Technical Report for H_2O_2-Based N95 Reuse Risk Management

1. Overview

Hydrogen peroxide (H_2O_2) vapor and plasma decontamination is an established industrial decontamination method used in research settings, pharmaceutical and medical industries, and by police and fire departments (Mickelsen et al., EPA Report, 2017). Many hospitals use hydrogen peroxide vapor (wet HPV or dry VHP™) or hydrogen peroxide gas plasma (HPGP) for decontamination. HPV, VHP™ and HPGP inactivate highly resistant pathogens, including nosocomial bacterial spores and viruses. A number of studies (Battelle, 2016, 2020; Bergman, 2010, Viscusi, 2009) have demonstrated that certain N95 Filtering Facepiece Respirators (N95 FFRs or respirators, often colloquially called 'N95 masks') can be safely decontaminated with proper use of HPV. Most of these studies used the Bioquell HPV system.

For the purpose of bulk decontamination of N95 masks, a whole-room decontamination system with controlled air-flow will allow carts with trays filled with N95 masks to be wheeled in and out, similar to a protocol developed by Duke University Medical Center. This would provide for a capacity of 700 N95 masks in a 12 ft x 12 ft room per cycle. Alternatively, an air-flow controlled BSC could be temporarily outfitted with a HPV system, which would then have capacity for 100–120 N95 masks, depending on BSC size per cycle. Typically, a decontamination plus off-gassing cycle takes 6 to 8 hours.

The Battelle study demonstrated that up to 20 cycles of HPV treatments on N95 FFRs will not affect filter performance, pressure drop, fit, or elastic band quality.

H_2O_2 gas plasma (HPGP) reduces filter quality if applied for 3 cycles at high dose. However, at low dose, filter quality was reported to be preserved for 2 cycles (personal communication, J. Yarwood, ASP), but this has not yet been independently confirmed and exact protocol might be a major issue.

Method	Abbreviation	Description	Example Provider
Hydrogen Peroxide Vapor	HPV	Wet H_2O_2 Vapor, >3 hr. aeration	Bioquell
Hydrogen Peroxide Gas Plasma	HPGP	Ionized H_2O_2 plasma (not confirmed), 1 hr. aeration	ASP (STERRAD)
Vaporized Hydrogen Peroxide	VHP™	Dry H_2O_2 Vapor, >3 hr. aeration	Steris

2. Status of Federal Guidance

CDC released guidance on the decontamination and reuse of N95s on March 31, 2020, including the use of vaporized H_2O_2 (Decontamination and Reuse of Filtering Facepiece

Respirators, 2020). The Battelle Decontamination System, an HPV system for decontaminating N95 masks, received emergency use authorization from the FDA on March 28, 2020. They report that the system can process at least 80,000 N95 masks per day (Battelle, 2020). "In preliminary studies, H_2O_2 vapor decontamination has been found to be a highly effective method of eradicating MRSA, *Serratia marcescens, Clostridium botulinum spores and Clostridium difficile* from rooms, furniture, surfaces and/or equipment; however, further investigation of this method to demonstrate both safety and effectiveness in reducing infection rates are required." (Rutala et al. 2019). An FDA-supported study by Battelle (2016) found that Bioquell HPV, with the established decontamination cycle parameters, achieved a 6-log reduction in organism viability and maintained high filtration and low resistance to air-flow of N95 masks following exposure to up to 50 cycles of HPV decontamination. Mask users should perform a seal check after they don the mask. Please refer to current CDC guidelines that are updated regularly as well as N95Decon's Full Legal Disclaimer (Decontamination and Reuse of Filtering Facepiece Respirators, 2020).

3. Mode of Action

HPV methods are used for terminal decontamination of hospital rooms, biosafety cabinets, and medical equipment and materials that are intolerant to heat or have diffusion-restricted space. Sterilizing units use liquid H_2O_2 that is vaporized by heat and released into the room until the effective concentration is detected by the HPV system. Individual, unwrapped, contaminated objects are placed in a room or biosafety cabinet. Typically, the HPV treatment involves a conditioning phase to change room humidity (dry or wet treatment); a gassing phase to saturate the room (approximately 15 min); a dwell phase to maintain a certain concentration (approximately 125 min); and an aeration or clearance phase for off-gassing and breakdown of HPV into oxygen and water vapor (approx. 4–6 h). Inactivation of microorganisms and viruses is achieved primarily by the combined actions of H_2O_2 gas and the generation of hydroxyl and hydroperoxyl free radicals (Finnegan et al. 2010).

HPGP machines are often used in hospitals for rapid sterilization of surgical tools that are wrapped or in Tyvek pouches. The plasma penetrates the material even when bagged, and also more rapidly eliminates any condensed H_2O_2.

4. Potential for SARS-CoV-2 Inactivation

HPV, VPH™, and HPGP destroy influenza viruses and other viruses and pathogens that are more resistant than SARS-CoVs, such as spores from *G. stearothermophilus*, nosocomial *C. difficile*, and *mycobacterium tuberculosis* (Battelle, 2016; EPA, 2004; Heckert et al. 1997; Kenny et al. 2020; Rudnick et al. 2009; Hall et al. 2007).

Bergman et al. (2010) studied 6 different N95 masks (industrial N95s: 3M 8210; 3M 8000; Moldex 2200 and surgical N95: KC PFR95-270; 3M 1870; 3M 1860; N=6 for each of the models tested) and applied 3 cycles of decontamination using HPGP, HPV and other methods. The HPV decontamination method (Clarus R™, Bioquell) involved a gassing phase of 15 min, a dwell phase of 125 min in a 64 m^3 room to achieve a room concentration of 8 g/m^3 (5700 ppm). Biological indicators containing *Geobacillus stearothermophilus* spores were placed in five locations inside the room and a 6-log spore reduction was measured after each treatment.

The 2016 Battelle Report prepared for the FDA summarized a study on the effects of HPV on N95 filter quality and microorganism attenuation. An HPV treatment (Clarus C™, Bioquell) of a 20 min gassing phase (2 g/min) followed by a 150 min dwell phase (0.5 g/min) completely inactivated *Geobacillus stearothermophilus* spores inoculated by droplet or aerosol onto N95 masks (3M 1860). The authors further showed that decontamination was achieved across 50 cycles of repeated treatments. Although the N95 masks were shown stacked up against each other in the exposure chamber (Figure 11, Battelle 2016), this resulted in variable sensor readings and is not recommended (personal communication, B. Heimbuch).

Kenny et al. (2020), in a non-peer reviewed report, evaluated viral decontamination of HPV (BQ-50, Bioquell) after inoculating N95 masks (3M 1870) with 3 different types of aerosolized bacteriophages. A single cycle of HPV completely eradicated phages from the N95 mask. N95 masks were suspended by their elastic strap on racks in a 33 m^3 room for a 30–40 min gas phase at 16 g/min, a 25 min dwell phase, and a 150 min aeration phase.

Heckert et al. (1997) inoculated glass and stainless steel with 9 exotic animal viruses. After HPV treatment, virus titer was reduced to 0 (except for hog cholera virus in whole blood). A VH™ P1000 Steris machine was used to generate a gas phase of 2 g/min for 30 min to maintain a H_2O_2 concentration of 1.73 mg/L (1211 ppm).

5. Integrity of N95 Filtering Facepiece Respirators

Viscusi et al. (2009) evaluated 6 different N95 masks (same as Bergman et al. 2011) and applied 1 cycle of treatment of 5 decontamination methods including high dose HPGP. There was no effect of HPGP on filter quality. However, 3 cycles of the same high dose HPGP decontamination process reduced filtering efficiency by > 5% for 4 of 6 different masks tested, bringing the efficiency below the FDA-advised 95% threshold (Bergman et al. 2010).

Three cycles of HPV treatment in the Bergman et al. (2010) study did not reduce filter quality (filter efficiency > 98% and no change in airflow resistance) nor were there observable physical changes to the N95 masks.

The 2016 Battelle Report phase 2 study evaluated filter quality and fit. The same exposure was applied as described in the SARS-CoV-2 Inactivation section above, with the addition of 300 min of aeration, to eighty-five N95 masks for 10, 20, 30, 40 and 50 cycles of decontamination, 15 N95 masks per cycle set. After decontamination, both inert and bioaerosol collection efficiency remained >99% and no degradation of airflow resistance was found for all eighty-five of the N95 masks. After 30 cycles, strap degradation was observed through strap length elongation and loss of elasticity, which could negatively impact respirator fit. There was no degradation in mannequin fit testing up to 20 cycles of decontamination (no fit testing was done beyond 20 cycles). Only the 3M 1860 N95 model was tested.

Duke University & Health HPV system (Schwartz et al., 2020) incorporates results from the Battelle study. N95 masks (3M 1860) were either suspended by their elastic straps or layed individually onto stainless steel racks in wheeled carts. An existing disinfection system was used in a room (12 ft X 12 ft) of their NIAID Regional Biocontainment Laboratory. The room was treated (Bioquell Clarus™ C system with a 35% H_2O_2 solution and distribution system to disperse HPV uniformly) to attain a 480+ ppm concentration of HPV with a gas time of 25 min

and dwell time of 20 minutes. One hundred N95 masks (3M 1860) were treated with HPV for 1 cycle. Air concentration near the N95 masks was measured during the aeration period to determine time until the concentration was below the OSHA Permissible Exposure Limit (1 ppm, 1.4 mg/m^3). At 4 hours, the concentration was below the limit of detection of the device (below detectable limit measured with a PortaSens II™ sensor). A qualitative test was conducted on the N95 masks by 3 individuals who detected no noticeable odors. There was no physical nor performance (not described) degradation of the N95 masks. They are currently evaluating the Bioquell Z-2 and Bioquell ProteQ™ system with >10 repeated treatment cycles for fit.

Although no measurements of filter performance were made in the non-peer reviewed Kenny et al. (2020) study, after 5 cycles of HPV treatment, the 3M 1870 N95 masks "appeared similar to new with no deformity."

On March 16, 2020, the Dutch National Institute for Public Health reported that 3M 8822 masks with up to 4 cycles of HPGP (STERRAD NX100, Express cycle with AllClear™) and found that 2 cycles of treatment did not deform masks or compromise fit, but 3 or 4 cycles compromised the fit. There was no test of filter efficiency. They also noted that used masks sterilized with this process did not support SARS-CoV-2 growth when in medium for 72 hours.

Data are suggested to show that Sterrad units for HPGP, when used with the proper settings, can decontaminate 10 individually Tyvek-wrapped N95s in a 25 or 27 min. cycle without compromising filtration or airflow for 2 cycles (personal communication, J. Yarwood, ASP). Treatment is followed by 60 min aeration (after opening Tyvek bag) to allow outgassing of residual H_2O_2 gas. HPGP can reduce filter quality if applied with intensive treatments (Bergman et al., 2010) and so the exact protocol for this treatment used is a concern. This method is not recommended in the updated CDC report (2020).

It is important to note that HPV, VHP™ and HPGP are not compatible with cellulose, which is not a component listed in 3M model 1860 N95 masks (3M 1860 Data Sheets), but may be present in other PPE. The presence of cellulose in PPE is an important consideration in the adoption of H_2O_2-based strategies.

6. Data Summary Tables

Table 1. Impact of HPV on *G stearothermophilus* spores and viruses

Author	Media	Dose	Time (min)	Strain(s)	Effectiveness (log reduction)
A	Biological indicators in room (N=5)	8 g/m^3	gas 15; dwell 120	*G stearothermophilus spores*	≥6
B	3M 1860 N95 masks inoculated with aerosol (N=15)	2 g/min then 0.5 g/min	gas 20; dwell 150	*G stearothermophilus spores*	≥6
C	3M 1860 N95 masks inoculated with droplets (N=3)	2 g/min then 0.5 g/min	gas 20; dwell 150	*G stearothermophilus spores*	≥6

D	3M 1860 N95 masks inoculated with aerosol then treated with VHP™ for 50 cycles (N=5)	2 g/min then 0.5 g/min	gas 20; dwell 150	*G stearothermophilus spores*	≥6 (50 cycles)
E	3M 1860 inoculated N95 masks (N=3 for each phage)	16 g/min	gas 30–40; dwell 25	Phage phi-6 Phage T7 Phage T1	≥6
F	Glass and stainless steel inoculated with 9 viruses	2 g/min	gas 30	Avian influenza African swine fever virus Bluetongue virus Hog cholera virus Newcastle disease virus Pseudorabies virus Swine vesicular disease virus Vesicular exanthema virus Vesicular stomatitis virus	≥6

A: (Bergman et al., 2010), B: (Battelle, 2016) Phase 1 aerosol inoculated filters, C: (Battelle, 2016) Phase 1 droplet inoculated filters, D: (Battelle, 2016) Phase 3 aerosol inoculated filters, E: (Kenny et al., 2020), F: (Heckert et al. 1997).

Table 2. Impact of HPV on N95 FFRs

Author	N95 masks	Dose	Time (min)	# cycles	Filtration efficiency	Respirator damage
G	6 different N95 models (N=6 for each model)	8 g/m^3	gas 15; dwell 120	3	>97%	None noted
H	3M 1860 (N=85 total)	2 g/min then 0.5 g/min	gas 20; dwell 150; aeration 300	10, 20, 30, 40, 50	>99% for all N95 masks	> 30 cycles straps fragmented when stretched
I	3M 1860 (N=100)	Goal: 480+ ppm	gas 25; dwell 20; aeration 240	1	N/A	None noted

G: (Bergman et al., 2010), H: (Battelle, 2016), I: (Schwartz et al., 2020).

7. Strategies

Commercial systems are available from companies such as Bioquell, ASP (STERRAD), Steris, Battelle, and Halosil, though they differ in the method of delivering and sustaining H_2O_2 concentrations in a room/cabinet and in the solvents used. [Note: Bioquell uses the term H_2O_2 vapour (HPV) while Steris uses the term vaporized H_2O_2 (VHP™).] Bioquell HPV includes a generator to produce HPV, a module to measure the concentration of HPV, temperature, and relative humidity in the enclosure, and an aeration unit to catalyse the breakdown of HPV into oxygen and water vapour after HPV exposure. A control pedestal is set outside the enclosure to provide remote control. HPV is delivered until the air in the enclosure becomes saturated and H_2O_2 begins to condense on surfaces (Hall et al., 2007; Ray et al., 2010). Steris VHP™ systems have a generator inside the room with an integral aeration unit and dehumidifier designed to achieve a set humidity level prior to the start of the cycle. Alternatively, Steris units

may be connected to an existing biosafety cabinet in the hospital and operated by Steris-trained personnel remotely from outside the enclosure. The system delivers 'non-condensing' VHP™ by drying the vapour stream as it is returned to the generator. Bioquell systems do not control the H_2O_2 air concentration while the Steris systems hold a steady H_2O_2 air concentration throughout the exposure period.

The Halosil system uses the HaloMist solution which contains a low percentage (5%) peroxide with a biocidal silver nitrate additive at a low dose (0.01%) that converts to low ppb's once the product is aerosolized. Halosil foggers generate a 100-120 ppm H_2O_2 vapor content through initial water evaporation from microdroplets that concentrate both the peroxide and the silver. This effectively increases the peroxide concentration in the vapor phase above the initial 5%. While this very low silver concentration is unlikely to impact the electrostatically-charged membrane of an N95 mask, the filtering function of the N95 masks should be determined after multiple cycles of decontamination.

The Bioquell systems [used by Duke, Bergman (2010) and Battelle (2016)] uses a 35% peroxide solution without additives. It is important that such high concentrations of peroxide be handled safely; high concentrations are toxic and can be explosive. The manufacturer uses RFID-chipped bottles so use of third party peroxide is not an option.

The Duke Medical Center has developed a standard operating procedure (SOP) for decontamination of N95 masks using the Bioquell Clarus™ C system and has performed qualitative testing on more than one hundred N95 masks (see above; Schwartz et al., 2020). Masks are checked before and after decontamination and prior to each use. Soiled N95 masks with visible blood, hair or damage are discarded and not decontaminated. N95 masks are separated into 4 streams based on size of N95 mask (common: 3M 1860 or small: 3M 1860s) and the visible presence or absence of facial cosmetics. After decontamination, the integrity of the straps (evaluate for elongation), nose bridge, and nose foam are checked for integrity. The Duke Medical Center protocol does not return the mask back to the same user while the Battelle protocol does. A reason for returning the mask to the same user is because the mask was initially fit and fit tested to that user, and also in case the former user applied any non-compatible wipe (alcohol or surfactant; Viscusi et al., 2007 but see Heimbuch et al. 2014 for efficacy of bleach wipe option) that could disrupt the hydrophobic coating or electrostatic charge. In either case, users should perform a seal check before reuse.

8. Primary Risks and Unknowns

Dosing protocol is complex and could result in incomplete decontamination or explosion risk. Therefore only trained personnel should operate HVP, VHP™ or HPGP equipment. H_2O_2 gas is a strong oxidizer that may cause fire or explosions, and is a corrosive irritant that may cause skin, eye or lung damage. H_2O_2 gas may interact with N95 mask components to form a toxic residue - analytical chemistry tests for H_2O_2 can test for this. The OSHA permissible exposure limit is 1 ppm over an 8-hour Time Weighted Average (TWA). During the decontamination process, room concentrations may be higher than 100 ppm.

Detection of odor does not provide adequate warning of hazardous residual concentrations in the N95 mask. Quantitative tests for sufficient decontamination (*G.*

stearothermophilus spore growth) and aeration should be done on sentinel N95 masks (PortaSens-II test). Probability of N95 mask straps failure increases with more than 20 cycles of decontamination; this will vary with the decontamination method and N95 mask model. Straps should be examined after decontamination and prior to each use.

9. Conclusions

Multiple studies have confirmed that N95 masks contaminated with aerosol or droplets containing *G. stearothermophilus* spores were successfully decontaminated with H_2O_2 with a 6-log reduction in spore level. Furthermore, N95 mask filter efficiency did not degrade with up to 50 cycles of decontamination. However, after 20 cycles of HPV decontamination the N95 mask (3M 1860) straps showed degradation and were permanently deformed when stretched. Disadvantages are that N95 masks must be individually placed or hung and cannot be bagged during or after HPV treatment in order to attain complete exposure and aeration. Typical decontamination durations, including aeration, are 4 to 8 hours.

Some hospitals have HPGP systems (H_2O_2 gas plasma; Sterrad, Irvine, CA). Three cycles of high dose treatments (Sterrad 100S) reduces N95 filter efficiency to an unacceptable level (Bergman, 2010). According to ASP (personal communication, J. Yarwood), 3 cycles of treatment with lower dose (STERRAD 100NX, AllClear, Express cycle) does not reduce filter efficiency, but this awaits independent confirmation. H_2O_2 gas plasma provides rapid clearing of toxic H_2O_2 vapor (55 minute total processing time) and individual tyvek bagging of masks.

Many hospitals already have HPV systems in-house for use in full room terminal decontamination. These could be deployed in dedicated decontamination rooms. Processing carts filled with trays of N95 masks could be wheeled in and out of the room. Alternatively, existing biosafety cabinets within the hospital can be connected to a VHP™ (pending data for dry H_2O_2 vapor efficacy and safety) or HPV unit. Another solution is to send the respirators to an outside service-provider (e.g., Battelle) for decontamination. To achieve an appropriate concentration of H_2O_2 vapor during the gas and dwell phase the specification of the HPV system should be matched to the treated volume. The long (300 min) aeration phase could be conducted in an adjacent room where H_2O_2 vapor is converted to inert O_2 and water vapor. The concentration of HPV should be measured during decontamination to confirm adequate treatment level and that at the end of aeration workers who enter the room are protected. The OSHA Permissible Exposure Limit is 1 ppm (1.4 mg/m^3). HPV can be detected by smell and irritation, but may pose respiratory dangers at levels below user detection.

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