Technical Report for Hydrogen Peroxide Methods for Decontaminating N95 Respirators

Much of the available literature on decontamination of N95 FFRs reviewed in this document is a result of recent efforts to relieve the shortage of N95 FFRs during the SARS-CoV-2 outbreak. Because of this, many of the research papers cited in this document are not yet peer reviewed. **For clarity, wherever non-peer-reviewed results are cited in this report, the citation is preceded by a *.**

Summary of Updates in v2.1 Report: Update adds 3 FDA EUAs, adds new findings on N95 mask decontamination (*Wigginton et al. 2020), updates Appendix A, and adds Appendix B which presents data on the use of liquid hydrogen peroxide submersion for decontaminating N95 masks.

Summary of Updates in v2.0 Report: Update includes more in-depth description of hydrogen peroxide systems, new findings on effects of decontamination on N95 masks inoculated with SARS-CoV-2 (*Kumar et al. 2020; *Oral et al. 2020; *Fischer et al. 2020); and two new FDA EUAs for hydrogen peroxide decontamination methods.

Executive Summary

Hydrogen peroxide (H_2O_2) is well known for eradicating resistant microorganisms such as *Serratia marcescens, Clostridium botulinum spores and Clostridium difficile*. In vapor or gaseous form, it can be used to decontaminate whole rooms, biosafety cabinets and medical instruments made of metal or plastic. Recent studies demonstrate that hydrogen peroxide vapor decontaminates N95 filtering facepiece respirators (FFRs) inoculated with SARS-CoV-2 virus with greater than 3-log attenuation. There are many types of hydrogen peroxide delivery systems that vary in humidity, temperature, hydrogen peroxide concentration, and duration of exposure, depending on whether the hydrogen peroxide is delivered as a true gas, liquid, vapor, aerosol, or ionized gas. This makes it particularly important for hospitals to make sure that the proper protocol for N95 FFR decontamination matches the available equipment. For example, the Bioquell process, used by Battelle, will not damage N95 FFRs with up to 20 repeated decontamination cycles. But the STERRAD (ASP) process will damage N95 FFRs with very few decontamination cycles. The FDA has granted Emergency Use Authorization for three different hydrogen peroxide decontamination processes for N95 FFRs, including one mail-out process.

1. Overview

The novel coronavirus (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) has led to a global shortage of N95 Filtering Facepiece Respirators (N95 FFRs, also referred to as ‘N95 masks’). While new N95 FFRs should be used with every change in activity, in this emergency, it may be necessary to decontaminate N95 FFRs. In this document, we review the use of hydrogen peroxide vapor to decontaminate N95 FFRs with the goal of increasing the useful lifetime of N95 FFRs worn by healthcare providers during the COVID-19 pandemic.

Effective decontamination requires inactivation of the SARS-CoV-2 virus and maintenance of both the fit and filtration efficiency of the N95 FFR while minimizing the risk of cross-contamination.

Hydrogen peroxide (H_2O_2) is an established industrial decontamination method used in hospitals, research settings, research animal facilities, pharmaceutical and medical industries, and by police and fire departments (Mickelsen et al., EPA Report, 2017) for terminal decontamination of biosafety cabinets (BSC) and whole rooms. Hydrogen peroxide vapor/vapor phase hydrogen peroxide (H_2O_2 vapor/VPHP or VHP™), aerosolized hydrogen peroxide (aHP), or hydrogen peroxide gas plasma (HPGP) inactivate highly resistant pathogens, including nosocomial bacterial spores and viruses. The hydrogen peroxide decontamination method is incompatible with cellulose, which is a component in some N95 FFRs (Appendix A).

A number of studies (Battelle et al., 2016, 2020; Bergman et al., 2010, Viscusi et al., 2009; *Oral et al., 2020) have demonstrated that some N95 FFRs can be safely decontaminated with proper use of H_2O_2. Most of these studies used H_2O_2 delivered as a gas or vapor using either the Bioquell or Steris systems. The Battelle study demonstrated that up to 20 cycles of H_2O_2 vapor treatments on N95 FFRs will not compromise filter performance, pressure drop, fit, or elastic band quality. For the purpose of bulk decontamination of N95 FFRs, a whole-room decontamination system with controlled air-flow, allows carts filled with N95 FFRs to be wheeled in and out; see protocol developed by Duke University Medical Center (*Schwartz et al., 2020). This would provide for a capacity of up to 2000 N95 FFRs per day using a 12 x 12 ft room. Alternatively, an air-flow controlled chamber or BSC could be temporarily outfitted with a Steris VHP™ system (e.g., *Oral et al., 2020), which would then have capacity for 100–120 N95 FFRs/cycle, depending on the BSC size. The duration of a decontamination cycle varies from 4.5 to 8 hours depending on the system and volume of the chamber or room.

Method	Abbreviation	Description	Example Providers
Hydrogen Peroxide Vapor/Vapor Phase Hydrogen Peroxide	HPV/VPHP	H_2O_2 vapor, >500 ppm	Bioquell (Claris); Battelle CCDS™
Hydrogen Peroxide Gas Plasma	HPGP	HPV, low frequency power, H_2O_2 plasma	ASP (STERRAD™)
Ionized Hydrogen Peroxide	iHP®	H_2O_2 microdroplets, ionization arc	Tomi™ (SteraMist®)
Vaporized Hydrogen Peroxide™	VHP™	H_2O_2 vapor	Steris (V-PRO)
Aerosolized Hydrogen Peroxide	aHP	H_2O_2 microdroplets, 100-120 ppm; additive:	Halosil

		silver nitrate (0.01%)	
Aerosolized Hydrogen Peroxide	aHP	H_2O_2 microdroplets, 80-150 ppm	Curis®

2. Status of Federal Guidance

In this unprecedented COVID-19 pandemic, due to a limited supply of N95 FFRs, the Centers for Disease Control and Prevention (CDC) has provided guidance that healthcare workers can practice extended use or limited reuse of N95 FFRs (CDC, 2020b). In addition, the CDC has provided guidance to hospitals on methods for decontaminating N95 FFRs during a crisis (CDC, 2020c).

The Occupational Safety and Health Administration (OSHA) states that cosmetics or other barriers should not be present during respirator use (OSHA, n.d.). Emergency use authorizations (EUAs) that the FDA has granted for N95 FFR decontamination during the COVID-19 pandemic also stipulate that cosmetics should not be present on respirators sent for decontamination (Battelle, 2020).

After decontamination, the CDC recommends that a user seal check is performed when the respirator is donned to ensure adequate seal (CDC, 2020c). A user seal check after every decontamination cycle is especially important because there is evidence that the fit factor of N95 FFRs decreases with numerous don/doffs (Bergman et al., 2012).

Per FDA guidelines for N95 FFR decontamination EUAs, virucidal decontamination requires ≥ 3-log reduction (corresponding to a 99.9% reduction) in viral activity (FDA, 2020). Based on this guideline, we describe a process as sufficiently "decontaminating" or "inactivating" only when it leads to a ≥ 3-log reduction in viral activity. Note that our definition of decontamination in this report, unless otherwise specified, only considers virucidal activity and does *not* consider mycobactericidal or sporicidal activity, for which the FDA has other guidelines (FDA, 2020). N95 FFR decontamination processes for SARS-CoV-2 may not result in sterilization (≥ 6-log reduction) of the N95 FFR.

The March 31, 2020 CDC guidance on the decontamination and reuse of N95 FFRs includes the use of vaporized H_2O_2 (Decontamination and Reuse of Filtering Facepiece Respirators, 2020). On March 28, 2020 the Battelle H_2O_2 vapor/VPHP Critical Care Decontamination System™ (CCDS) received FDA EUA for decontamination of N95 FFRs for reuse for a maximum of 20 decontamination cycles (single-user reuse or pooled use not specified). Battelle reports that each of their six facilities can process at least 80,000 N95 FFRs per day (Battelle, 2020). On April 9, 2020 the STERIS VHP™ system (V-PRO, maX, and maX2) received FDA EUA for decontamination of N95 FFRs for single-user reuse for a maximum of 10 decontamination cycles. On April 11, 2020 the ASP STERRAD process (100S, NX Standard, 100NX Express Cycles) received FDA EUA for decontamination of N95 FFRs for single-user reuse for a maximum of 2 decontamination cycles. **[Note: The 100NX Standard process (different than the 3 authorized processes) damages N95 filters and should not be used for decontamination (*Kumar et al., 2020)]** In a single-user reuse process, the decontaminated FFR is returned to the original user, while in a pooled reuse process, the

decontaminated FFR goes into a large pool and can be used by anyone. On April 14, 2020 the Stryker Instrument's Sterizone VP4 Sterilizer received FDA EUA for decontamination of N95 FFRs for single-user reuse for a maximum of 2 decontamination cycles. On April 20, 2020 the Sterilucent HC 80TT Hydrogen Peroxide Sterilizer received FDA EUA for decontamination of N95 FFRs for single-user reuse for up to 10 decontamination cycles. On May 7, 2020 the Duke Decontamination System (Bioquell; Duke University Health System) received FDA EUA for decontamination of N95 FFRs for a maximum of 10 decontamination cycles.

CDC guidance for disinfection or sterilization in health care facilities states that "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 H_2O_2 vapor, 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 FFRs following exposure to up to 50 cycles of H_2O_2 vapor/VPHP decontamination.

3. Mode of Action

H_2O_2 vapor 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 or aerosolized and released into the room or chamber. Individual, unwrapped, contaminated objects are placed in a room or biosafety cabinet. Typically, H_2O_2 vapor treatment involves a conditioning phase to change room humidity; a gassing phase to saturate the room with hydrogen peroxide gas or vapor (approximately 15 min); a dwell phase to maintain a constant concentration (approximately 125 min); and an aeration or clearance phase during which H_2O_2 is removed by evaporation and converted to oxygen and water vapor (approx. 2–6 h). The target concentration is closely tied to volume of the room/chamber and internal surface area (load density). In this process, 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).

Aerosols and vapors are essentially the same, except one starts as a liquid and partially evaporates, while the other starts as a gas and partially condenses. There are multiple technologies that do not boil the liquid H_2O_2 solution but rather use a nebulizer to convert the solution into microdroplets. These microdroplets are then distributed into the space in an aerosolized liquid that forms via a visible wet fog. Some percentage of the microdroplets evaporate and achieve equilibrium with the environment, forming a combination of liquid aerosol, gas or vapor phase H_2O_2. Viral inactivation is achieved through deposition of the liquid microdroplets and condensation of the vapor onto surfaces.

HPGP systems (e.g, STERRAD) and iHP (e.g., SteraMist) use ionization to accelerate the generation of hydroxyl radicals and are used in hospitals for rapid sterilization of BSCs and surgical tools. The gas plasma penetrates the material even when bagged in Tyvek pouches,

and also rapidly eliminates any condensed H_2O_2. The FDA EUA limits STERRAD decontamination of N95 FFRs to a maximum of 2 decontamination cycles.

4. SARS-CoV-2 and Other Pathogen Inactivation

H_2O_2 vapor, VHP™, HPGP and iHP 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* (Heckert et al. 1997; EPA, 2004; Hall et al. 2007; Rudnick et al. 2009; Battelle, 2016; Jiang et al., 2017; *Kenny et al. 2020;).

Three recent pre-publications demonstrated that VHP™ inactivates SARS-CoV-2. Kumar et al. (2020) inoculated four N95 FFR models (3M 1860, 3M 1870, 3M 1804, AO 1054) with SARS-CoV-2 and vesicular stomatitis virus Indiana serotype (VSV). VHP™ (ARD system, Steris, peak H_2O_2 concentration >750 ppm) inactivated both SARS-CoV-2 and VSV on all FFR inoculated cutouts (*Kumar et al. 2020). Oral et al. (*2020) inoculated cutouts of a N95 FFR (3M 1860S, N=3) with SARS-CoV-2 and treated the cutouts with VHP™ (Steris LTS-V ARD1000) at 410 ppm for three hours; the treatment inactivated SARS-CoV-2. Fischer et al. (2020) inoculated cutouts from a N95 FFR (AO N9504C) with SARS-CoV-2 and treated the cutouts with VHP (1000 ppm, process not identified) for 10 min; the treatment inactivated SARS-CoV-2.

The Dutch National Institute for Public Health report (March 16, 2020) stated that 3M 8822 FFRs treated with HPGP (STERRAD NX100, Express cycle with AllClear™) did not support SARS-CoV-2 growth when in medium for 72 hours. HPGP (STERRAD 100NX applied for a standard 47 min cycle) also inactivated VSV on all FFR inoculated cutouts (*Kumar et al. 2020).

Bergman et al. (2010) studied six different N95 FFRs (industrial N95 FFRs: 3M 8210; 3M 8000; Moldex 2200 and surgical N95 FFRs: KC PFR95-270; 3M 1870; 3M 1860; N=6 for each of the models tested; FFRs models listed in Bergman et al. (2011)) and applied 3 cycles of decontamination using H_2O_2 vapor. The H_2O_2 decontamination method (Clarus R™, Bioquell) involved a pre-conditioning 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). After each treatment, a 6-log spore reduction was measured using a biological indicator of *G. stearothermophilus* spores placed inside the room.

The 2016 Battelle Report, prepared for the FDA, summarized a study on the effects of H_2O_2 vapor on N95 FFR filter quality and microorganism attenuation. In Phase 1 of the study, H_2O_2 treatment (Clarus C™, Bioquell) of a 20 min pre-conditioning phase (2 g/min) followed by a 150 min dwell phase (0.5 g/min) completely inactivated *G. stearothermophilus* spores inoculated by droplet or aerosol onto N95 FFRs (3M 1860). The authors further showed that decontamination was achieved across 50 cycles of repeated treatments. Although the N95 FFRs were shown to be 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 H_2O_2 vapor (BQ-50, Bioquell) after inoculating N95 FFRs (3M 1870) with three different types of aerosolized bacteriophages. A single cycle of H_2O_2 completely eradicated phages from the N95

FFRs. N95 FFRs were suspended by their elastic strap on racks in a 33 m^3 room for a 30–40 min pre-conditioning phase at 16 g/min, a 25 min dwell phase, and a 150 min aeration phase.

Cramer et al. (*2020) found that the standard *G. stearothermophilus* spore biological indicator, when placed under or near N95 FFRs in a SteraMist-equipped room that had iHP mist delivered to a total of 90 mL/min for 15 min for a concentration of 17.7 mL/m^3, showed an inactivation of > 6-log.

Derr et al. (*2020), evaluated viral decontamination using the Curis aHP system on five models of N95 FFRs (3M 1860, 1870+, 8511, 9211+ and Honeywell Sperian N11125) inoculated with herpes simplex virus 1, Coxsackie virus B3, and phage phi6. When the decontamination room HP exposure killed the biological index (G *stearothermophilus*) then all viruses were inactivated to > 6-log.

Wigginton et al. (*2020) treated 3M 1860 FFRs with VHP (Bioquell Q10, Condition 1: gassing 446 or 495 ppm, dwell 490 ppm for 20 min, aeration 68 min; Condition 2: gassing 659 ppm, dwell 647 ppm for 150 min, aeration 80 min). FFR cutouts were inoculated with phage (MS2, Phi6), influenza, MHV, *E. coli, S. aureus, G. stearothermophilus,* and *Aspergillus niger.* Microorganisms were inactivated by the VHP process to between 2 to 5-log depending on the organism and media used.

Heckert et al. (1997) inoculated glass and stainless steel with 9 exotic animal viruses. After VHP™ treatment, virus titer was reduced to 0 (except for hog cholera virus in whole blood). A VHP 1000 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

As noted, there are a variety of H_2O_2 methods and equipment available, and the methods evaluated to date, H_2O_2 vapor/VPHP, HPGP, and VHP™ have different effects on N95 FFR function. Filter function can be assessed with a quantitative fit test (e.g., PortaCount) or the NIOSH method (e.g. TSI 8130) which quantifies filter efficiency and airflow resistance. The filter efficiency rating for a N95 FFR should be above 95%.

The 2016 Battelle Report phase 2 study evaluated filter quality and fit with Bioquell H_2O_2 vapor/VPHP. The same exposure was applied as described above, but with the addition of 300 min of aeration, to 85 N95 FFRs (3M 1860) for 10, 20, 30, 40 and 50 cycles of decontamination, 15 N95 FFRs per cycle set. After decontamination, both inert and bioaerosol collection efficiency remained >99% and no degradation of airflow resistance was found for any of the 85 N95 FFRs (NIOSH method). Mannequin fit quality did not degrade when tested up to 20 cycles of decontamination (no fit testing was done beyond 20 cycles). After 30 cycles, strap degradation was observed with strap length elongation and loss of elasticity, which could negatively impact FFR fit (3M 1860).

The Duke University & Health H_2O_2 vapor system (*Schwartz et al., 2020) incorporates results from the Battelle study. N95 FFRs (3M 1860) were either suspended by their elastic straps or layed individually onto stainless steel racks in a disinfection room (12 X 12 ft) of their NIAID Regional Biocontainment Laboratory. The room was treated with H_2O_2 vapor (Bioquell Clarus™ C system with a 35% H_2O_2 solution) to attain a 480+ ppm concentration of H_2O_2

vapor with a gas time of 25 min and dwell time of 20 min. One hundred N95 FFRs (3M 1860) were treated with H_2O_2 vapor for 1 cycle. Air concentration near the N95 FFRs was measured during the aeration period to determine the time when 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 (0.2 ppm) of the device (PortaSens II™ sensor). A qualitative test was conducted on the N95 FFRs by 3 individuals who detected no noticeable odors. There was no physical nor performance (not described) degradation of the N95 FFRs. They are currently evaluating the Bioquell Z-2 and Bioquell ProteQ™ system with >10 repeated treatment cycles for fit.

Bergman et al. (2011) found that three cycles of H_2O_2 vapor decontamination (Bioquell) did not compromise filter efficiency (> 98%) in the six different N95 FFR models tested (see above) nor were there observable physical changes to the N95 FFRs. Although no measurements of filter performance were made in the Kenny et al. (*2020) study, after 5 cycles of H_2O_2 vapor treatment, the 3M 1870 N95 FFRs "appeared similar to new with no deformity." Fischer et al. (*2020) evaluated an N95 FFR (3M 9211) with 2 hours of wear and VHP decontamination, for 3 rounds and found no decline in quantitative (PortaCount) fit testing.

Viscusi et al. (2009) evaluated six different N95 FFRs (same FFRs as Bergman et al. 2011) and applied one cycle of treatment of HPGP (STERRAD 100S 55-min short cycle, with FFRs in Tyvek pouches) and found no effect on filter efficiency. However, in a follow up study by the same research group, three cycles of HPGP decontamination reduced filtering efficiency by > 5% for 4 of 6 different FFRs tested (NIOSH method; TSI 8130) (Bergman et al. 2010). This is below the FDA-required 95% threshold.

Kumar et al. (*2020) treated 4 different N95 models (3M 1860, 3M 1870, 3M 1804, AO 1054) with multiple cycles of HPGP (STERRAD 100NX 47 min). After 1 cycle of HPGP treatment, qualitative fit testing (PortaCount) demonstrated no reduction in filter quality. However, after 5 cycles of treatment, all 4 FFRs failed qualitative fit testing. The Dutch National Institute for Public Health report (March 16, 2020) summarized the effects of decontamination of 3M 8822 FFRs with up to 4 cycles of HPGP (STERRAD NX100, Express cycle with AllClear™). One or 2 cycles of decontamination did not deform FFRs or compromise fit (qualitative PortaCount method), but 3 cycles compromised fit and 4 cycles deformed the FFRs.

Oral et al. (*2020) treated N95 FFRs (3M 1860S, N=5) with a single cycle of VHP™ and found no reduction in filter efficiency. Kumar et al (2020) also used VHP™ (ARD Steris) to treat 4 different N95 FFRs (3M 1860, 3M 1870, 3M 1804, AO 1054). They found no degradation of filter fit test (PortaCount) after 1, 3, 5, or 10 cycles of treatment.

Cramer et al., (*2020) treated N95 FFRs for 5 cycles (3M 1860, N=3 and Halyard 46767, N=3) or 2 cycles (Gerson 2130, N=1 and 3M 8210, N=2) of SteriMist iHP room decontamination. Filter efficiency remained above 97% in all cases. They also tested 3M 1860 and Halyard 46767 and found no degradation in quantitative fit testing after 5 cycles. UCSF (personal communication, 2020) treated 3M 1860 and Halyard Fluidshield N95 FFRs with 0, 1, 2, and 5 cycles of decontamination with Halosil fogger. Filter efficiency remained above 95% after all treatments.

Wigginton et al. (*2020) treated 5 different 95 FFRs (3M 1860, 8210, 8210c, 8511, Moldex 1511) with VHP (see above for dose) and HPGP and evaluated filter efficiency with a modified-NIOSH method and non-quantitative fit testing. The VHP method was applied to 3 masks (3M 1860, 8210, Moldex 1511) for 5 to 10 cycles and filter efficiency remained >99% and all fit tests passed. The HPGP process and (Sterrad 100NX express cycle with respirators in Tyvek pouches) decreased filter efficiency for the 2 masks tested (3M 1860 and 8210) to below 95% after 3 cycles and was therefore not studied further for viral inactivation.

6. Data Summary Tables

Table 1. Impact of H_2O_2 decontamination methods on *G. stearothermophilus* spores and viruses

Author	Media	Dose	Phase Times (min)	Strain(s)	Effectiveness (log reduction)
A	Biological indicators in room (N=5)	HPV (Bioquell) (8 g/m^3	gas 15; dwell 120	*G stearothermophilus spores*	≥6
B	3M 1860 N95 FFRs inoculated with aerosol (N=15)	HPV (Bioquell) 2 g/min then 0.5 g/min	gas 20; dwell 150	*G stearothermophilus spores*	≥6
C	3M 1860 N95 FFRs inoculated with droplets (N=3)	HPV (Bioquell) 2 g/min then 0.5 g/min	gas 20; dwell 150	*G stearothermophilus spores*	≥6
D	3M 1860 N95 FFRs inoculated with aerosol (N=5)	HPV (Bioquell) 2 g/min then 0.5 g/min	gas 20; dwell 150	*G stearothermophilus spores*	≥6
E	Biological indicators near N95 FFRs (N=30)	SteraMist (TOMI)	gas 15; dwell 20	*G stearothermophilus spores*	≥6
F	3M 1860 inoculated N95 FFRs (N=3 for each phage)	HPV (Bioquell) 16 g/min	gas 30–40; dwell 25	Phage phi-6 Phage T7 Phage T1	≥6
G	3M 1860, 1870, 1804 and AO 1054 FFRs (N=1) inoculated with VSV and SARS-CoV2	VHP^{TM} (Steris) 5 g/min then 2.2 g/min	gas 3; dwell 30	Vesicular stomatitis virus; SARS-CoV-2	≥6
H	3M 1860S FFR pieces inoculated with SARS-CoV2	VHP^{TM} (Steris) 410 ppm	3 h	SARS-CoV-2	≥6
I	AO N9504C FFR pieces inoculated with SARS-CoV2	VHP 1000 ppm	10 min`	SARS-CoV-2	≥6
J	3M 1860, 1870+, 8511, 9211, HW N11125 FFRs	Curis	gas 12; dwell 50	Herpes Simplex Virus 1 Coxsackievirus B3 Phage phi6	≥6

	inoculated with virus species				
K	Glass and stainless steel inoculated with 9 viruses	VHP™ (Steris) 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: (*Cramer et al., 2020), F: (*Kenny et al., 2020), G: (*Kumar et al. 2020), H: (*Oral et al. 2020), I: (*Fischer et al. 2020), J: (*Derr 2020), K: (Heckert et al. 1997).

Table 2. Impact of H_2O_2 decontamination methods on N95 FFR filter efficiency by NIOSH method

Author	N95 FFRs	Dose	Time (min)	# cycles	Filtration efficiency	FFR damage
L	6 different N95 FFR models (N=6 for each model)	HPGP (STERRAD 100S)	55	1	>99.2%	Not evaluated
M	6 different N95 FFR models (N=6 for each model)	HPV (Bioquell) 8 g/m^3	gas 15; dwell 120	3	>97%	None noted
M	6 different N95 FFR models (N=6 for each model)	HPGP (STERRAD 100S)	55	3	<95% (4 of 6 models of N95 FFRs tested)	Not evaluated
N	3M 1860 FFRs (N=85 total)	HPV (Bioquell) 2 g/min then 0.5 g/min	gas 20; dwell 150	10, 20, 30, 40, 50	>99%	> 30 cycles, straps fragmented when stretched
O	3M 1860S FFRs (N=5)	VHP™ (Steris) 410 ppm	3 h	1	>98.8%	
P	3M 1860, Halyard Fluidshield N95 FFRs	Halosil	gas 15; dwell 120	0, 1, 2, 5	>99.3 (3M) >95.5 (HF)	

L: (Viscusi et al. 2009), M: Bergman et al., 2010), N: (Battelle, 2016), O: (*Oral et al. 2020), P: (UCSF, personal comm.)

7. Strategies

Commercial systems are available from companies such as ASP, Bioquell, Steris, Battelle, Curis, TOMI, and Halosil, most of which differ in the method of delivering and sustaining H_2O_2 concentrations in aerosolized liquid, gas, vapor or gas plasma phases by temperature, humidity, concentration of H_2O_2 /H_2O and the presence of other additives. Bioquell uses the term hydrogen peroxide vapor (HPV) and Battelle uses the term vapor phase hydrogen peroxide (VPHP) for the same method, Steris uses the term vaporized hydrogen peroxide (VHP™) for a slightly different method. ASP (STERRAD) applies a different method termed hydrogen peroxide gas plasma (HPGP).

The Bioquell wet-HPV systems [used by Duke, Bergman et al. (2010) and Battelle (2016)] includes a generator to produce H_2O_2 vapor, a module to measure the concentration of H_2O_2 vapor, temperature, and relative humidity in the enclosure, and an aeration unit to catalyse the breakdown of H_2O_2 vapor into oxygen and water vapor after H_2O_2 vapor exposure. The Bioquell systems do not control the H_2O_2 air concentration. H_2O_2 vapor 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). The manufacturer uses RFID-chipped bottles with a 35% peroxide solution without additives. It is important that such high concentrations of peroxide be handled safely; high concentrations are toxic irritants. Under certain circumstances, high concentration peroxide may be explosive, but this has not been reported for peroxides used for sterilization.

The Steris VHP™ systems have a generator inside the room with an integral aeration unit and dehumidifier to a set humidity level prior to the start of the cycle. The system delivers VHP™ by drying the vapor stream as it is returned to the generator. The Steris systems monitor the H_2O_2 concentration in the air throughout the exposure period.

The STERRAD system (ASP) generates a H_2O_2 gas plasma (HPGP) from H_2O_2 vapor by applying low frequency power. In this process, ionized H_2O_2 vapor inactivates virus and other pathogens and the low frequency power converts H_2O_2 vapor to plasma which converts rapidly to water and carbon dioxide. Some of the HPGP processes degrade N95 FFR filter efficiency with 3 cycles of decontamination (Bergman et al., 2010; *Wigginton et al. 2020). It is not known why some of the HPGP processes, compared to other H_2O_2 vapor processes, reduce N95 filter efficiency. One possible explanation is that the low frequency power directly impacts the electrostatic properties of the polypropylene electret filter (Dr. Peter Tsai, personal communication).

The Curis® system uses a solution of 7% hydrogen peroxide with a proprietary blend to create a fog of aerosolized liquid H_2O_2 and vapor with concentration between 80-150 ppm. The aerosolized droplets form a micro-condensation which contributes to the efficacy of the process. This treatment is based on delivery of solution combined with microdroplets within the treatment area. Although the manufacturer of the system has internal studies showing a 6-log reduction of *G. stearothermophilus* spores in HEPA and N95 FFR applications and no failures in fit testing**,** its impact on filtration efficiency is unknown (*Derr et al., 2020).

The Halo system (Halosil) aerosolizes hydrogen peroxide (aHP). It uses the HaloMist solution which contains a low percentage (5%) hydrogen peroxide with a biocidal silver nitrate additive at a low concentration (0.01%). Halo 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%. It is not known whether the low silver nitrate concentration negatively impacts the electrostatically-charged filter of N95 FFRs. An independent 3rd party study is underway to assess this.

The Battelle process involves the hospital collecting FFRs labeled with hospital number, unit number (and user name), shipping them to one of 6 sites around the country, processing them and returning them to the same hospital. Up to 80,000 FFRs can be processed per day at one site. The turn around time is approximately 10 days.

The Duke Medical Center has developed a procedure for decontaminating N95 FFRs using the Bioquell Clarus™ C system and has performed qualitative testing on more than one hundred N95 FFRs (see above; *Schwartz et al., 2020). Soiled N95 FFRs with visible blood, hair or damage are discarded and not decontaminated. N95 FFRs are separated into 4 streams based on size of N95 FFR (common: 3M 1860 or small: 3M 1860s) and the visible presence or absence of facial cosmetics. After decontamination, the integrity of the straps (evaluated for elongation), nose bridge, and nose foam are checked for integrity. The Duke Medical Center protocol does not return the FFRs back to the same user while the Battelle protocol can depending on how the hospital manages the FFRs. N95 FFRs are fit to a person's face and fit tested to confirm that there is no side leakage. A theoretical benefit of returning the FFR to the same user is to preserve the fit. When users receive their decontaminated N95 FFRs they should don the FFR and perform a self seal check to ensure that there is no side leakage.

8. Primary Risks and Unknowns

Dosing protocols are complex and could result in incomplete decontamination. Standard spore attenuation tests can be incorporated during decontamination. H_2O_2 is a strong oxidizer that can be an explosion hazard under certain conditions. Therefore, only trained personnel should operate H_2O_2 vapor, VHP™ or HPGP equipment. H_2O_2 gas is a corrosive irritant that can cause skin, eye and lung damage. H_2O_2 gas may interact with N95 FFR 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 and personnel access is strictly prohibited.

Detection of odor does not provide adequate warning of hazardous residual concentrations in the N95 FFRs. Complete off-gassing of sentinel FFRs should be confirmed with quantitative H_2O_2 testing (e.g., PortaSens-II test).

Probability of N95 FFRs straps failure increases with more than 20 cycles of decontamination; this will vary with the decontamination method and N95 FFRs model. Straps should be examined after decontamination and prior to each use to ensure that they are not elongated.

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

Repeated donning of a N95 FFR will gradually reduce the fit. For some N95 models a study found that fit declines to below acceptable after 5 donnings and for other models this does not occur until 15 donnings (Bergman et al., 2012).

9. Conclusions

Multiple studies have confirmed that N95 FFRs contaminated with aerosol or droplets containing *G. stearothermophilus* spores were successfully decontaminated with H_2O_2 with a 6-log reduction in spore level. Recent studies confirm that SARS-CoV-2 on N95 FFRs are eradicated with VHP™ (*Kumar et al. 2020; *Oral et al. 2020; *Fischer et al. 2020). N95 (3M 1860) FFRs filter efficiency did not degrade with up to 50 cycles of H_2O_2 vapor decontamination. However, after 20 cycles of H_2O_2 vapor decontamination the N95 FFRs straps showed degradation and were permanently deformed when stretched. A disadvantage of H_2O_2 processes is that the N95 FFRs must be completely aerated after treatment and this may take 4 to 8 hours.

Many hospitals already have H_2O_2 vapor/VPHP systems in-house for use in full room terminal decontamination. These could be deployed to dedicated N95 FFR decontamination rooms. Processing carts filled with spaced out N95 FFRs could be wheeled in and out of the room. Alternatively, a hermetically sealed biosafety cabinet (e.g., BSC type 3) could be connected to a VHP™ or H_2O_2 vapor unit and used for decontamination. Another solution is to send the FFRs to an outside service-provider (e.g., Battelle) for decontamination. To achieve an appropriate concentration of H_2O_2 vapor the specification of the H_2O_2 vapor system should be matched to the treated volume. The long 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 H_2O_2 vapor should be measured during decontamination to confirm adequate treatment level and also at the end of aeration to protect workers who enter the room. The OSHA Permissible Exposure Limit is 1 ppm (1.4 mg/m^3). H_2O_2 vapor can be detected by smell and irritation, but may still pose respiratory dangers at levels below user detection.

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Appendix A. List of N95 FFRs with or without cellulose based on information from Battelle and 3M Personal Safety Division (9 April 2020).

Cellulose-free N95 FFRs listed by Battelle and/or 3M Personal Safety Division

3M 1860 (+S)
3M 1870
3M 1870+
3M 8110S
3M 8205J

3M 8210
3M 8210Plus
3M 8210V
3M 8211
3M 8240
3M 8246
3M 8247
3M 8271
3M 8510
3M 8511
3M 8515
3M 8516
3M 8576
3M 8577
3M 8612F
3M 8670F
3M 8801H
3M 9205+

3M 9210(+)
3M 9211(+)
3M 9320+(BR)
3M 9322+
3M 9501+ (NOTE: 9501 does contain cellulose)
3M 9501V+
3M 9502+
3M 9502V+
3M 9505+
3M 9541 (+V)
3M 9542 (+V)
3M 9552 (+V)
3M 9820 Br
3M 9820+BR
3M 9920H

Honeywell N1105
Honeywell N1125
Honeywell NBW95

Moldex 1512
Moldex 2200

Kimberly Clark 46727
Kimberly Clark 46767
Kimberly Clark 46867
Kimberly Clark 62126
Kimberly Clark 62355

Cellulose-containing N95 FFRs listed by 3M Personal Safety Division
3M 9105 (+S)
3M 1804 (+S)
3M 1805 (+S)
3M 8000
3M 8200
3M 8212
3M 8214
3M 8233
3M 8293
3M 8511P
3M 8512
3M 8514
3M 8822 (+KV +AUS)
3M 9001
3M 9002 (+CN)
3M 9010 (+CN, +V,+MX)
3M 9105 (+S)
3M 9132
3M 9501
3M 9502

Protech NON27501

Appendix B: Liquid H_2O_2 (LHP) Decontamination of N95 FFRs

Many types of hydrogen peroxide delivery systems are reviewed above. Here we also review the low-cost method of submerging N95 FFRs into a solution of 6% hydrogen peroxide (LHP). While the FDA has granted Emergency Use Authorization for five different hydrogen peroxide decontamination processes for N95 FFRs, they have not at this time granted EUA for a device and process using LHP.

However, hydrogen peroxide solutions have been used as a sterilant for medical and dental tools for many years, and LHP has been approved as a high level disinfectant by the FDA (FDA-Cleared Sterilants and High Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices, FDA, 4/28/2020). It is used in clinical settings for disinfection of semi-critical devices (e.g., endoscopes) where devices are submerged for 8 to 30 min at 20°C. The solutions can be reused for up to 21 days.

A detailed summary of the literature on the effectiveness of LHP for inactivating viruses and other microorganisms and methods of action has been compiled by the CDC: https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html. Although all H_2O_2 decontamination methods described above in the main document have been shown to inactivate N95 FFRs inoculated with SARS-CoV-2 and other microorganisms, there are no studies of the effectiveness of LHP for inactivating microorganisms on N95 FFRs. It is likely that the findings from studies of VPHP and aHP are applicable to decontamination of N95 FFRs by LHP, assuming viral particles on the N95 FFR come into contact with LHP. There is a concern that during submersion in a solution, air bubbles may be trapped between the liquid and mask, especially when considering a surgical N95 FFR that has a fluid barrier layer. Lack of direct contact between LHP and the N95 FFR may diminish the virucidal effect, making the air bubbles a concern. Currently whether or not air bubbles form in LHP has not been well studied, and this should be an area of caution for anyone implementing an LHP method.

Filtration performance of N95 FFRs has been shown to be preserved following immersion in 3–6% liquid hydrogen peroxide. Viscusi et al. (2007) studied two different N95 FFRs (3M 1860 & 3M 8000) and 4 FFRs were used for each test condition. For one test condition, N95 FFRs were submerged for 30 min in 3% LHP. For the second test condition, the FFRs were submerged for 30 min in 6% hydrogen peroxide. Following exposure, the FFRs were hung on a peg board and air dried for 72 hours before measuring filter efficiency (NIOSH method). The filter efficiency was greater than 99.2% after treatment for both FFRs. A slight fading of the ink on the fabric was observed.

Bergman et al. (2010) studied six different N95 FFRs (industrial N95 FFRs: 3M 8210; 3M 8000; Moldex 2200 and surgical N95 FFRs: KC PFR95-270; 3M 1870; 3M 1860 [FFRs models listed in Bergman et al. (2011)]. Six of each of the models were submerged for 30 min in a 6% solution of hydrogen peroxide. Following each exposure, the FFRs were hung on a laboratory peg board and dried for a minimum of 16 hours with the aid of a fan before the treatment was repeated for 3 cycles of decontamination. The filter efficiency test (NIOSH method) following the third treatment was greater than 95% for all models and the mean filter airflow resistance was not increased. Some staples were observed to oxidize. Together, these two studies indicate that N95 FFR filtration efficiency remains acceptable after at least 3 cycles of LHP

immersion. However, there are no studies that measure the effect of LHP immersion on N95 FFR fit performance. Nor were there studies to determine how long a decontaminated N95 FFRs needs to dry in order to remove all H_2O_2.

To achieve the desired concentration, liquid hydrogen peroxide can be diluted with deionized water. The solution should not contain surfactants which may decrease N95 FFR filter efficiency (Viscusi et al. 2007).

In conclusion, LHP is a promising, low cost decontamination method that does not damage N95 FFR filters. However, it has not yet been proven to inactivate viruses or other organisms on masks, and N95 FFR fit performance was not tested after LHP. In addition, it is not known how long it takes for N95 FFRs to dry after submersion in H_2O_2. Health care workers should be cautioned not to bring masks home for decontamination due to the risk of contaminating others in their household with hospital acquired microorganisms.