Final Report for the Bioquell Hydrogen Peroxide Vapor (HPV) Decontamination for Reuse of N95 Respirators

FINAL REPORT

for

Bioquell HPV Decontamination for Reuse of N95 Respirators

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FDA Contracting Officer's Representative
Brenda Brooks

Prepared by
Battelle
Columbus, Ohio 43201

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List of Acronyms

BI	biological indicator
°C	degrees Celsius
CFR	Code of Federal Regulations
CFU	colony forming units
cm	centimeter
CMD	count median diameter
E	collection efficiency
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
FFR	filtering facepiece respirator
GSD	geometric standard deviation
HEPA	high efficiency particulate air
HPV	hydrogen peroxide vapor
IOM	Institute of Medicine
L	liter
m	meter
µL	microliter
µm	micrometer
mL	milliliter
MMAD	mass median aerodynamic diameter
min	minute
mm	millimeter
NaCl	sodium chloride
NIOSH	National Institute for Occupational Safety and Health
NPPTL	National Personal Protective Technology Laboratory
OPS	optical particle sizer
OSHA	Occupational Safety and Health Administration
P	aerosol penetration
PEL	permissible exposure limit
PPE	personal protective equipment
ppm	parts per million
psig	pounds per square inch gauge
PSL	polystyrene latex
RH	relative humidity
s	second
SMPS	scanning mobility particle sizer
SOP	standard operating procedure
StAH	static advanced headform
SWPF	simulated workplace protection factor
TSA	tryptic soy agar
TSB	tryptic soy broth

DISTRIBUTION LIST

Mr. William Richter
Mr. Kent Hofacre
Mr. Zachary Willenberg
Battelle
505 King Avenue
Columbus, OH 43201-2693

Ms. Brenda Brooks
FDA Office of Counterterrorism and Emerging Threats
10903 New Hampshire Avenue, WO Building 1, RM4314
Silver Spring, MD 20993-0002

1.0 Executive Summary

Filtering facepiece respirators (FFRs) are a common form of personal protective equipment (PPE) used throughout the medical care industry and by first responders. In the event of a pandemic (e.g. influenza), large numbers of FFRs will be used by healthcare workers for protection. It was estimated that during a 42-day influenza pandemic over 90 million N95 FFRs will be needed to protect healthcare workers [1], resulting in a shortage of FFRs. In 2009, an interagency working group of the US government published a comprehensive report, Project BREATHE, which included recommendations towards the development of improved respiratory equipment for healthcare workers and included considerations for research in the area of reuse and repeated decontamination of N95 FFRs [2]. Consensus Statements 6 and 7 of the Project BREATHE report recommends that respirators should be capable of being repeatedly decontaminated during a crisis for up to 50 cycles without causing damage to the respirator.

This project investigated a potential approach to decontaminate and reuse FFRs in an emergency scenario. Hydrogen peroxide vapor (HPV) decontamination is an industry standard decontaminant used in research, pharmaceutical, and medical facilities. The low toxicity combined with the ease of catalytic reduction to oxygen and water makes HPV a suitable choice for the decontamination of FFRs. The Bioquell Clarus C HPV generator is a commercially-available technology that can fumigate spaces from small chambers to a large room, allowing the end user implementation flexibility as well as maximizing the number of FFRs that can be decontaminated at one time. The objectives of this project were to assess the efficacy of HPV decontamination of a selected N95 FFR and to characterize the impact of HPV exposure on the mechanical integrity and performance of the FFR. This project comprised three phases: 1) determination of decontamination parameters, 2) impact of repeat decontamination cycles on functional performance of the FFR, and 3) assessment of repeated decontamination efficacy for up to 50 decontamination cycles. As this was a pilot study, only one brand of N95 FFR, the Model 1860 (3M, St. Paul, MN), was used to assess the feasibility of the approach.

Phase I established the parameters of the HPV decontamination cycle to ensure a 6-log reduction in organism viability. Swatches of FFR material were inoculated with liquid droplets containing *Geobacillus stearothermophilus* spores, selected due to its known resistance to hydrogen peroxide vapor decontamination, at a loading level of $1x10^6$ colony forming units (CFU) per swatch and exposed to the HPV cycle. Swatches were removed at regular intervals and assayed to determine the exposure time required to achieve the 6-log reduction (i.e., complete inactivation of the organism). Testing in Phase I also demonstrated that the HPV cycle achieved a 6-log reduction when the spores were inoculated as an aerosol instead of liquid droplets. The final HPV cycle included a 10 min conditioning phase, 20 min gassing phase at 2 g/min, 150 min dwell phase at 0.5 g/min, and a 300 min aeration phase such that no “off-gassing” of hydrogen peroxide from the

FFR was detected. Thus, the total cycle duration was 480 min (8 hr). A shorter aeration phase is possible as the measured hydrogen peroxide concentration "off-gassing" from the FFR was already below the Permissible Exposure Limit (PEL) of 1 ppm when the first measurement was made at 210 min.

Phase II evaluated the mechanical integrity and performance of the FFR following exposure to up to 50 cycles of the HPV decontamination. Performance tests included inert aerosol collection efficiency, biological aerosol collection efficiency, inhalation resistance, and respirator fit on a manikin head form. No visible degradation was observed after exposure to 10 or 20 HPV cycles. However, after 30 HPV cycles, it was observed that that elastic material in the straps fragmented when stretched. This strap degradation could have a negative impact on the fit of the respirator or cause complete failure if the strap broke. It is recommended to identify alternative materials for the straps that would have more resistance to the HPV exposure. The aerosol collection efficiency (both inert and biological) and the air flow resistance were not affected over the 50 cycles of HPV exposure. Further testing is recommended with other brands/models of N95 FFRs as different types of media may be affected differently by the HPV cycle.

Phase III confirmed that decontamination of the N95 FFR was still achieved even after 50 repeated cycles of biological aerosol exposure/HPV decontamination. The FFRs were loaded with an aerosol containing *G. stearothermophilus* (target loading of $1x10^6$ CFU per 4 cm^2) and then underwent decontamination using the Bioquell Clarus C HPV system. Complete inactivation was demonstrated following 50 repeat aerosol inoculation/decontamination cycles.

This project offered a comprehensive pilot-scale study that evaluated the efficacy of HPV for decontamination of N95 respirators using the Bioquell Clarus C HPV decontamination system. This project evaluated efficacy against a single organism and the structural and functional integrity of the selected N95 FFR by measuring the filter efficiency, fit, and differential pressure after exposure to up to 50 decontamination cycles. It is recommended to characterize the impact of the HPV decontamination cycle on the performance of other N95 FFR brands/models, especially because different respirators may have filtration media that are affected differently. In addition, testing could be performed to demonstrate the efficacy of the HPV decontamination cycle against organism of interest within the healthcare community. This project successfully demonstrated the feasibility of a test approach to evaluate FFR reuse and establish testing methods for future investigation of additional decontamination technologies.

2.0 Introduction

2.1 Background

Filtering facepiece respirators (FFRs) are a form of personal protective equipment (PPE) to reduce exposure to airborne particles. While there are several types of FFRs available, N95 FFRs are most commonly used by healthcare workers to prevent exposure to infectious aerosols. Current practices require the disposal of the FFR immediately after use. However, in circumstances such as an emergency response resulting from an emerging infectious disease (for example pandemic influenza) where the demand for FFRs exceeds the available supply, the reuse of FFRs may be required. It was estimated that during a 42-day influenza pandemic over 90 million N95 FFRs will be needed to protect healthcare workers, resulting in a shortage of FFRs [1]. While studies to characterize the persistence of influenza on N95 FFRs are few, the limited data demonstrate influenza can remain infectious for days and that the persistence is a function of several factors such as the influenza subtype and environmental conditions [3, 4].

In 2006, the Institute of Medicine (IOM) published a report addressing strategies that could extend the N95 FFR supply in emergency situations [1]. One option considered was decontamination followed by reuse, and while this was not a viable approach at that time, it was recommended that further investigations be conducted to determine the effects of simple decontamination techniques on the performance of FFRs. In 2009, an interagency working group of the US government published a comprehensive report, Project BREATHE, which built upon the recommendations of the IOM and proposed the next steps that should be taken towards the development of improved respiratory equipment for healthcare workers [2]. As part of the report, the working group issued a list of consensus statements to be considered in development. The issues of reuse and repeated decontamination durability were addressed in Consensus Statements 6 and 7, summarizing that respirators should be capable of reuse for up to 50 decontamination cycles (each ideally being 60 seconds or less), while maintaining a simulated workplace protector factor (SWPF) greater than 100. Both Consensus Statements were given a Priority Designation of 1 (highest priority) relative to other consensus statements, highlighting the importance of this issue.

2.2 Objective

The objective of this project was to assess efficacy of hydrogen peroxide vapor (HPV) decontamination of a selected N95 FFR and characterize the impact of HPV exposure on FFR mechanical integrity and performance.

2.3 Scope

The objectives of the project align with the U.S. Food and Drug Administration (FDA) technical objectives to identify and evaluate methods to improve the availability and reuse of PPE. The

project was a pilot-scale assessment of HPV, using the Bioquell Clarus C decontamination system, as an approach to achieve decontamination of N95 disposable FFRs for reuse. Up to 50 decontamination cycles were evaluated to determine whether a change in filter efficiency, differential pressure drop, and/or fit occurred.

As this was a pilot-scale study, only one brand of N95 FFR was used to assess the feasibility of the approach. The Model 1860 N95 FFR (3M, St. Paul, MN), shown in Figure 1, was selected for this project. It is approved by the National Institute for Occupational Safety and Health (NIOSH) as an N95 and is also cleared by the FDA as a surgical mask. It has a cup-shape design and uses an advanced electrostatic media to reduce breathing resistance [5]. The basic physical design of the 3M Model 1860 is representative of other manufacturer's N95 FFRs, but may differ with the type of filtration media, strap material, and/or sealing interface materials.

Figure 1. 3M 1860 N95 Filtering Facepiece Respirator

Bioquell's HPV method is registered with the Environmental Protection Agency (EPA) as a sterilant, which has rapid action and is residue free (EPA registered sterilant: 72372-1-86703). The Bioquell Clarus C decontamination system, shown in Figure 2, generates hydrogen peroxide vapor that is uniformly distributed over surfaces. This technology has been used for approximately 15 years in life sciences, pharmaceutical, biodefense and healthcare applications. The technology has been applied in enclosures ranging from small glove boxes to entire buildings [6-8]. HPV has been

used in healthcare settings for the past decade to disinfect clinical areas and remove environmental reservoirs of nosocomial pathogens. The use of HPV in hospitals has been associated with reduced rates of infection [9] and improved control of outbreaks [10,11]. HPV has demonstrated biological efficacy against a range of bacterial endospores, vegetative bacteria, viruses and fungi, including influenza viruses, *Mycobacterium tuberculosis* and *Bacillus anthracis* Ames spores.[6,10,12,13].

Figure 2. Bioquell Clarus C Decontamination System

The Bioquell Clarus C is a candidate for the treatment of N95 FFRs to facilitate reuse by health care workers and emergency responders. Indeed, a preliminary study of three repeat cycles of HPV concluded that there were no effects on the laboratory performance and physical integrity of N95 FFRs [14]. This same study noted reduction in filter efficiency following exposure to a hydrogen peroxide gas plasma technology. Based on these preliminary data, Battelle investigated a vapor-phase hydrogen peroxide generating system (i.e., the Bioquell Clarus C) to mitigate any adverse effects noted with using a hydrogen peroxide gas plasma technology.

This project was divided into three phases as summarized in Figure 3. Phase I focused on establishing the HPV decontamination cycle parameters and demonstrating a 6-log reduction of the

biological indicator, *Geobacillus stearothermophilus*. This spore-forming organism was selected because it represents a worst case scenario due to its resistance to hydrogen peroxide vapor decontamination [6], as well as a culture temperature (55-60°C) that will reduce/mitigate the growth of potential endogenous contaminants. Phase II characterized the performance of the FFR after exposure to up to 50 decontamination cycles to determine whether the HPV exposure adversely affected respirator function. Phase III assessed the efficacy of the decontamination cycle after 50 cycles of biological aerosol exposures/HPV decontamination.

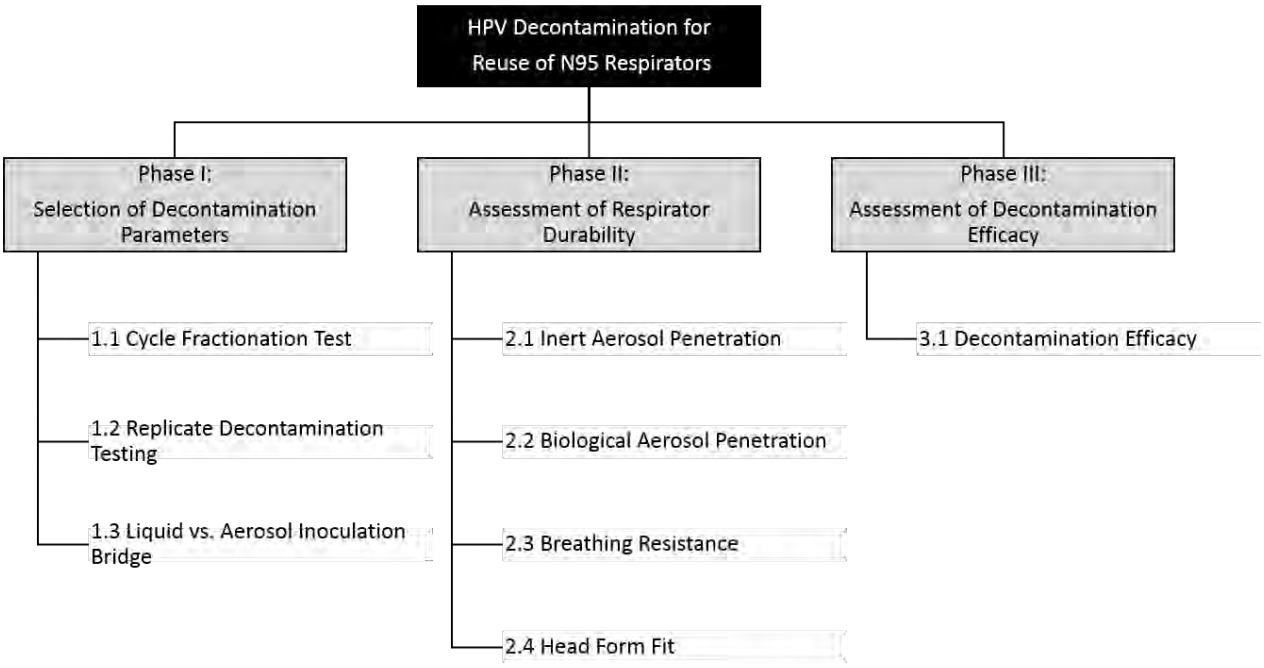

Figure 3. Work Breakdown Structure

3.0 Phase I: Selection of Decontamination Parameters

The objective of Phase I was to determine the parameters required to achieve decontamination of N95 FFRs by HPV exposure with the Bioquell Clarus C system. Phase I consisted of three tasks summarized below:

- Cycle Fractionation Testing (Task 1.1): Establish the decontamination cycle parameters needed to achieve a 6-log reduction in organism viability when spiked onto swatches of N95 FFR material. The AOAC International validated Method 2008.05 [15], requires 6-log reductions for products with sporicidal claims; therefore, the 6-log reduction was used as a benchmark value for the project. The expected range of viral loading on a FFR during actual use in a healthcare environment is not known and will depend on a variety of factors.
- Replicate Decontamination Testing (Task 1.2): Confirm decontamination cycle parameters still achieve a 6-log reduction in organism viability when a whole, intact N95 FFR is contaminated with a liquid challenge and allowed to dry.
- Liquid versus aerosol Inoculation Bridge (Task 1.3): Confirm decontamination cycle parameters still achieve a 6-log reduction in organism viability when the N95 FFR is contaminated with an aerosol containing the biological indicator instead of liquid droplets.

All tasks were conducted using *G. stearothermophilus* spores as the biological indicator. For each task, complete decontamination (i.e. 6-log reduction) was qualitatively determined in liquid culture, eliminating potential detection limitations with a quantitative method. Key aspects of the HPV decontamination cycle are described in Section 3.1. The three tasks of Phase I are described in Sections 3.2 through 3.4.

3.1 Overview of HPV Cycle

All industry standard hydrogen peroxide decontamination systems use similar cycle patterns with variations such as starting relative humidity (RH), temperatures, and contact times. A typical decontamination process is shown in Figure 4 and consists of four steps: (1) conditioning, (2) gassing, (3) dwell (or contact time), and (4) aeration. In the conditioning phase, air is circulated from the decontamination exposure chamber through a dehumidification process. The next phase is a gassing mode where a preset rate of hydrogen peroxide is vaporized and injected into the decontamination chamber. Each proprietary technology on the market, which is designed to convert hydrogen peroxide to a vaporized state, achieves this end and delivers the gaseous hydrogen peroxide via propriety methods. The goal of the Bioquell technology is to achieve micro-condensation (i.e., thin film of hydrogen peroxide) on the exposed surfaces as quickly as possible. Once micro-condensation is uniform throughout the chamber, the injection rate of hydrogen peroxide is reduced during the dwell or contact phase. This phase is used to maintain a plateau of

hydrogen peroxide concentration for the remainder of the decontamination process. Once the pre-specified amount of hydrogen peroxide has been delivered, the unit switches to aeration phase where the hydrogen peroxide vapor is catalytically converted into oxygen and water.

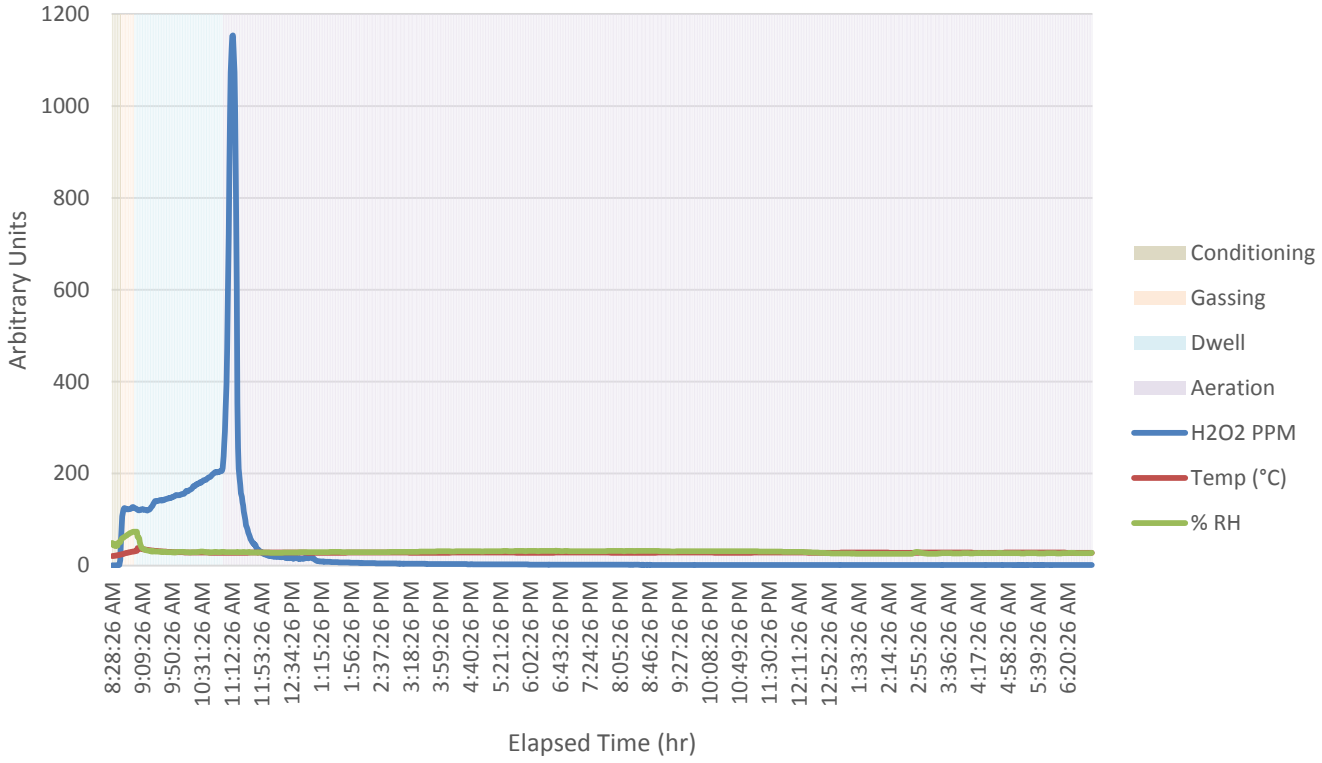

Figure 4. Representative HPV Decontamination Cycle Profile

3.2 Task 1.1: Cycle Fractionation Testing

Testing was conducted to determine the decontamination cycle parameters for complete inactivation of *G. stearothermophilus* spores spiked onto swatches of FFR material. The swatches (2 cm x 2 cm) were excised from the N95 FFR, as shown in Figure 5, and contained all of the material layers of the respirator. The swatches were inoculated with 10^6 total colony-forming units (CFU) of *G. stearothermophilus* spores suspended in water and allowed to dry overnight. Inoculm was applied the the FFR swatch as a single 100 µL droplet of spore suspension. Bioquell HPV biological indicators (BI), also containing $1x10^6$ CFU *G. stearothermophilus* spores, were tested in parallel with the contaminated N95 swatches as controls. The test swatches and control BIs were suspended along a rack within the decontamination chamber. The chamber, shown in Figure 6, was a static glove-box (Model No. 830-ABC, Plas-Labs, Inc., Lansing, MI) with dimensions of 71 cm x 59 cm x 74 cm and an internal volume of about 310 L that has been extensively used for fumigant efficacy studies at Battelle.

Figure 5. 3M 1860 N95 FFR with 2 cm x 2 cm Swatch Excised

Figure 6. HPV Exposure Chamber

Three trials were completed with a goal to determine the shortest decontamination cycle that achieved the 6-log, complete inactivation of the target organism. The cycle parameters for each trial are summarized in Table 1. Note that the aeration phase was evaluated separately and will be discussed separately. Initial test parameters were selected based on a previous project which examined the efficacy of the Bioquell Clarus C unit against *B. anthracis* and *G. stearothermophilus* utilizing the same Plas Labs exposure chamber [6]. Every 2 to 5 minutes during dwell phase and depending on the test duration, coupons were removed and placed into tubes containing tryptic soy broth (TSB), capped and sealed. These tubes were incubated at 60°C and assessed for turbidity after one and seven days. Turbid cultures indicated the presence of the viable target organism (Figure 7). The test materials were not considered sterile, so even though there was a possibility that a contaminant (*i.e.*, not the target organism) could propagate in the TSB, the high temperature-incubation required for *G. stearothermophilus* mitigates growth of typical endogenous contaminants.

Table 1. Summary of HPV Cycle Parameters Evaluated in Task 1.1

Trial ID	Conditioning	Gassing	Dwell
1	10 min	20 min @ 2 g/min	30 min @ 0.5 g/min
2	10 min	20 min @ 2 g/min	120 min @ 0.5 g/min
3	10 min	20 min @ 2 g/min	120 min @ 0.5 g/min

Figure 7. Representative Fractionation Test Results Comparing Turbid (Growth) and Clear (No-Growth) Samples

The results of the cycle fractionation testing are summarized in Table 2. A "positive" in the table indicates the presence of the viable organism as the broth was turbid. A "negative" indicates that the swatch was effectively decontaminated (i.e., 6-log reduction) as the broth remained clear. In Trial #1, all N95 swatches spiked with *G. stearothermophilus* were positive for growth (i.e., turbid), even after the 30 min dwell phase. In comparison, all of the control BIs were negative for growth. The reason for the observed difference is not known but may be attributed to the fibrous nature of the filter material. The dwell time was increased from 30 to 120 min in Trials #2 and #3, respectively. In Trial #2, the first spiked swatch that was negative was observed 60 min into the dwell phase; however, there were intermittent positive samples observed through 100 min. While Trial #3 used the same HPV cycle as Trial #2, no growth was observed in all samples after 45 min into the dwell phase. Therefore, based on the results of Trials #2 and #3, a dwell time of 120 min was recommended for complete decontamination.

Table 2. Summary of Cycle Fractionation Testing Results

Dwell Time (min)	Trial #1		Trial #2		Trial #3	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
≤30	Positive[(a)]	Positive[(a)]	(b)	(b)	(b)	(b)
35	(c)	(c)	Positive	Positive	Negative	Positive
40	(c)	(c)	Positive	Positive	Positive	Positive
45	(c)	(c)	Positive	Positive	Negative	Negative
50	(c)	(c)	Positive	Positive	Negative	Negative
55	(c)	(c)	Positive	Positive	Negative	Negative
60	(c)	(c)	Negative	Negative	Negative	Negative
65	(c)	(c)	Positive	Positive	Negative	Negative
70	(c)	(c)	Negative	Negative	Negative	Negative
75	(c)	(c)	Positive	Positive	Negative	Negative
80	(c)	(c)	Negative	Negative	Negative	Negative
85	(c)	(c)	Negative	Negative	Negative	Negative
90	(c)	(c)	Negative	Negative	Negative	Negative
95	(c)	(c)	Negative	Negative	Negative	Negative
100	(c)	(c)	Positive	Positive	Negative	Negative
105	(c)	(c)	Negative	Negative	Negative	Negative
110	(c)	(c)	Negative	Negative	Negative	Negative
115	(c)	(c)	Negative	Negative	Negative	Negative
120	(c)	(c)	Negative	Negative	Negative	Negative

[(a)] All samples collected at or before 30 min in Trial #1 were positive.

[(b)] Samples not collected until 35 min into dwell phase.

[(c)] Trial #1 was stopped after 30 min dwell phase.

The results described above were used to establish the conditioning, gassing, and dwell cycle parameters. Since the N95 FFRs are intended for reuse, the aeration phase is a critical factor to reduce the potential for exposure to hydrogen peroxide during wear. Assessment of the off-gassing period after treatment was conducted to determine how long the aeration cycle should be to achieve a non-detect when sampling at the surface of the N95 FFR material with a low-level hydrogen peroxide monitor (Portasens II, International, Inc. DeMotte, IN). The range of the sensor for hydrogen peroxide was 0 to 10 ppm in increments of 0.1 ppm. In comparison, the OSHA Permissible Exposure Limit (PEL) is 1 ppm. Five N95 FFRs were exposed to a single HPV decontamination cycle. During aeration, the facility HVAC system was connected to the test chamber and a high efficiency particulate air (HEPA) filtered port was opened to allow room air to flush the chamber to aid in the removal or breakdown of the hydrogen peroxide. The "off-gassing" from the FFR was measured three, four, and five hours into the aeration phase. The off-gassing was measured by placing the FFR onto a "chuck" and drawing air through the mask. The air that passed through the mask was sampled using the hydrogen peroxide monitor. A total of five hours was required to achieve a non-detect for hydrogen peroxide. Hydrogen peroxide readings taken from the masks at the tested time points are shown in Table 3. Note that even after only 3 hours of aeration the hydrogen peroxide concentration was below the PEL so a shorter aeration phase may be possible.

Table 3. Aeration Phase Hydrogen Peroxide Readings from Whole N95 FFRs

Sample ID	**Aeration Phase Duration (hr)**		
	3	4	5
	H_2O_2 Concentration (ppm)		
N95 FFR Dräger	0.2	0.2	0.0[(a)]

[(a)] Once one FFR read 0.0, all 5 FFRs were tested and read 0.0.

Based on these results, the recommended HPV decontamination cycle parameters for Task 1.2 are summarized in Table 4. These cycle parameters were sufficient to provide a 6-log reduction of *G. stearothermophilus* inoculated onto N95 FFR swatches and allowed sufficient time for FFR to off-gassing to below PEL. The Project BREATHE report recommended a decontamination cycle that was less than 60 seconds. Although the cycle recommended in this report is longer, the approach is viable as large numbers (>50) of FFRs can be decontaminated simultaneously. Thus, this decontamination approach is not anticipated to be used by individuals at the point of use. Rather, this approach may be used to decontaminate FFRs in bulk at the end of a work shift.

Table 4. Decontamination Parameters Determined by Cycle Fractionation Testing

Phase	Duration (min)	Rate of HPV Injection (g/min)
Conditioning	10	NA
Gassing	20	2.0
Dwell	120	0.5
Aeration	300	NA

3.3 Task 1.2: Replicate Decontamination Testing

This task focused on decontaminating whole, intact N95 FFRs instead of swatch material. The reason for placing the entire N95 FFR inside the exposure chamber (*i.e.*, for HPV decontamination) was to allow the HPV to absorb into the entire respirator rather than a representative swatch. Therefore, absorption and off-gassing of HPV were therefore accounted for in this evaluation. While testing swatches in Task 1.1 provided an efficient approach to screen the HPV cycle parameters, testing was needed to confirm the decontamination of whole FFRs.

The completed test matrix for Task 1.2 is provided in Table 5. Each trial included a total of 10 N95 FFRs inoculated with *G. stearothermophilus* spores, five for HPV decontamination and five controls. A known concentration of purified *G. stearothermophilus* spores (10^6 total CFU in sterile water) was inoculated onto a pre-determined area of the N95 FFR surface as represented in Figure 8. Similar to Task 1.1, a 100 µL droplet was applied to the marked area (Figure 8), and allowed to dry overnight at ambient conditions before the N95 FFR was subjected to HPV exposure. The controls were placed into a chamber that was controlled at a similar temperature and RH as the decontamination chamber. The control chamber consisted of a 9-L lock'n'lock container (Locknlock USA, Aneheim, CA) that was placed into an incubator to control temperature. A saturated sodium chloride (NaCl) solution was prepared and placed at the bottom of the chamber to control relative humidity within the chamber. Post-HPV exposure, the inoculated area on each N95 FFR was excised from the respirator, placed in TSB, incubated at 55-65 °C, and observed for turbidity after four and seven days. After observation on day seven, all samples were plated onto nutrient agar to verify the presence of the target organism via morphological verification and to rule out any potentially bactriostatic components resulting in a false negative.

Table 5. Completed Task 1.2 Test Matrix

Trial	HPV Cycle Parameters				Number of FFRs	
	Conditioning	Gassing	Dwell	Aeration	HPV	Controls
1	10 min	20 min @ 2 g/min	120 min @ 0.5 g/min	300 min	5	5
2	10 min	20 min @ 2 g/min	150 min @ 0.5 g/min	300 min	5	5
3	10 min	20 min @ 2 g/min	150 min @ 0.5 g/min	300 min	5	5
4	10 min	20 min @ 2 g/min	150 min @ 0.5 g/min	300 min	5	5

Figure 8. N95 FFR with Marked Area for Contamination

In the first trial, one of the five FFRs exposed to the HPV was positive for growth. Streak plate analysis and growth of this sample confirmed morphology to be *G. stearothermophilus* and not a contaminant. Thus, it was recommended to increase the dwell phase by 30 min to a total time of 150 min. This dwell time was used for Trials 2 through 4 in which no positive results were observed in the liquid cultures or subsequent streak plates following expousre to the adjusted HPV cycle. Based on these results, the decontamination cycle parameters recommended to carry forward into subsequent tests are summarized in Table 6.

Table 6. Decontamination Parameters Determined by Replicate Decontamination Testing

Phase	Duration (min)	Rate of HPV Injection (g/min)
Conditioning	10	NA
Gassing	20	2.0
Dwell	150	0.5
Aeration	300	NA

3.4 Task 1.3: Liquid VS. Aerosol Inoculation Bridge

The final assessment in Phase I investigated the successful decontamination of N95 inoculated via aerosol exposure. The test matrix is provided in Table 7. A total of 10 FFRs were loaded with *G. stearothermophilus* spores in each trial. This included five that were subsequently exposed to the HPV cycle and five controls. The control respirators were not exposed to the decontaminant but rather were placed into a chamber where they were exposed to temperature and RH conditions similar to those experienced in the HPV cycle. Three trials were completed for a total sample size of 15 for both the HPV and control FFRs.

Table 7. Proposed Liquid vs. Aerosol Inoculation Bridge Test Matrix

Treatment	HPV FFRs	Control FFRs
Trial	**Number of FFRs Tested**	
1	5	5
2	5	5
3	5	5
Total	15	15

Contamination of N95 respirators was conducted in the custom chamber shown in Figure 9. The challenge aerosol containing *G. stearothermophilus* spores was generated using a 6-Jet Collison nebulizer (BGI, Inc., Waltham, MA) operated at 20 psig from an aqueous suspension containing $5x10^8$ CFU/milliliter (mL) spores. The aerosol exiting the nebulizer was mixed with HEPA filtered air and delivered to the exposure chamber. The chamber was equipped with a mixing fan to ensure a homogeneous challenge. The flow rate through each FFR during contamination was about 20 L/min (constant flow), representative of a light work respiration rate [16].

The size distribution of the viable organisms associated with the aerosol was measured using a cascade impactor. The mass median aerodynamic diameter was about 1 micrometer (μm). Thus, the spores were aerosolized predominantly as single spores. The aerosol concentration (CFU/L) was measured during each trial by collecting 47-mm gelatin filters for subsequent bioassay. The measured aerosol concentration was nominally $1x10^5$ CFU/L. The loading was determined based on the product of the measured aerosol challenge concentration, flow rate through the FFR, and

exposure duration. The typical loading duration was 20 min to target a loading of $1x10^6$ CFU per 4 cm^2 swatch. The surface area of the FFR was estimated to be about 150 cm^2 (size small FFRs were used for this task).

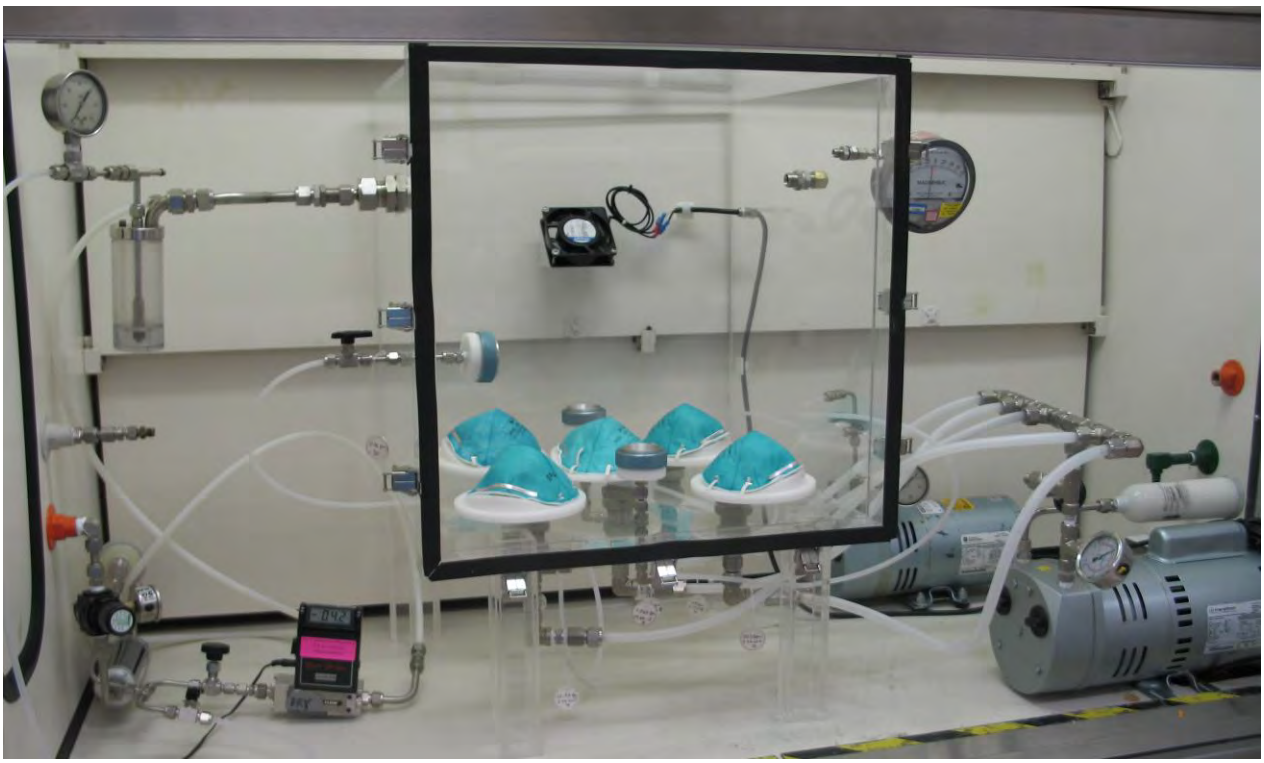

Figure 9. Aerosol Contamination Test System

Following contamination, the five HPV FFRs were placed in the decontamination chamber as shown in Figure 10 and exposed to a single HPV cycle as determined in Task 1.2. Note, however, the FFRs were removed from the decontamination chamber immediately following the dwell phase, therefore the aeration phase was not evaluated as part of this task. Following decontamination, the efficacy of the HPV exposure was determined by removing one 4 cm^2 (2 x 2 cm) swatch from each FFR, placing the swatch in 10 mL TSB, and observing for turbidity following incubation at 55-65 °C on days four and seven. All samples were streaked on tryptic soy agar (TSA) to confirm sterility and if positive, to confirm colony morphology consistent with *G. stearothermophilus* following the seven day incubation period. The excised swatches were estimated to be loaded with $1x10^6$ CFU, such that a 6-log reduction was observed if the broth remained clear. In addition, the loading level (CFU/cm^2) on the control FFRs was determined after each trial by excising a swatch, extracting into buffer solution, and plating for quantification.

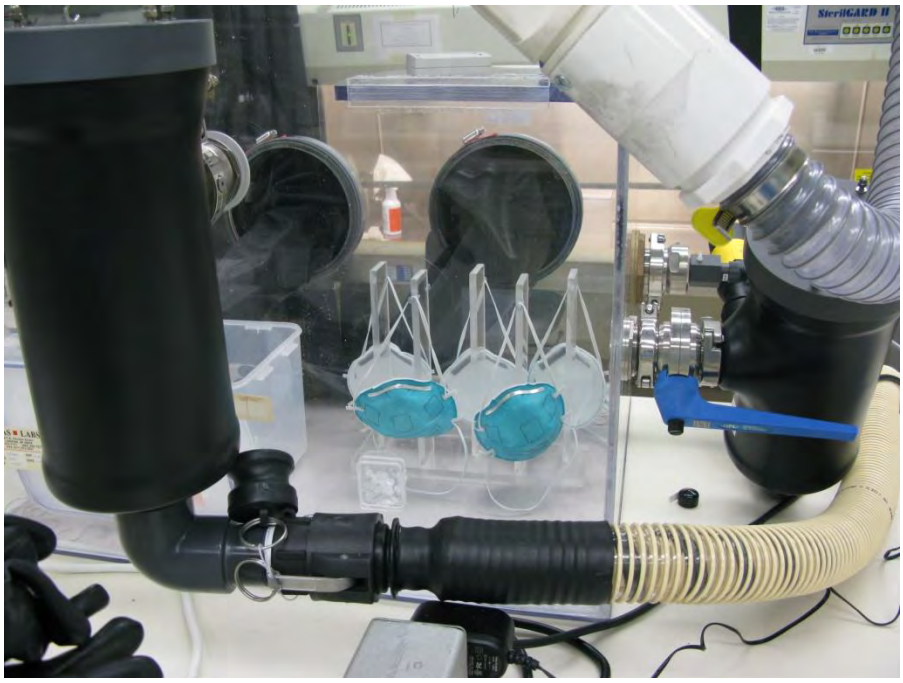

Figure 10. Orientation of N95 FFRs Contaminated by Aerosol Exposure in the HPV Decontamination Chamber

The measured loading levels of *G. stearothermophilus* spores on the HPV and control FFRs are summarized in Table 8. The target loading level was $1x10^6$ CFU/swatch to allow determination of a 6-log inactivation by exposure to the HPV process. The loading levels across trials for the HPV and control FFRs ranged from $7.3x10^5$ to $2.4x10^6$ CFU/swatch as measured based on the 47-mm reference filters, exposure duration, and air flow rate through the FFR. For the control filters, the results based on extraction of the swatches excised from the FFRs following the T/RH exposure were very similar (within a factor of two). These data suggest that spore viability was not affected by the control exposure conditions.

Table 8. Summary of *G. stearothermophilus* Loading Levels on HPV N95 FFRs

FFR	Measured Loading Level[a] (CFU/swatch)		
	Trial #1	Trial #2	Trial #3
HPV FFRs[b]	$7.3x10^5$	$2.4x10^6$	$2.0x10^6$
Control FFRs[b]	$9.3x10^5$	$2.0x10^6$	$2.2x10^6$
Control FFRs[c]	$9.5x10^5$	$3.4x10^6$	$1.2x10^6$

[a] Measured loading level per 4 cm^2 swatch. Target loading level was $1x10^6$ CFU/swatch.
[b] Based on measured aerosol challenge concentration, exposure duration, flow rate, and FFR surface area.
[c] Average (n=5) based on extraction of excised swatches from the five control FFRs following T/RH exposure.

The decontamination efficacy results are summarized in Table 9. All samples exposed to HPV were negative (i.e., no turbidity observed), thus, indicating a 6-log inactivation of the *G. stearothermophilus* spores, even when loaded onto the FFR as an aerosol. As expected, all control samples were positive (i.e., turbidity observed). All samples were streaked on TSA for confirmation on day 7 and results were consistent with the growth/no growth observations.

Table 9. Summary of Decontamination Results Following Aerosol Inoculation with *G. stearothermophilus*

Trial	HPV FFRs	Day 4	Day 7	Streak Plate	Control FFRs	Day 4	Day 7	Streak Plate
1	#1	Negative	Negative	Negative	#1	Positive	Positive	Positive
	#2	Negative	Negative	Negative	#2	Positive	Positive	Positive
	#3	Negative	Negative	Negative	#3	Positive	Positive	Positive
	#4	Negative	Negative	Negative	#4	Positive	Positive	Positive
	#5	Negative	Negative	Negative	#5	Positive	Positive	Positive
2	#1	Negative	Negative	Negative	#1	Positive	Positive	Positive
	#2	Negative	Negative	Negative	#2	Positive	Positive	Positive
	#3	Negative	Negative	Negative	#3	Positive	Positive	Positive
	#4	Negative	Negative	Negative	#4	Positive	Positive	Positive
	#5	Negative	Negative	Negative	#5	Positive	Positive	Positive
3	#1	Negative	Negative	Negative	#1	Positive	Positive	Positive
	#2	Negative	Negative	Negative	#2	Positive	Positive	Positive
	#3	Negative	Negative	Negative	#3	Positive	Positive	Positive
	#4	Negative	Negative	Negative	#4	Positive	Positive	Positive
	#5	Negative	Negative	Negative	#5	Positive	Positive	Positive

[(a)] 10 μL streaked on plate to confirm positive or negative response at end of 7 days.

3.5 Phase I: Summary

The HPV cycle was established for a 6-log inactivation of a selected N95 FFR (3M Model 1860) using the Bioquell Clarus C system and a static glove-box (Model No. 830-ABC, Plas-Labs, Inc.) with an internal volume of about 310 L as the decontamination chamber. The FFRs were loaded with *G. stearothermophilus* spores at a level of $1x10^6$ CFU per 4 cm^2 swatch. This spore-forming organism was selected because it represents a worst case scenario due to its resistance to hydrogen peroxide vapor decontamination [6], as well as a culture temperature that will reduce/mitigate the growth of potential endogenous contaminants. The HPV cycle was demonstrated to provide a 6-log inactivation (complete kill observed) for both liquid droplet and aerosol inoculation. Based on these results, the HPV cycle defined in Table 6 was recommended for testing in Phase II to assess the durability of the respirator following exposure to multiple decontamination cycles.

4.0 Phase II: Assessment of Respirator Durability after Multiple Cycle Decontamination

The objective of this phase was to determine whether exposure to up to 50 HPV decontamination cycles adversely affected FFR performance (i.e., inert and biological aerosol collection efficiency, inhalation resistance, and facial fit as assessed on a manikin head form). A subset of the FFRs was exposed to HPV using the Bioquell Clarus C and the parameters determined in Phase I. The performance of the FFRs was evaluated after exposure to 10, 20, 30, 40, and 50 cycles. Control FFRs were also evaluated after exposure to similar temperatures and RH, but not HPV. Control respirator filters were tested likewise after 10, 20, 30, 40, and 50 cycles. A set of as-received FFRs was also tested to establish the baseline performance. The completed test matrix is shown in Table 10.

Table 10. Completed Phase II Test Matrix

Test	Inert Aerosol Collection Efficiency		Biological Aerosol Collection Efficiency		Head Form Fit	
Treatment	**Control[a]**	**HPV**	**Control**	**HPV**	**Control**	**HPV**
Cycles	**Number of FFRs Tested**					
0	5[b]	0	5[b]	0	5[b]	0
10	5	5	5	5	5	5
20	5	5	5	5	5	5
30	5	5	5	5	0[c]	0[c]
40	5	5	5	5	0[c]	0[c]
50	5	5	5	5	0[c]	0[c]

[a] Exposed to similar T/RH conditions of decontamination cycle but not HPV.

[b] Control respirators tested as-received (i.e., out of box).

[c] Strap degradation observed after exposure to 30 HPV cycles; head form testing not performed.

4.1 HPV Exposure Observations

A total of 85 FFRs were exposed to HPV in the same exposure system used in Phase I. A rack was fabricated to maintain spacing between the FFRs in the chamber. The orientation of the contaminated N95s in the HPV exposure chamber is shown in Figure 11. Fifteen of the FFRs were removed from the chamber after exposure to 10 cycles for performance testing. These FFRs were disposed after performance testing. A second set of 15 FFRs were removed from the chamber after 20 HPV cycles and so on. The HPV cycle included a 10 min conditioning phase, 20 min gassing phase at 2 g/min, 150 min dwell phase at 0.5 g/min, and 300 min of aeration.

Figure 11. HPV Exposure Chamber

The FFRs removed from the HPV chamber for performance testing were visually inspected following exposure to 10, 20, 30, 40, and 50 HPV cycles. The observations are summarized in Table 11. No visible degradation was observed after exposure to 10 or 20 HPV cycles. However, after 30 HPV cycles, it was observed that the elastic straps within the straps fragmented when stretched as shown in Figure 12. No degradation was observed for any of the control FFRs.

Table 11. Observed Degradation Following Exposure to HPV and Control Cycles

Number of Cycles	Summary of Observations	
	HPV Cycles	Control Cycles
0	N/A	No degradation observed
10	No degradation observed	No degradation observed
20	No degradation observed	No degradation observed
30	Straps degrade when stretched	No degradation observed
40	Straps degrade when stretched	No degradation observed
50	Straps degrade when stretched	No degradation observed

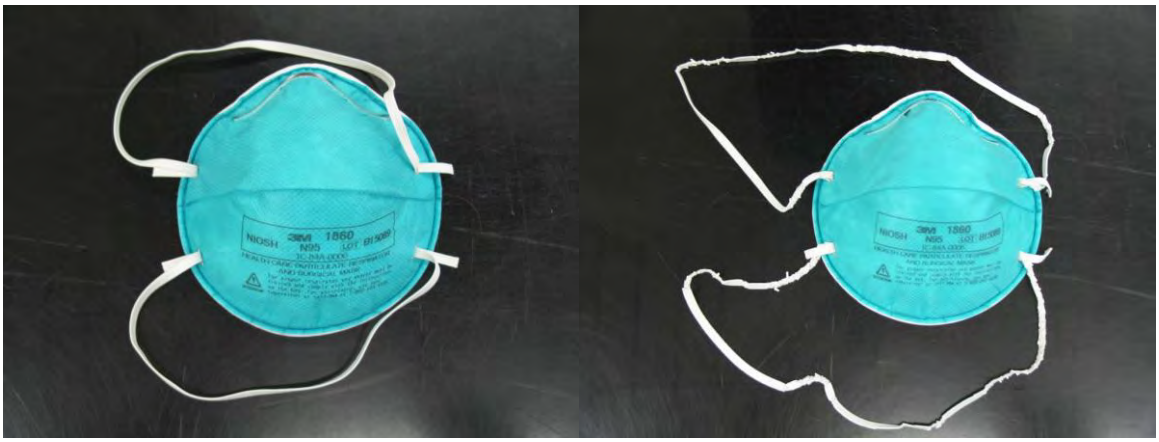

Figure 12. Comparison of N95 FFR Straps after Exposure to 30 Control (Left) and HPV (Right) Cycles (After Stretching Straps)

4.2 Task 2.1: Inert Aerosol Collection Efficiency Testing

The test parameters and requirements for the inert aerosol collection efficiency testing were based on those described in 42 Code of Federal Regulations (CFR) Part 84 [17] (i.e., the NIOSH N95 certification test method). The two most important factors affecting aerosol collection efficiency are the flow rate through the FFR and the challenge aerosol size distribution. The flow rate through the FFR was 85±1 L/min (constant). The challenge aerosol was NaCl with a count median diameter (CMD) of 0.075±0.02 µm and a geometric standard deviation (GSD) not exceeding 1.86. The temperature was 25±5°C and the RH was 30±10%. The test conditions are summarized in Table 12. As this was a pilot study to assess the feasibility of the decontamination approach, the testing did not assess the impact of aerosol loading as only the initial collection efficiency was measured. This approach was consistent with previous research by NIOSH [18].

Table 12. Test Conditions for Inert Aerosol Collection efficiency Testing of FFRs

Parameter	Target	Tolerance Range
Temperature	25°C	±5°C
Relative Humidity	30% RH	±10 [(a)]
Flow Rate	85 L/min	± 1 L/min
Aerosol Size	0.075 µm[(a)]	±0.02 µm
Aerosol Challenge Concentration	10 mg/m^3[(b)]	±5 mg/m^3

[(a)] Count median diameter.

The test system used to measure the aerosol collection efficiency is shown in Figure 13. The FFR was sealed to the holder using hot melt glue to prevent leakage at the periphery such that all aerosol detected downstream penetrated through the filtration media. Leakage at the face seal was assessed

by the manikin head form testing described in Section 4.5. The exposure chamber housed the FFR/holder and contained the aerosol challenge. The aerosol was generated using the Model 8118A salt generator (TSI, Inc., Shoreview, MN). This generator is used in the TSI Automated Filter Tester Model 8130 and is designed to produce an aerosol that meets that specified in 42 CFR part 84. The challenge aerosol passed through a charge neutralizer (Model 3012, TSI, Inc.) prior to being delivered into the test chamber. The size distribution of the challenge aerosol was measured using a Scanning Mobility Particle Sizer (SMPS, TSI, Inc.). The chamber contained the aerosol challenge and was equipped with ports for sampling the challenge aerosol and measuring the temperature/RH. The tubing downstream of the FFR also contained a sampling port. The challenge and effluent aerosol concentrations were measured using a photometer (Model 8530 DustTrak, TSI, Inc.). The aerosol penetration (P) was defined as the ratio of the measured effluent to challenge concentration. The collection efficiency (E) was defined as E(%)=(1-P)*100. The collection efficiency of N95 filters is required to be greater than 95%.

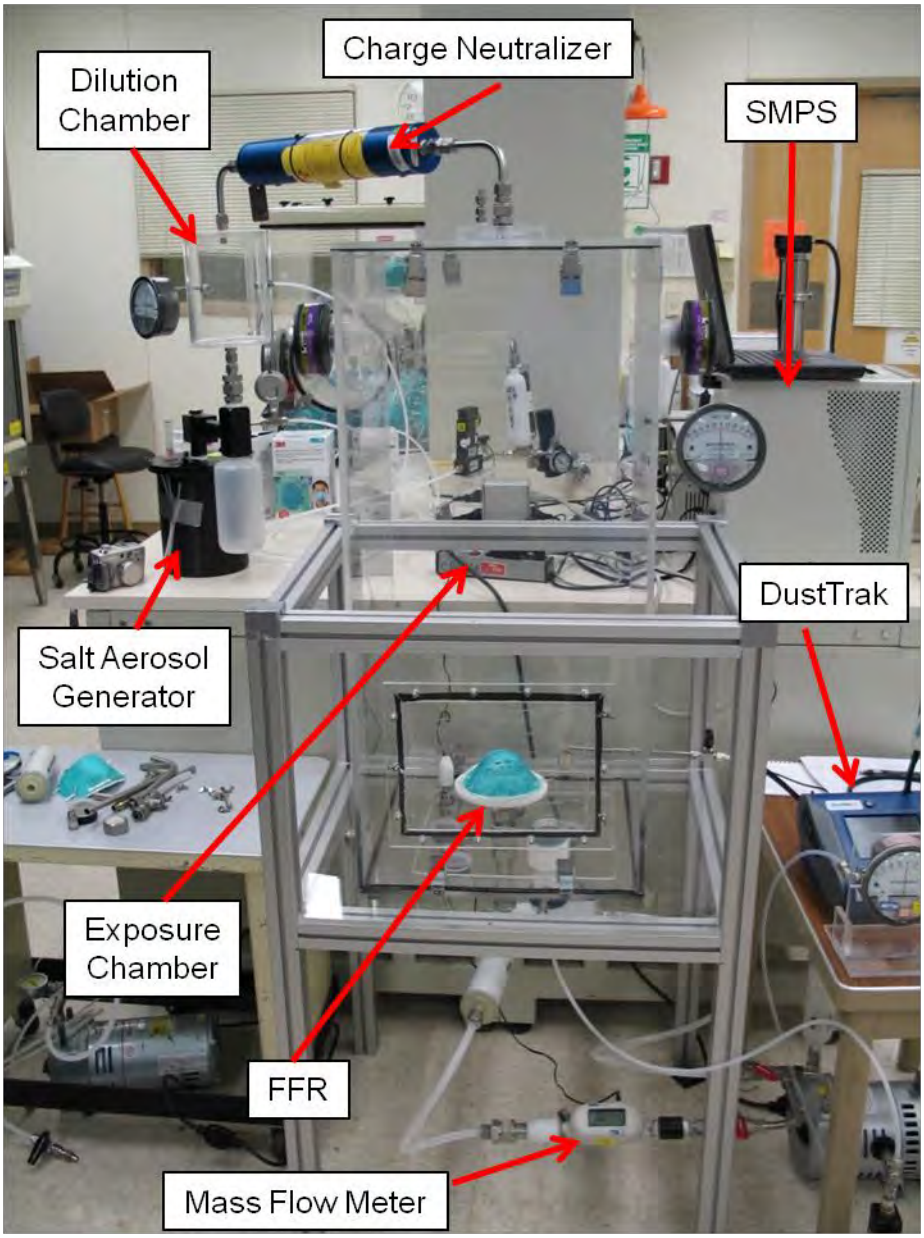

Figure 13. Inert Aerosol Collection Efficiency Test System

The inert aerosol collection efficiency results are summarized in Tables 13 and 14 for the HPV and control FFRs, respectively. The average measured collection efficiencies are compared in Figure 14 at 10 cycle increments. The error bars represent one standard deviation. As shown, the collection efficiencies for all of the N95 FFRs exposed to the HPV cycle were greater than 99%, exceeding the requirement of 95% for N95 FFRs, even after 50 cycles. The average collection efficiencies were similar (within 0.2%) for the HPV and control FFRs. Thus, exposure to the HPV cycles did not degrade the performance of the aerosol filtration media for this specific FFR model. Further

testing is recommended with other models/brands of N95 FFRs as different types of media may be affected differently by the HPV cycle.

Table 13. Summary of Inert Aerosol Collection Efficiency Results (HPV Samples)

HPV Cycles	Collection Efficiency (%)					
	#1	#2	#3	#4	#5	Average
10	99.3	99.7	99.8	99.6	99.6	99.6±0.2
20	99.3	99.5	99.6	99.6	99.5	99.5±0.1
30	99.6	99.3	99.7	99.6	99.5	99.5±0.2
40	99.8	99.6	99.8	99.7	99.3	99.6±0.2
50	99.4	99.6	99.6	99.7	99.8	99.6±0.2

Table 14. Summary of Inert Aerosol Collection Efficiency Results (Control Samples)

T/RH Cycles	Collection Efficiency (%)					
	#1	#2	#3	#4	#5	Average
0[(a)]	99.7	98.8	99.5	99.5	99.4	99.4±0.4
10	99.6	99.6	99.6	99.6	99.5	99.6±0.03
20	99.6	99.5	99.6	99.6	99.5	99.6±0.05
30	99.6	99.6	99.7	99.6	99.7	99.6±0.08
40	99.8	99.6	99.8	99.6	99.7	99.7±0.07
50	99.6	99.6	99.6	99.6	99.6	99.6±0.03

[(a)] As-Received FFRs.

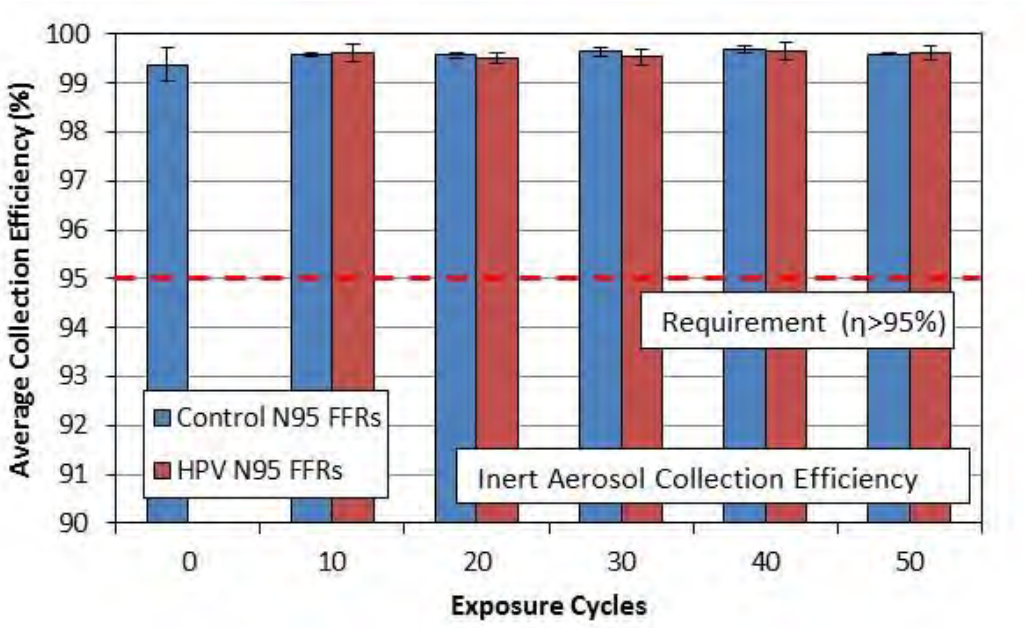

Figure 14. Comparison of Average Measured Collection Efficiency of the HPV and Control N95 FFRs

4.3 Task 2.2: Biological Aerosol Collection Efficiency Testing

The test parameters for the biological aerosol collection efficiency testing are summarized in Table 15. The challenge aerosol was spores of *Bacillus atrophaeus*. The viable size distribution was measured using a six-stage cascade impactor during system characterization. The mass median aerodynamic diameter (MMAD) was 1.2 μm (with a geometric stanadard deviation of 1.9) suggesting predominantly single spores were aerosolized. The flow rate through the N95 FFR was 28 L/min (constant flow) to match the flow rate used in the bacterial filtration efficiency test method [19]. There were no requirements on the temperature and RH of the challenge. Both were measured and recorded during each trial.

Table 15. Test Conditions for Bioaerosol Collection efficiency Testing of FFRs

Parameter	Target	Tolerance Range
Temperature	No requirement	N/A
RH	No requirement	N/A
Flow Rate	28 L/min (constant)	± 1 L/min
Aerosol Size	1.2 μm[(a)]	N/A
Aerosol Challenge Concentration	$1x10^6$ CFU/m^3	±50%

[(a)] Mass median aerodynamic diameter measured during system characterization using cascade impactor.

The bioaerosol collection efficiency test system is shown in Figure 15, which was fundamentally the same as that for inert aerosol collection efficiency tests described in Section 4.2. Primary components included an aerosol generator, exposure chamber with filter holder, bioaerosol samplers, and a vacuum pump to pull air through the filter. Similar to the inert aerosol collection efficiency, the FFR was sealed to a test plate using hot melt glue. The spores were aerosolized from a liquid (sterile distilled water) suspension using a Collison nebulizer. The Collison nebulizer delivered a continuous flow of aerosol laden air to the chamber to ensure a stable challenge aerosol was maintained. The challenge laden air exiting the generator was mixed with HEPA filtered dilution air to evaporate the droplets, yielding the dry challenge bioaerosol. The aerosol was then passed through a charge neutralizer (Model 3012, TSI, Shoreview, MN) and delivered to the test chamber. The chamber was equipped with sampling probes for collecting bioaerosol samples to quantify the challenge concentration. A vacuum pump was connected downstream of the FFR and was used to draw a constant flow through the FFR. The challenge and downstream aerosol concentrations were quantified by collecting 47-mm gelatin filters for subsequent bioassay. The collected samples were extracted and analyzed to quantify the number of organisms collected per unit volume of air sampled. As with the inert aerosol testing the aerosol penetration (P) was defined as the ratio of the measured downstream to challenge concentration and the collection efficiency (E) was defined as E(%)=(1-P)*100.

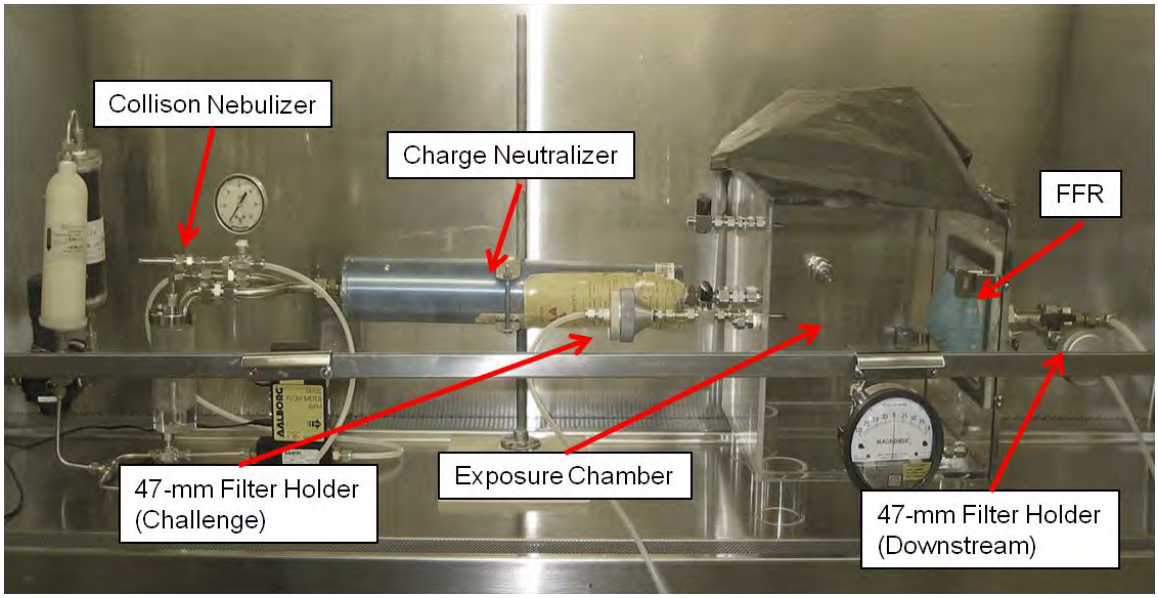

Figure 15. Bioaerosol Collection Efficiency Test System

The bioaerosol collection efficiency results are summarized in Tables 16 and 17 for the HPV and control FFRs, respectively. As shown, the collection efficiencies for all of the N95 FFRs exposed to the HPV cycle were greater than 99%, with the majority reported as greater than 99.9%. In these

instances, the downstream concentration was below the minimum limit of quantification of the method (< 30 CFU/plate). Exposure to 50 HPV cycles did not degrade the performance of the aerosol filtration media under the conditions tested. Efficiencies were generally higher than as measured against the inert aerosol. This was attributed to the larger particle size of the biological aerosol as compared to the inert aerosol.

Table 16. Summary of Bioerosol Collection Efficiency Results (HPV FFRs)

HPV Cycles	Collection Efficiency (%)					
	#1	#2	#3	#4	#5	Average
10	99.5	>99.9	99.7	99.9	99.6	>99.5
20	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9
30	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9
40	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9
50	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9

Table 17. Summary of Bioaerosol Collection Efficiency Results (Control FFRs)

T/RH Cycles	Collection Efficiency (%)					
	#1	#2	#3	#4	#5	Average
0[(a)]	99.7	99.7	99.8	99.8	>99.9	>99.7
10	99.8	99.3	>99.9	99.4	>99.9	>99.3
20	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9
30	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9
40	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9
50	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9

[(a)] As-Received FFRs.

4.4 Task 2.3: Airflow Resistance Testing

The inhalation resistance was measured by sealing the FFR to a fixture connected to a vacuum pump that pulled a continuous flow rate of 85 ± 2 L/min through the FFR as specified in 42 CFR Part 84 [17]. The resistance of the FFR was measured using a calibrated pressure gauge. Per 42 CFR Part 84, the inhalation resistance is required to be less than 35 mm H_2O. The inhalation resistance was measured for each FFR tested for inert or biological aerosol collection efficiency. The measurements were made prior to the aerosol test. The results are summarized in Tables 19 and 20 for the HPV and control FFRs, respectively. The measured resistances ranged from 8 to 11 mm H_2O with no definitive trends with increased cycles of HPV exposure.

Table 18. Summary of Inhalation Resistance Results (HPV Samples)

HPV Cycles	Inhalation Resistance (mm H2O)										
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	Average
10	10	9	9	9	8	11	10	10	10	11	10±0.8
20	9	9	9	9	8	8	9	9	8	9	9±0.3
30	9	9	9	9	9	9	9	9	9	9	9±0.3
40	9	9	9	9	9	8	9	9	9	9	9±0.3
50	8	9	9	9	9	9	9	8	8	8	9±0.4

Table 19. Summary of Inhalation Resistance Results (Control Samples)

Control Cycles	Inhalation Resistance (mm H2O)										
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	Average
0[(a)]	9	9	9	8	9	11	10	11	11	11	10±1
10	9	9	9	9	8	10	10	10	10	11	10±0.8
20	8	9	9	10	9	9	9	8	9	9	9±0.4
30	9	9	9	10	9	10	10	9	9	9	9±0.6
40	9	10	9	9	8	9	9	9	9	9	9±0.4
50	9	9	9	8	9	9	9	9	9	8	9±0.3

[(a)] As-Received FFRs.

4.5 Task 2.4: Head Form Testing

A preliminary assessment was completed to determine whether HPV exposure degraded respirator fit by donning the exposed FFRs onto a manikin head form and measuring the amount of leakage into the mask. The Project BREATHE report states that a SWPF greater than 100 is required after up to 50 cycles. In this project, manikin head form testing was used as a preliminary assessment of respirator fit after exposure to the decontamination cycles. These data were reviewed to determine whether there was any degradation in fit after exposure to the HPV. Future testing will be needed with human subjects to confirm a fit factor of greater than 100 is maintained after decontamination.

For the fit testing, the FFRs were donned onto the Static Advanced Headform (StAH) developed by the NIOSH National Personal Protective Technology Laboratory (NPPTL) and loaned to Battelle [20]. This head form is shown in Figure 16 and has a surface intended to simulate the properties of skin. The head form was connected to a breathing machine to simulate breathing flow through the FFR. The minute volume was 20 L/min, representative of a light workload. The FFRs were challenged with an inert aerosol, nominal 1.0 µm polystyrene latex (PSL) spheres. This particle diameter was selected as the N95 filtration media collects these particles very efficiently (>99%) and, thus, particles in the mask can be attributed to leakage and not penetration through the filtration media. The PSL challenge was generated from a liquid suspension using a Collison nebulizer. The respirators were equipped with flush-mounted probes (Model 8025-N95, TSI, Inc.)

positioned forward of the oral/nasal region. The challenge and in-mask aerosol concentrations were measured using an Optical Particle Sizer (OPS, Model 3330, TSI, Inc.). The OPS was selected because it measures the aerosol concentration within specific size bins over a range of 0.3 to 10 μm. This permitted use of only those size bins that classify particles ranging from 0.9 to 1.4 μm to determine the fit factor. Thus, smaller particles that may penetrate the filter media more readily were excluded from the fit factor measurement. The fit factor was determined based on the ratio of the challenge to in-mask aerosol concentration (#/cc).

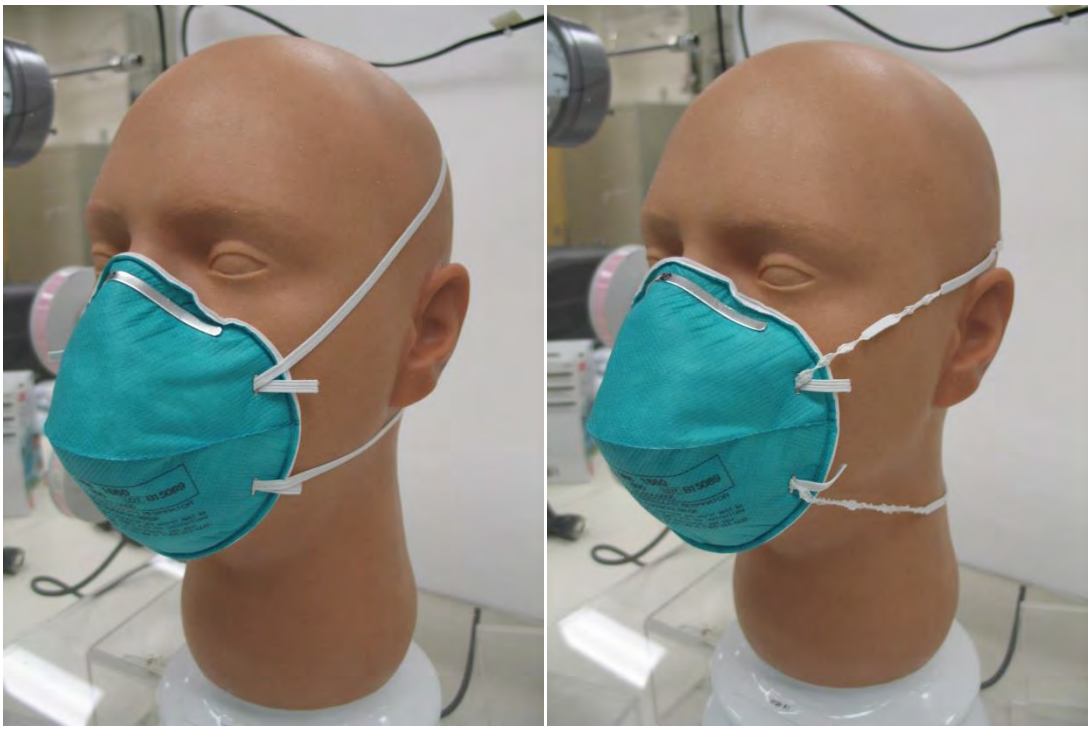

Figure 16. Static Advanced Head Form used for Preliminary Respirator Fit Assessment Loaned to Battelle by NIOSH/NPPTL

During an actual fit test, a human subject performs a user seal check after donning the mask to increase the likelihood of a proper fit. This was not possible on the manikin head form so an approach similar to that used in previous NIOSH research was used [20]. During donning, in-mask and ambient aerosol concentrations were monitored to perform a real-time leak check. The FFR was donned on the head form and adjustments were made in an effort to obtain a passing fit factor (>100). If a passing fit factor could not be obtained, the FFR was removed from the head form and a second or third donning performed. If a passing fit factor was not measured on the third donning, the test proceeded regardless.

The measured fit factors on the manikin head form are summarized in Tables 20 and 21 and compared in Figure 17 for the HPV and control FFRs, respectively. Five replicates were completed

and the geometric mean (GM) and GSD are provided. The head form results suggest that fit was unaffected for up to 20 HPV exposure cycles as similar fit factors were measured on the HPV, control, and as-received FFRs. Note that some measured fit factors were less than 100 for both the HPV and control FFRs. Thus, the low fit factors (i.e., less than 100) were not attributed to the HPV exposure. These results should not be interpreted as being equivalent to a standard OSHA-accepted fit test method. Human subject testing is needed to truly assess respirator fit.

Table 20. Summary of Head Form Fit Factor Results (HPV FFRs)

HPV Cycles	Fit Factor							Fit Factor Pass %(a)
	#1	#2	#3	#4	#5	GM	GSD	
10	112	128	100	92	110	108	1.1	80
20	185	114	99	95	100	115	1.3	60
30	Not tested, Strap degradation							
40	Not tested, Strap degradation							
50	Not tested, Strap degradation							

(a) Assumes pass criterion of ≥100.

Table 21. Summary of Head Form Fit Factor Results (Control FFRs)

T/RH Cycles	Fit Factor							Fit Factor Pass %(a)
	#1	#2	#3	#4	#5	GM	GSD	
0(b)	97	130	220	85	139	127	1.4	80
10	115	70	98	159	119	109	1.3	60
20	134	68	84	83	64	84	1.3	20
30	Not tested, Strap degradation in HPV samples							
40	Not tested, Strap degradation in HPV samples							
50	Not tested, Strap degradation in HPV samples							

(a) Assumes pass criterion of ≥100.
(b) As-Received FFRs.

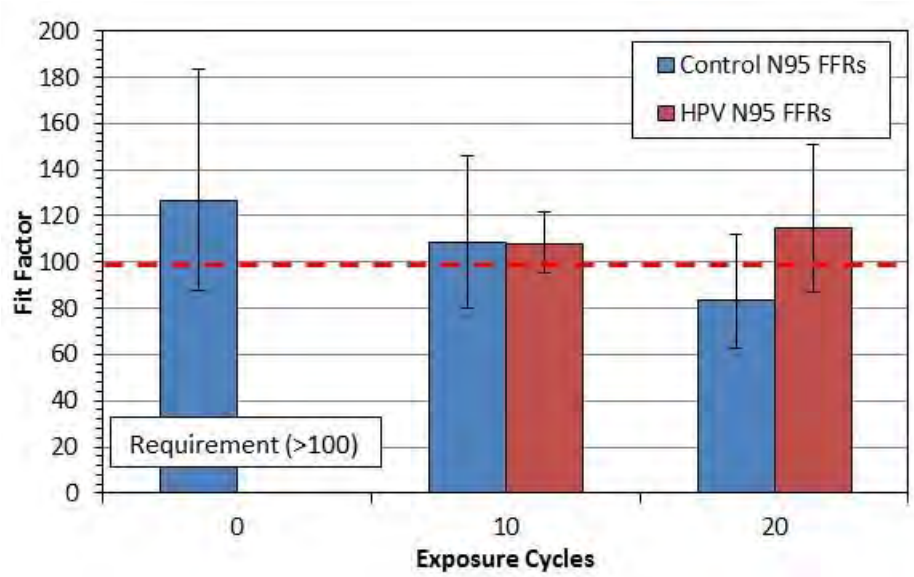

Figure 17. Comparison of Measured Fit Factors of Control and HPV FFRs after Exposure to up to 20 Cycles

As described above, strap degradation was observed following exposure to 30, 40, and 50 HPV cycles. A FFR exposed to 30 HPV cycles was shown donned on the head form in Figure 16. As shown, the straps did not pull the respirator tight to the face after stretching during donning. Gaps were observed between the FFR and the head form. Thus, the FFRs exposed to 30, 40, and 50 HPV cycles (and the corresponding controls) were not tested for respirator fit.

Mechanical tests were performed on the straps since they were observed to be affected by the HPV exposure. Two types of tests were performed using an Instron Model 5564: (1) tensile pull to failure and (2) ten tensile cycles from 0 to 100% strain (to simulate multiple donning of the same respirator). Average strain on the bottom strap is about 35% and strain on top strap is about 75% during wear of 3M 1860S N95 FFR [21]. Thus, a strain range of 0 to 100% likely covers the typical range for wear and donning/doffing activities. The mechanical tests were performed on as-received, control, and HPV-exposed straps. Control and HPV straps were tested after 10 and 20 exposure cycles.

A sample of an elastic strap exposed to 50 HPV cycles is shown being stretched using the Instron in Figure 18. The initial distance between the two clamps (i.e., length of strap at 0% strain) was 2.5 cm. As shown, the elastic strap exposed to 50 HPV cycles began to fragment at strains as low as 25%. Similar observations were made regarding the HPV straps exposed to 30 and 40 HPV cycles. A control strap is shown in Figure 19 for comparison, which does not break at applied strains up to 100%.

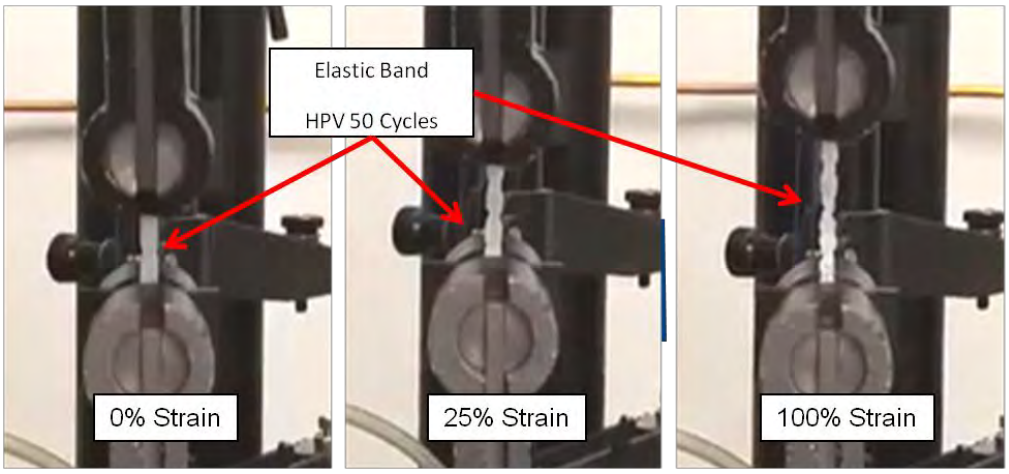

Figure 18. Straining of Elastic Strap Exposed to 50 HPV Cycles using Instron Model 5564

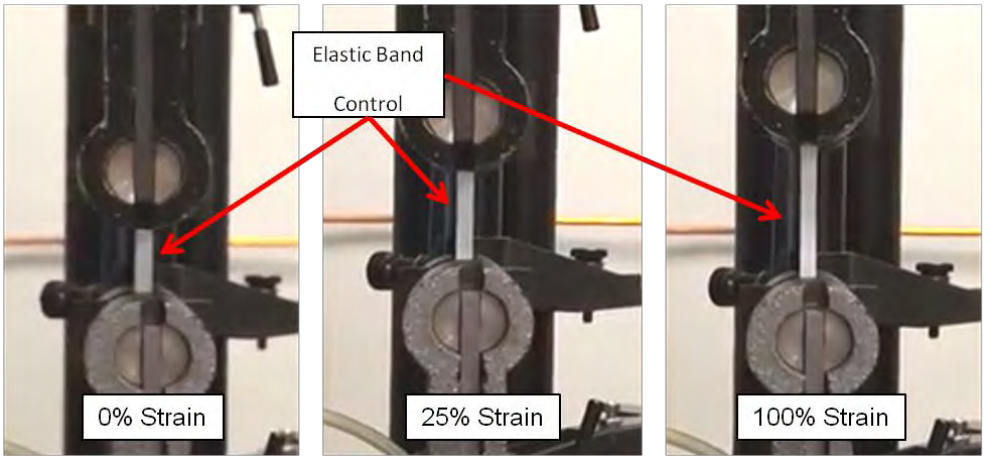

Figure 19. Straining of Elastic Strap Exposed to 50 Control Cycles using Instron Model 5564

Figure 20 compares the average tensile strain at failure of the HPV-exposed and control straps. The HPV-exposed straps tended to break at lower tensile strain. Figure 21 compares the average maximum load measured during the tenth tensile cycle for the HPV-exposed and control straps. The values represent the average of up to five measurements and the error bars represent one standard deviation. The load measured for the control straps were similar to the as-received straps even after 50 cycles. The HPV-exposed straps exhibited increased stiffness after exposure to the HPV cycles. Beyond 20 HPV cycles, the elastic fragmented during stretching on the first cycle and, thus, the drop in maximum load observed on the tenth pull. These differences between the control and HPV straps may potentially impact the fit or comfort of the respirator exposed to the HPV cycles. Human subject testing would be needed to assess the impact, if any, on fit and comfort.

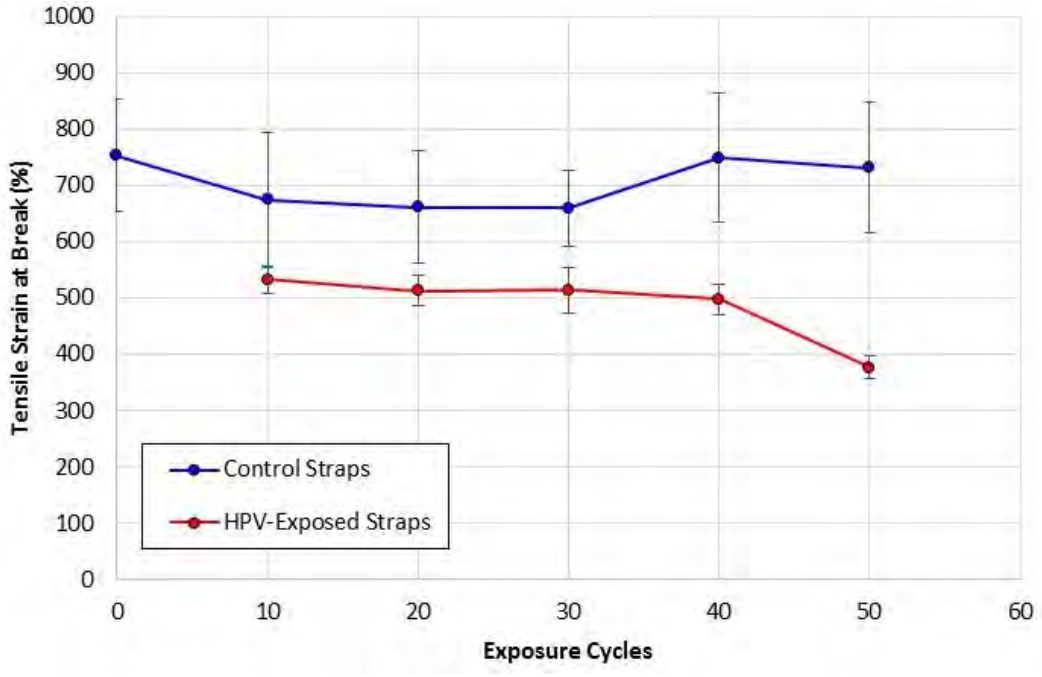

Figure 20. Comparison of Maximum Load Generated by Control and HPV Straps during the Tensile Pull to Failure Testing

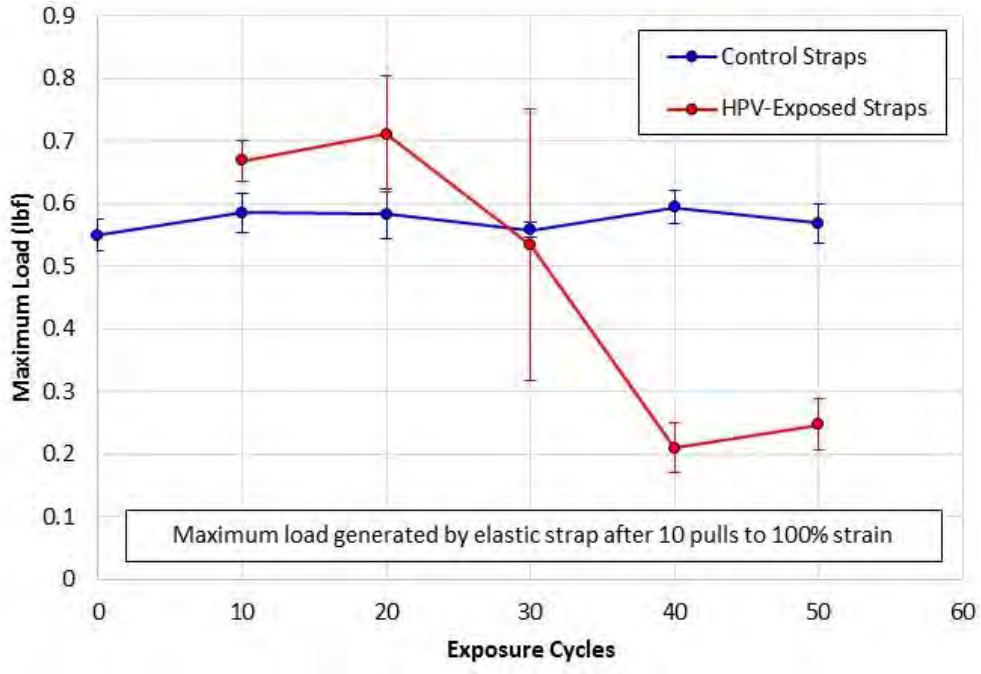

Figure 21. Comparison of Maximum Load Measured for Control and HPV Straps after 10 Tensile Cycles to 100% Strain

4.6 Phase II: Summary

The primary findings and recommendations regarding the durability and performance of the N95 FFR following exposure to up to 50 HPV decontamination cycles are summarized below:

- Exposure to up to 50 HPV cycles did not degrade the filtration media contained in the FFR tested. The filtration efficiency was in excess of 99% for both the inert and biological aerosol tests performed even after 50 HPV cycles.
- The inhalation resistance was also not affected by the HPV exposure.
- The exposure to HPV did degrade the elastic material within the straps. Mechanical testing demonstrated that the elastic straps were stiffer after exposure to up to 20 HPV cycles. However, fit factors measured on a static head form demonstrated similar fits for the HPV, control, and as-received FFRs. Human subject testing is recommended to further assess respirator fit and comfort following HPV exposure.
- The elastic straps exposed to at least 30 HPV cycles were observed to fragment when stretched to simulate donning. Alternative materials or protective coatings will need to be identified for the strap.

5.0 Phase III: Assessment of Multiple Cycle Decontamination Efficacy

Consensus Statement 7 of Project BREATHE states that respirators should be capable of being repeatedly decontaminated during a crisis for up to 50 cycles. The objective of Phase III testing was to determine whether decontamination of N95 FRRs is still achievable after 50 repeat cycles of biological aerosol exposures/HPV decontamination using the loading and decontamination parameters determined in Phase I.

Decontamination efficacy was evaluated by loading respirator filters with an aerosol of *G. stearothermophilus* as described previously in Section 3.0 (Phase I). Consistent with the previous tests, the target loading level was 1.0×10^6 CFU per excised swatch (4 cm^2). Again, the AOAC International validated Method 2008.5 requires 6-log reductions for products with sporicidal claims; therefore, the 6-log reduction was used as a benchmark value for this project. The expected range of viral loading on a FFR during actual use in a healthcare environment is not known and will depend on a variety of factors.

During each contamination, 47-mm filter samples were collected to quantify the challenge concentration for estimation of organism loading onto the FFR based on the measured challenge concentration, loading duration, and flow rate through the FFR. Once loaded, FFRs then underwent decontamination using the Bioquell Clarus C HPV system using parameters defined in Section 3.0 (Phase I). Decontamination efficacy was determined following 50 repeat aerosol inoculation/decontamination cycles by removing one 2 x 2 cm coupon from each FFR, placing each coupon in 10 mL TSB and observing for turbidity following incubation at 55-65 ℃ on days four and seven. All samples were streaked on TSA to confirm sterility or, if positive, to confirm colony morphology consistent with *G. stearothermophilus* following the seven day incubation period.

The test matrix is provided in Table 22. A total of ten N95 FFRs were loaded with the aerosol of *G. stearothermophilus* each cycle. Five of these were exposed to the HPV decontamination cycle and five of them were positive controls that were not exposed to the HPV decontaminant. These controls were placed into a chamber where they were exposed to temperatures and RH similar to that generated during the HPV cycle.

Table 22. Completed Phase III Test Matrix

Treatment	Control	HPV
Number of Loading/Decontamination Cycles	**Number of FFRs Tested**	
50	5	5

The results of the multiple cycle decontamination testing are summarized in Table 23. All samples exposed to HPV were negative (i.e., no turbidity observed). All control samples were positive (i.e., turbidity observed). All samples were streaked on TSA for confirmation on day 7 and results were consistent with the growth/no growth observations. Based on the 47-mm reference filters collected during loading, the estimated average loading level on the FFRs after each cycle was $1.7x10^6$ CFU/swatch (standard deviation $=8.0x10^5$ CFU /swatch) for the FFRs exposed to HPV treatment and $2.0x10^6$ CFU/swatch (standard deviation $=8.7x10^5$ CFU/swatch) for the control FFRs.

Table 23. Summary of Results from the Multiple Cycle Decontamination Efficiency Testing

FFR	Growth/No Growth Observations		Streak Plate[(a)]
	Day 4	Day 7	
HPV #1	Negative	Negative	Negative
HPV #2	Negative	Negative	Negative
HPV #3	Negative	Negative	Negative
HPV #4	Negative	Negative	Negative
HPV #5	Negative	Negative	Negative
Control #1	Positive	Positive	Positive
Control #2	Positive	Positive	Positive
Control #3	Positive	Positive	Positive
Control #4	Positive	Positive	Positive
Control #5	Positive	Positive	Positive

[(a)] 10 μL streaked on plate to confirm positive or negative response at end of 7 days.

6.0 Conclusions and Recommendations

Battelle successfully established a HPV decontamination process, applied to N95 FFRs, and implemented test methods to demonstrate the feasibility of using HPV. This project offered a comprehensive pilot-scale study which evaluated the efficacy of HPV for decontamination of N95 respirators for reuse using the Bioquell Clarus C system. This project evaluated decontamination efficacy against a single organism and characterized the mechanical integrity and function performance of the selected N95 FFR following HPV exposure. Complete inactivation (a 6-log reduction) was demonstrated on whole, in-tact FFRs of a biological indicator, *G. stearothermophilus* spores, when contaminated using either liquid droplets or aerosol exposure. In fact, the ability to decontaminate the respirator was demonstrated even after multiple cycles (up to 50) of biological exposure/decontamination. The recommended HPV decontamination cycle had a duration of 480 min. Thus, this decontamination approach is not anticipated to be used by individuals at the point of use. Rather, this approach may be used to decontaminate FFRs at the end of a work shift, and is a viable approach to decontaminate large numbers (>50) of FFRs simultaneously.

It is important that the mechanical integrity and performance of the FFR is maintained following exposure to the HPV cycle. Thus, the performance of the FFR was evaluated after exposure to up to 50 HPV cycles in increments of 10 cycles. Performance tests included inert aerosol collection efficiency, biological aerosol collection efficiency, inhalation resistance, and respirator fit on a manikin head form. No visible degradation was observed after exposure to 10 or 20 HPV cycles. However, after 30 HPV cycles, it was observed that that elastic material in the straps fragmented when stretched. This could impact the fit of the respirator. It is recommended to identify alternative materials for the straps that would have more resistance to the HPV exposure. Conversely, the aerosol collection efficiency (both inert and biological) and the air flow resistance were not affected by the HPV exposure.

It is recommended to characterize the impact of the HPV decontamination cycle on the performance of other N95 FFR brands/models. The ability to reduce the aeration phase should also be further explored to reduce the overall cycle trim. In addition, testing could be performed to demonstrate the efficacy of the HPV decontamination cycle against organism of interest within the healthcare community. This project represented a pilot scale test approach to demonstrate the feasibility of FFR decontamination and reuse and establish testing methods for future investigation of additional decontamination technologies, verification of organism inactivation.

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