and the pack is labeled with load information. Masks are then transported to the central sterile reprocessing area and handed off to staff wearing gloves and standard PPE to be loaded into the reprocessor.

We used the V-PRO maX Low Temperature Sterilization System by Steris to test and implement the viability of VHP decontamination of N95 respirators. Based on the technical dossier that accompanies

| UNIT TO CENTRAL STERILE TRACKING LOG |                        |               |                            |                |
|--------------------------------------|------------------------|---------------|----------------------------|----------------|
| Unit/Department:                     |                        |               |                            |                |
| Contact Name:                        |                        |               |                            |                |
| Contact Information:                 |                        |               |                            |                |
| Unit Inventory                       |                        | CS Inventory  |                            | Notes/Comments |
| Date Sent                            | Quantity of Masks Sent | Date Received | Quantity of Masks Received |                |
|                                      |                        |               |                            |                |
|                                      |                        |               |                            |                |
|                                      |                        |               |                            |                |
|                                      |                        |               |                            |                |

Fig 1. Above is a sample template of the log sheet that is completed by the transporter before delivering dirty respirators to the decontamination area.

the unit, this apparatus is intended for use of sterilization of heat- and moisture-sensitive nonmetal and nonmetal medical devices and operates at low temperatures and pressures.<sup>21</sup>

Peel packs are placed on the 2 shelves of the sterilizer. Approximately 10–15 masks can be placed into each load. A biological indicator (BI) containing *Geobacillus stearothermophilus* is placed into the center of each load. The masks are then processed on a 28-minute nonlumen cycle per instructions provided with the V-PRO maX Low Temperature Sterilization System. The 28-minute Non Lumen Cycle is intended for surface sterilization, such as defibrillator paddles, cables, and cords.<sup>21</sup> After each cycle, packaged masks are cooled to room temperature prior to being subjected to a new sterilization cycle.

The VPRO cycle consists of 3 stages: sterilizing, conditioning, and aeration. In the sterilization phase, VHP is injected into 4 separate segments (“pulses”), with this stage lasting about 18 minutes. After each pulse, hydrogen peroxide vapor is removed from the chamber through a catalytic converter which decomposes the VHP into water and oxygen. The last stage of the process, aeration, lasts 8 minutes. Per the manufacturer’s technical dossier, testing of medical devices established that level of residues were “well below the established residue limits proving that the V-PRO maX Sterilizer effectively eliminates toxic process residuals.”<sup>21</sup> Devices reprocessed in V-PRO maX are considered ready for immediate use by STERIS, therefore off-gassing was not further evaluated in our study.

After completion of cycle, the peelpacks with decontaminated respirators are placed in a clean container for pick-up by the appropriate department and personnel. The decontaminated masks are retrieved by HCPs at the designated “clean area” at determined times. When the HCP has retrieved his/her decontaminated mask, he/she must ensure fit and mark a check on their assigned log to keep track of the number of decontamination cycles that their mask has gone through

(Fig 2). The HCP is responsible for verifying the fit of the mask upon donning/doffing per CDC guidelines.

**DECONTAMINATION VALIDATION STUDY**

*Quantitative fit testing*

The masks underwent quantitative fit testing to evaluate fit and seal, as per OSHA’s ambient aerosol condensation nuclei counter quantitative fit test protocol.<sup>27</sup> TSI Inc Portacount PRO 8030 Respirator Fit Tester was used to perform the quantitative fit. Fit testing was administered by an experienced occupational health nurse. The test subjects were healthcare workers who volunteered to evaluate the process. The quantitative fit test condensation nuclei counter method measures the microscopic aerosols, generated by the fit testing device, inside and outside the respirator. Ambient particles were counted and used in the fit factor (FF) formula to determine the quantitative fit.<sup>28,29</sup> A fit factor of at least 100 for half-mask respirators was used as the minimum fit factor pass level. Subjects that had confirmed fit for the specified masks were tested with an unprocessed mask (control) and masks that had been reprocessed but not used in the clinical setting at 5, 10, and 15 cycles.<sup>29</sup>

Masks were also inspected for changes in integrity. Weakening of straps was assessed using feedback from test subjects and observation by investigators. We subjectively assessed physical fit on the faces of the subjects as well as loosening or tightness of the straps.

*Respirator filtration study*

To validate the VHP decontamination protocol, the filtration efficiencies of 2 models of N95 respirators, 3M 8211 and 3M 9210, treated with 0 (ie, not sterilized), 5 and 10 cycles, were measured

| N95 REPROCESSING CYCLE TRACKING LOG |  |   |   |   |   |   |   |   |   |    |
|-------------------------------------|--|---|---|---|---|---|---|---|---|----|
| Employee Name:                      |  |   |   |   |   |   |   |   |   |    |
| Department/Unit:                    |  |   |   |   |   |   |   |   |   |    |
| SPECIFIC N95                        | <b>REPROCESSING CYCLE</b><br>Please document the reprocessing cycle and that you checked your respirator for integrity and fit after EACH reprocessing cycle.<br>Mask should not be misshaped, straps should stretch without breaking and fit securely in place with donning, and fits according to <a href="#">CDC protocol</a> |   |   |   |   |   |   |   |   |    |
|                                     | 1  | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|                                     |  |   |   |   |   |   |   |   |   |    |
|                                     |  |   |   |   |   |   |   |   |   |    |
|                                     |  |   |   |   |   |   |   |   |   |    |

Fig 2. Above is a sample template of the log sheet provided to each provider that utilizes an N95 that has been decontamination with VHP.

following a protocol based on NIOSH testing procedures. We evaluated 2 masks of each model that were treated with 5 or 10 decontamination cycles. Due to the limited supply of respirators, we tested only one of each model that was not sterilized, and we repeated the test to obtain technical duplicates. The masks were not worn between sterilization cycles.

For the test, particles were generated from a 2% sodium chloride solution using a Collison 3-jet nebulizer (BGI MRE-3) inside a 280 L polyethylene chamber (Sigma AtmosBag) at 22°C and 25%-35% RH. A small fan was used to promote mixing inside the chamber. The size distribution of the resulting polydisperse particles had a geometric mean aerodynamic diameter of 166 nm and geometric standard deviation of 141 (Fig 3), as measured using a scanning mobility particle sizer (TSI SMPS 3936), assuming a particle density of 2.165 g/cm<sup>3</sup>

(NaCl). A 25 mm diameter circular piece of the respirator was cut out and mounted in a stainless steel filter holder (Advantec) that was connected to a vacuum line whose flow rate was held at 2.7 L/min by a mass flow controller. The SMPS was also connected to this line and sampled at a rate of 0.3 L/min, producing a total flow rate of 3.0 L/min and a corresponding face velocity of 10 cm/s through the respirator. Clean make-up air flow to the chamber was provided through a high-efficiency particulate air filter. Particle concentrations and size distributions over the range of 40-1,000 nm were measured through 2 different pieces cut from each mask and compared to those measured through an empty filter holder for calculation of the filtration efficiency. We compared the filtration efficiencies of masks sterilized for 0 cycles, 5 cycles, and 10 cycles using a *t* test with a significance level of 0.05.

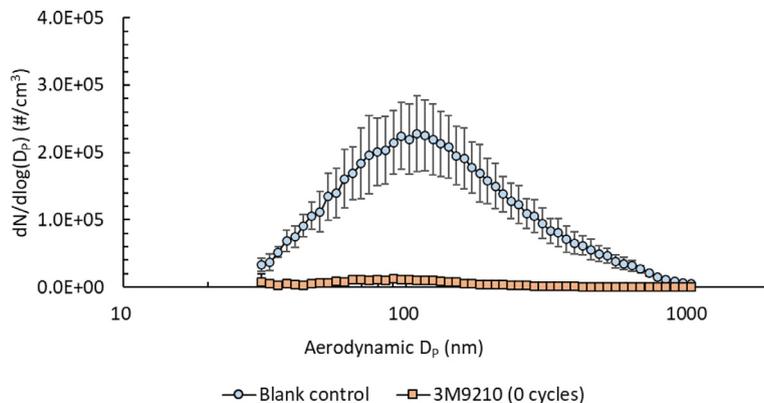


Fig 3. The size distribution of the NaCl particles, shown as a function of aerodynamic diameter ( $D_p$ ), used to challenge the respirators (Blank control) had a geometric mean of 166 nm and geometric standard deviation of 141, and the amount of particles that penetrated a 3M9210 respirator was very low.

**Table 1**  
Table of quantitative fit testing results using the TSI Inc Portacount PRO 8030 Respirator Fit Tester for each model of N95 respirator after decontamination cycles

| Decontamination cycles |              | 0 (control) | 5 cycles   | 10 cycles | 15 cycles |
|------------------------|--------------|-------------|--|-----------|-----------|
| N95 Respirator Model   | 8211 (n = 1) | 393         | Overall fit factor after decontamination using 59% VHP |           |           |
|                        | 9210 (n = 1) | 120         | 203  | 143       | 735       |
|                        |              |             | 166  | 133       | 187       |

**RESULTS**

The overall fit factor for either the 3M 8211 and 9210 respirators using quantitative fit testing were consistently above the minimum fit factor pass level of 100 needed for half-mask respirators as shown in (Table 1) prior to and following VHP sterilization cycles. Individuals who were fit tested did not report odor or facial irritation. Straps remained functional for both models and at all cycles. The masks reprocessed for 15 cycles were reported to be tighter and uncomfortable on the face and because of this, were not sent to be included for validation in the respirator filtration efficient study.

VHP sterilization did not demonstrate an effect on filtration efficiency. There was a minor drop observed for particles smaller than 70 nm with the 3M 8211 that was sterilized with 10 cycles (Fig 4). However, the difference was not significant ( $p > .05$ ) between this respirator and an untreated one (0 cycles). The 3M 8211 respirator treated for 5 cycles demonstrated significantly higher filtration efficiency than the other 2 for particles smaller than 100 nm ( $P < .05$ ), though the difference was still small. The 3M 9210 model treated with 5 cycles or 10 cycles demonstrated no significant difference when compared to the untreated one ( $P > .05$ ; Fig 5).

**DISCUSSION AND OTHER CONSIDERATIONS**

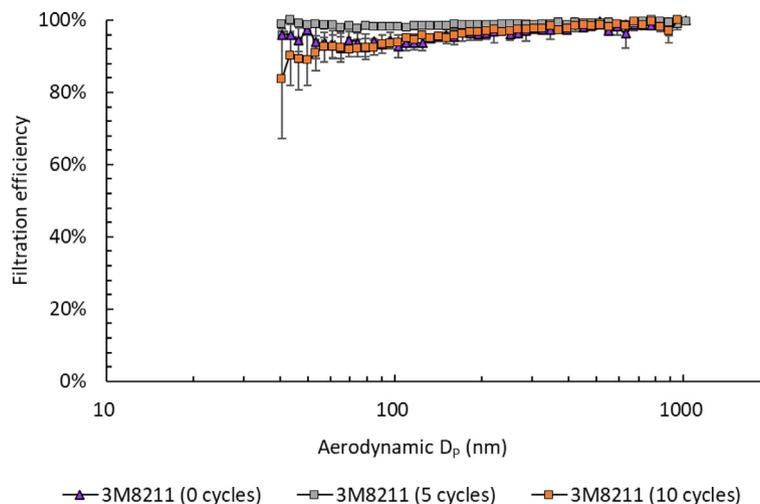
Our study using 59% VHP to decontaminate single use respirators in a setting of “crisis capacity” for N95 masks during a period of national shortage on account of the COVID-19 pandemic, builds on earlier studies that demonstrate the virucidal activity of this decontamination process<sup>14,11,19</sup> while adding to the dearth of knowledge that mask fit and filtration efficiency are not affected using this high concentration of VHP and for up to 10 cycles of decontamination.

Battelle and Duke University Medical Center have demonstrated decontamination and quantitatively validated filtration of masks

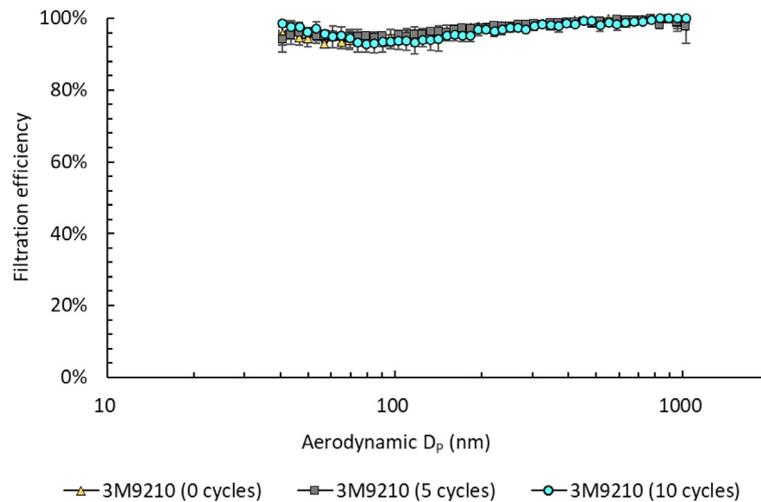
treated with multiple cycles with 30% and 35% hydrogen peroxide.<sup>13,23</sup> Other studies supporting the implementation of VHP to prolong N95 respirator supplies during the COVID-19 pandemic have since been published.<sup>16,17</sup> Our findings were consistent with previous studies that have demonstrated the resilience of N95 filtration efficiency after multiple treatments with VHP.<sup>12,8</sup>

Though an Emergency Use Authorization was announced by the US Food and Drug Administration regarding the use of the V-PRO maX Low Temperature Sterilization System by Steris, there continues to be a considerable paucity of data regarding the implementation of VHP decontamination of N95 respirators with this system.<sup>22</sup> We believe that our decontamination study has validated the maintenance of filtration efficiency of select N95 respirators with this particular sterilization system. When we first implemented VHP decontamination in our hospital system, the effects of VHP at concentrations higher than 35% on the filtration efficiency were largely unknown. Thus, we hope the results and outline of this study will aid other health systems that may, during crisis scenarios, have to implement VHP systems that utilize higher concentrations of vaporized hydrogen peroxide above 35% to decontaminate single-use N95 respirators.

A limitation of this study was our inability to perform our fit testing and filtration efficiency experiments in replicates due to the limited supply of the respirators. We used technical duplicates in evaluating the filtration efficiency to mitigate this limitation. The respirators were not used in the “real-world setting” in between each sterilization cycle. This limitation merits further studies. We also evaluated certain N95 respirator models and hope to expand our evaluation to other respirator models to further generalize our conclusions. Our study also did not directly validate the sterilization efficacy of our specific VHP modality and concentration, though this validation has been done at lower concentrations<sup>8-11</sup> and we continue to use the spore-based bioindicator with each treatment.



**Fig 4.** VHP decontamination did not have a significant effect on filtration capacity. Though there was a small drop in the 3M 8211 model treated with 10 cycles, the difference was not significant.



**Fig 5.** There were no significantly significant differences in filtration efficiency of 3M 9210 respirator models treated with 5 and 10 cycles of VHP compared to the untreated control.

With successful validation and frontline provider education (including reinforcing appropriate donning/doffing of respirators on COVID-19/patients under investigation units), we hope to scale up decontamination of masks using VHP to increase throughput and extend our respirator supply while in crisis capacity for N95s. The rapid evaluation of feasibility and implementation at our institution was the result of cross-institutional and interprofessional collaboration, particularly with the researchers at the Virginia Tech College of Engineering. This methodology is relatively novel and continues to be subjected to strict quality assurance measures and frontline provider feedback.

We believe it is important to note that decontamination methodologies should only be used as crisis capacity as these respirators were designed for single-use. Any decontamination of single-use respirators will void NIOSH approval. Without appropriate expertise and logistics, we would not recommend respirator decontamination and would recommend only extended use of respirators per CDC guidelines.

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