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Development and validation of technologies suitable for the decontamination and re-use of contaminated N95 filtering facepiece respirators in response to the COVID-19 pandemic

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SUMMARY

Background: Coronavirus disease 2019 (COVID-19) has brought significant challenges to society globally, particularly in the area of healthcare provision. A pressing need existed in protecting those tasked with delivering healthcare solutions during the COVID-19 crisis by providing solutions for preserving adequate supplies of effective personal protective equipment (PPE).
Aim: To evaluate and validate available methods for the decontamination of N95 filtering facepiece respirators (FFRs) while maintaining functionality during re-use.
Methods: Multiple low-temperature steam and vaporized hydrogen peroxide (VHP) technologies were assessed for inactivation of *Mycobacterium* spp. and feline calicivirus (employed as representatives of the contamination challenge).
Findings: Virus ($\geq 3\log_{10}$) and *Mycobacterium* spp. ($\geq 6\log_{10}$) inactivation was achieved on various types of N95 FFRs using an array of heat (65–71°C), humidity (>50% relative humidity) and VHP without affecting the performance of the PPE.
Conclusion: The methods have been validated and were authorized by the US Food and Drug Administration under a temporary emergency use authorization. Based on the findings, opportunities exist for development and deployment of decontamination methods made from simple, general purpose materials and equipment should a future need arise.

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), and the resulting disease [coronavirus disease 2019 (COVID-19)], was declared a public health emergency of international concern by the World Health Organization (WHO) in early 2020 [1]. While previous outbreaks of coronaviruses

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(CoVs) had higher fatality rates (e.g. 40% for Middle East respiratory syndrome, as opposed to 2—3% for COVID-19 [2]), COVID-19 has a significantly higher transmission rate [3]. Like many virus particles, SARS-CoV-2 has relatively low resistance to chemical disinfection and other decontamination processes. The world's medical communities face intractable challenges to the containment and management of the SARS-CoV-2 outbreak, including aerosol spread, asymptomatic transmission and high transmission rate [1—4]. To protect healthcare workers and limit transmission, WHO recommends the use of N95 or FFP2 standard (or equivalent) facepiece respirators (FFRs) [5]. When in sufficient supply, FFRs are of critical importance in providing a safe work environment for delivering emergency care, preventative medicine and elective surgeries [6].

At the beginning of the SARS-CoV-2 outbreak, WHO and US officials estimated the need for approximately 100—300 million FFRs per month to supply the expected demand, with the USA having only 1% of this supply in the stockpile [7]. As shortages of FFRs were experienced, alternative strategies to mitigate this deficiency in supply required expedient consideration, with guidance based on that issued by the US Food and Drug Administration (FDA) for obtaining emergency use authorizations (EUA) to market devices (equipment) for the decontamination of N95 masks during the period of critical need [8].

During FFR shortages, healthcare providers are faced with limited options: (1) sessional use; (2) re-use; or (3) use of alternative PPE [5]. To address known shortages of N95 availability in the USA, the US Centers for Disease Control and Prevention published recommendations for extended use or limited re-use as potential mitigation strategies [9]. Decontamination has been proposed as another strategy to further extend the re-use of masks.

Decontamination strategies must meet all of the following criteria: (1) include screening and replacement processes for those FFRs not suitable for re-use; (2) inactivate the virus and other relevant bioburden on the FFR; (3) be compatible with the FFR to avoid rendering it dysfunctional; (4) be available and practical in healthcare settings; and (5) minimize risk to operators of the decontamination equipment and end users alike. Not all available decontamination technologies are appropriate for FFRs and may present one or more shortcomings. For example, simple cleaning strategies using both inert and disinfection wipes are effective for decontamination but can damage key attributes of FFRs, such as the filtering effect on electrostatic polypropylene [10,11].

With the onset of FFR shortages during COVID-19, a diverse range of decontamination technologies have been explored, with many shown to be successful to address the need to provide safe FFRs following application of the decontamination method [12—14].

The aims of this study were to: (1) assess potential candidate technologies for decontaminating compatible FFRs; (2) validate the selected technologies with approved methods; and (3) demonstrate that the PPE remains fully functional. An additional aim of this study was to demonstrate methods that may be of consideration where sterilization equipment resources are limited. While the purpose of the research is applicable to the current challenges of COVID-19, future considerations may pertain to the environmental impacts of PPE and long-term sustainability [12]. It is estimated that some 44 million non-woven PPE items are utilized by front-line workers every day, resulting in some 15,000 tonnes of waste [12]. While many materials in PPE are recyclable, contamination influences disposal strategies significantly, and this is coupled with the natural resource consumption during manufacture, which collectively may be environmentally impactful [15,16].

Methods

Decontamination method

A number of decontamination technologies including ethylene oxide (EO), radiation, vaporized hydrogen peroxide (VHP) and moist heat were considered based on effectiveness, availability and safety. Radiation sterilization and heat processes >75°C were eliminated due to material incompatibility, and EO was omitted due to potential residues. All other candidate technologies described in Table I were investigated further using validated and calibrated decontamination equipment. As both VHP and steam sterilizer equipment are widely available in healthcare facilities, these technologies received particular focus for EUA submission to FDA.

Micro-organism challenge

Feline calicivirus (FCV) (Strain F9, ATCC # VR-782), a non-enveloped virus, was selected as the virus challenge as it provides a greater level of resistance than enveloped CoVs, is non-pathogenic to humans, and is stable at high titres. Virus titres were determined by a 50% tissue culture infectious dose ($TCID_{50}$) assay on the host cell line, Crandell Rees feline kidney (CRFK) cells (ATCC CCL-94).

Mycobacterium decontamination was evaluated with *Mycobacterium smegmatis* (ATCC 19420) or *Mycobacterium hassiacum* (DSM 44199 or ATCC 700660), which are non-pathogenic surrogates of *Mycobacterium tuberculosis*. *M. hassiacum* is a thermophilic species suitable to validate thermal disinfection such as moist heat [17].

Soil challenge

The processes were challenged with appropriate soil challenges comprised of mucin and saline based upon guidance from ASTM Standard E2721-16 [18].

For the viral challenge in all decontamination applications, 0.3% (m/v) of mucin (from porcine stomach, Sigma-Aldrich, St Louis, MO, USA), 0.45% (m/v) of inorganic salt (constituent of cell culture media, DMEM, Life Technologies, Waltham, MA, USA), and 2.5% (v/v) of fetal bovine serum (FBS, Life Technologies) were incorporated into the viral testing soil challenge that included the standard formulation of the virus stock media.

For the *M. smegmatis* challenge, a 42—54-h culture at 37°C in trypticase soy broth (Becton Dickinson, Franklin Lakes, NJ, USA) was aerated by shaking at 200 rpm, centrifuged at 1000 rpm for 1 min, decanted to remove the supernatant, and resuspended in the soil containing 0.3% mucin (from porcine stomach, Sigma-Aldrich) and 0.9% NaCl (Fisher Scientific, Waltham, MA, USA) for testing in the steam sterilizer, and 0.3% mucin and 0.3% NaCl for VHP atmospheric testing. Each suspension was then ground using a mortar and pestle, and five complete passages were performed to disperse the aggregated cells. At times, additional diluted cultures were made from

Table I
Decontamination methods used

Technology	Programmed parameters	Test method and sample numbers (replicate inoculated coupons individually pouched and decontaminated under simulated-use conditions, unless stated otherwise)
Moist heat via steam sterilizer (steam decon cycle in the AMSCO 400 and Century medium sterilizers)	Gravity cycle Temperature: 65°C RH: 100% (saturated steam) Exposure time: 30 min Drying time: 1 min	Number of trays per sterilizer size, with each tray containing 12 pouched FFRs 36" sterilizers — nine trays 48" sterilizers — 12 trays 60" sterilizers —15 trays
Moist heat via climate chamber (manufacturer: Memmert; model: HPP 110; size: 3.8 ft^3)	Temperature: 65°C RH: 40, 60 and 80% Exposure time: 30 min	Number of FFRs based on size of the climate chamber
Moist heat via warming cabinet (STERIS AMSCO DJ060 warming cabinet)	Temperature: 71°C RH: 90% Exposure time: 30 min	Coupons placed inside a quart-sized (4"x 7.625") sealable plastic bag (Ziploc DRK CB003103) containing FFR and 4"x4"12 ply gauze pad, folded to 2"x2" and pre-wetted with 3 mL of distilled water. Number of FFRs based on size of the warming cabinet chamber
Moist heat via drying phase of single-chamber washer-disinfector	Temperature: 70°C RH: 60—80% Exposure time: 40 min	Coupons placed inside a quart-sized sealable plastic bag containing an FFR and a gauze pad wetted with 3 mL distilled water. Number of FFRs based on loading arrangement and chamber dimension (single-chamber washer-disinfector: 48—72 pouched FFRs)
VHP via STERIS V-PRO sterilizer	V-PRO 1 Plus, maX and maX 2 sterilizers operating non-lumen cycle	Coupons decontaminated with chamber containing 10 pouched FFRs or 20 unpouched FFRs (five or 10 per shelf, respectively)
VHP via STERIS V-PRO sterilizer	V-PRO 60 and s2 sterilizers operating non-lumen cycle	Coupons individually pouched and decontaminated in chamber containing six pouched FFRs or 14 unpouched FFRs (three or seven per shelf, respectively)
Atmospheric VHP via STERIS Victory, 1000ED, 1000ARD and M100X biodecontamination units	N95 programmed cycle, except for the Victory unit which monitors parameters during the cycle	Replicate inoculated coupons were contained in uncovered Petri dishes placed through the room and decontaminated under simulated-use conditions. Number of FFRs based on size of the room, with the allowance of three FFRs per cubic foot of room space

VHP, vaporized hydrogen peroxide; RH, relative humidity; FFR, filtering facepiece respirator.

these suspensions and used in testing. The test organism was confirmed by colony morphology and growth on trypticase soy agar or the selective media Middlebrook 7H11 agar (Becton Dickinson).

For *M. hassiacum* DSM 44199, a sample of the microorganism was transferred from Middlebrook 7H11 stock agar plates (Becton Dickinson) to Middlebrook 7H9 broth (Becton Dickinson) and incubated with 200 rpm shaking (New Brunswick Scientific, Edison, NJ, USA) at 37°C for 3 days. The liquid culture was pelleted by centrifugation at 2000 rpm (Thermo Scientific, Waltham, MA, USA). The supernatant was removed and a 0.3% mucin and 0.3% NaCl solution was added to the pellet. The pellet was ground to break up clumps. An optical density of the *M. hassiacum* suspension was performed using an ultraviolet–visible spectrophotometer (Thermo Scientific), and the suspension titre was adjusted to achieve 10^8 colony-forming units (CFU)/mL. The test organism was confirmed by colony morphology and growth on the selective media Middlebrook 7H11 agar.

Washer-disinfector testing

M. hassiacum ATCC 700660 was grown in Middlebrook 7H9 broth with Tween (Hardy Diagnostics, Santa Maria, CA, USA) at 37°C for 7 days. The bacterial culture was mixed (1:1, v/v) with a suspension of 0.6% mucin (Sigma-Aldrich) + 0.9% NaCl (Fisher Scientific) to obtain an inoculum at $>10^8$ CFU/mL.

N95 FFR selection

At the recommendation of the N95 FFR manufacturer, efficacy testing was performed with masks currently in use within clinical settings, such as the 3M 8210 and 1860/S models. Each coupon used for efficacy evaluations included all layers of the mask under evaluation: the coverweb (patient-facing front side), the inner filter material and the innershell (user-facing back side).

FFR manufacturers also make N95 masks using similar materials that, for the sake of efficacy evaluations, are considered equivalent. An example of this is the polypropylene or polyester coverweb or innershell of each mask. For 3M FFRs, a polypropylene filter material is used within all masks, and minor differences (e.g. different filter fibre diameter) are not considered significant to an efficacy evaluation where the focus is on decontamination of the fibre surface.

With regards to FFR build configuration, the mask components are essentially the same according to a review of technical data sheets that identify the materials of composition of compatible 3M N95 FFRs [19,20]. Based on the evaluation of 3M FFR materials and supporting information provided by 3M, testing was conducted with 3M Healthcare N95 FFR models 8210, 1860/S and 1870+. In addition, limited testing was performed on model 8511 containing an exhalation valve.

Coupon/sample inoculation

The method of inoculation and recovery of virus was chosen based on previously published literature testing similar decontamination methods [9,21,22]. Coupons (3 cm^2) from each N95 FFR model were inoculated aseptically on one or both sides of the mask with 10 μL of challenge organism via numerous droplets per side, targeting 1–1.5 x 10^6 CFU mycobacterium/coupon or 6.5–7.8 $\log_{10}$ $TCID_{50}$ FCV/coupon. The exhalation valve of the 3M 8511 N95 FFR was evaluated separately from the mask fibre material. The intact valve was cut out of the mask, and its surface as well as visible portions of the internal membrane were inoculated with virus. Once dried in ambient conditions, individual coupons were sealed in a sterilization pouch (Vis-U-All, STERIS, Mentor, OH, USA) or placed in a Petri dish prior to decontamination processing.

Recovery of test organism post decontamination processing

Virus testing

Following decontamination, each coupon was transferred to 10 mL (100 mL for exhalation valve) of recovery media [Eagles Modified Essential Media (EMEM, ATCC 3002003) containing 100 units/mL penicillin, 100 μg/mL streptomycin (P/S) and, in the case of coupons processed with VHP, an additional 500 mg/L sodium thiosulfate] and vortexed at the highest setting for 10 min. A $TCID_{50}$ assay was performed by plating serial 10-fold dilutions of each suspension on to monolayers of CRFK cells maintained in EMEM containing 2% FBS. Cells were incubated at 37ºC, 5% CO_2 and ≥85% relative humidity for up to 7 days, and monitored periodically for viral cytopathic effect using an inverted microscope.

The recovery media of coupons left uninoculated and without exposure to decontamination was used to evaluate mask material cytotoxicity to the cell line associated with virus testing. Testing of all mask models demonstrated no detectable levels of cytotoxicity to CRFK cells.

Mycobacterium testing

Decontaminated coupons were transferred aseptically into separate test tubes of recovery media containing either 10 mL Middlebrook 7H9 broth or 10 mL PBS + 0.5% Tween 80 (for *M. hassiacum*), 20 mL tryptic soy broth (for *M. smegmatis*) or 20 mL tryptic soy broth with sodium thiosulfate (for coupons used in VHP testing). After transfer, all recovery tubes were incubated for ≥14 days at 37 ± 2°C and scored for growth/no growth, except for coupons recovered in 10 mL PBS + 0.5% Tween 80. These coupons were extracted as follows: 30 s vortex, 30 s sonication at 40 ± 5 kHz, 30 s vortex, 30 s sonication at 40 ± 5 kHz, 30 s vortex, and the recovery media filtered through a 0.45-μm membrane (Pall, Port Washington, NY, USA); next, the filter was placed on Middlebrook 7H11 agar (Becton Dickinson) and the plates were incubated for 14 days at 37°C. To demonstrate adequate growth and population of test organism applied to the masks that were not subject to the decontamination process (positive control), the inocula suspensions were enumerated using two methodologies. In the first method, the inoculum was serially diluted in the respective recovery media and various dilutions were subjected to pour-plating in or spread-plating on the organism's respective agar (i.e tryptic soy agar, Middlebrook 7H11 agar). Another set of inoculated coupons were serially diluted in tubes containing their respective recovery media.

Physical evaluations of N95 FFRs

After decontamination processing, N95 masks were sent to the FFR manufacturer for filtration and fit evaluations to verify performance with NIOSH N95 requirements.

Initial filter penetration and pressure drop at 85 L/min flow were determined for the FFR samples with a TSI 8130 automated filter tester (AFT). The test aerosol used was the standard NIOSH NaCl aerosol defined in NIOSH's test procedure TEB-APR-STP-0059. This aerosol and AFT are typically used by NIOSH in certification testing of N95 FFRs per 42 CFR, Part 84, Subpart K, §84.181. Based on these evaluations, N95 FFRs were rated a 'pass' for NIOSH filtration efficiency. Fit-related evaluations of the FFR samples were conducted, and included measurement of headband mechanical properties; inspection of nosefoam for degradation; and inspection of the innershell for deformation, shrinkage or change in texture. Headband mechanical evaluations were conducted (Instron, Norwood, MA, USA) with a 1-kN load cell to evaluate the headband force after processing. Three elongation cycles were applied to each sample in the following order and magnitude: 200%, 50% and 50% to simulate the donning and redonning of a disposable FFR with non-adjustable elastic straps. The maximum tension of the headband in the third cycle was evaluated to determine if the treatment of FFR samples affected the mechanical properties of the headband and thus the fit of the FFR.

Visual and tactile inspections were conducted by the manufacturer. Evaluations included an inspection of overall FFR integrity, nosefoam integrity (where applicable), and an overall shape evaluation of the FFR.

Sterilant residual analysis

For VHP decontamination processes, residual levels of hydrogen peroxide were determined by a validated spectrophotometric assay that uses xylenol orange to determine hydrogen peroxide concentration in the 1–10 ppm range. Immediately following VHP (atmospheric and V-PRO sterilizer) processing, multiple compatible FFRs were extracted individually in sterile water for 24 h at a rate of 0.2 g of FFR per 1 mL water according to ISO 10993-12 [23]. The extraction liquid was evaluated in a spectrophotometric assay with appropriate standards and controls to give a quantitative value in parts per million (ppm) for the residual levels of hydrogen peroxide extracted from each sample. This ppm value is converted to mg H_2O_2 residue on the mask.

Controls

Virus controls

Positive controls (inoculated coupons not subjected to the decontamination process) were extracted as the reference to calculate $TCID_{50}$ log_{10} reduction. Negative cell controls were included to demonstrate viability of the host cells and ensure the absence of contamination during assay. The $TCID_{50}$ of a sample represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The $TCID_{50}$ titres of the virus controls and treated samples were determined according to the method of Spearman-Kärber [24,25].

Mycobacterium controls

In order to show no inhibitory substances were produced from the mask material during the incubation period, inoculated coupons not subjected to decontamination processing were dispensed into tubes of the recovery media and evaluated for growth. Likewise, uninoculated coupons were diluted in tubes of media as a negative control. All samples were incubated at 37°C for 14 days.

Results

Microbiological inactivation

Results of microbiological inactivation of various FFRs using a number of decontamination technologies are described in Table II. All evaluated methodologies demonstrated a bioburden reduction of ≥3 log_{10} reduction of a non-enveloped virus and ≥6 log_{10} reduction of *Mycobacterium* spp. in the presence of a representative simulated-use soil in accordance with FDA guidance 'Enforcement Policy for Face Masks and

Table II
Results of microbiological inactivation testing performed

	Log_{10} virus inactivation				Log_{10} *Mycobacterium* spp. inactivation		
	3M 8210	3M 1860	3M 1870+	3M 8511 with exhalation valve[a]	3M 8210	3M 1860	3M 1870+
Decontamination using moist heat							
Steam sterilizer (65°C/100% RH/30 min)	≥3.5	≥3.5	≥3.5	≥5.0	≥6.0	≥6.0	NT
Climate chamber (65°C/>50% RH/30 min)	≥3.5	≥3.5	≥4.5	≥4.5	NT	NT	NT
Warming cabinet (71°C/90% RH/30 min)	≥3.5	≥3.5	≥4.5	≥4.5	≥6.0[b]	≥6.0[b]	NT
Washer-disinfector (70°C/60–80% RH/40 min)	NT	NT	NT	NT	≥6.0	≥6.0	NT
Decontamination using vaporized hydrogen peroxide							
V-PRO 1 Plus, maX, maX2 (non-lumen)	≥3.0	≥3.0	≥4.0	NT	≥6.0	≥6.0	NT
V-PRO 60 and s2 (non-lumen)	≥3.5	≥3.5	≥4.0	NT	≥6.0	≥6.0	NT
Victory biodecontamination unit	≥3.5	≥4.0	≥3.5	NT	≥6.0	≥6.0	≥6.0
1000ARD, 1000ED, and M100X biodecontamination units	≥3.5	≥3.5	≥3.5	NT	≥6.0	≥6.0	≥6.0

RH, relative humidity; NT, not tested; ≥, inactivation exceeded the limit of detection.
Results (expressed as log_{10} reductions) from triplicate coupons tested in triplicate cycles of each decontamination technology (with the exception of single coupon tested in triplicate washer-disinfector cycles and triplicate 3M 1860 coupons tested in single climate chamber cycle).
[a] Minimum result from mask material and exhalation valve where differences in materials are observed.
[b] Samples exposed to treatment parameters for 60 min.

Table III
Physical performance testing of N95 filtering facepiece respirators (FFRs)

Technology	Number of cycles	NIOSH filtration efficiency	Fit-related evaluation
Moist heat – AMSCO medium steam sterilizer	10	Pass	Pass
Moist heat – in high-temperature self-seal pouches (1 FFR per pouch), temperature = 65±5°C, humidity = 50–80% RH, 30 min	10	Pass	Pass
V-PRO sterilizer non-lumen cycle (V-PRO 1 Plus, maX, maX 2, 60, s2 sterilizers)	10	Pass	Pass
VHP – Steris - Victory, 1000ED, 1000ARD and M100 biodecontamination units	20	Pass	Pass

RH, relative humidity; VHP, vaporized hydrogen peroxide.

Respirators During the Coronavirus Disease (COVID-19) Public Health Emergency' dated April 2020 [8]. Bioburden reduction was demonstrated to the limit of detection for each assay with potential to exceed beyond the reported $\log_{10}$ reduction.

Heat with some moisture and VHP-based technologies yielded comparable inactivation by organism type with a minimum 3 $\log_{10}$ reduction in virus and 6 $\log_{10}$ in *Mycobacterium* spp. Maximal inactivation of virus was achieved with the 1870+ FFR with both heat with some moisture and VHP, where up to 4.5 $\log_{10}$ virus was observed.

The inactivation achieved with a warming cabinet demonstrated >3 $\log_{10}$ virus inactivation by inserting an individual FFR into a closed plastic resealable bag along with approximately 3 mL of sterile water: This additional water was observed to provide an increase in relative humidity, necessary for decontamination efficacy. The closed bag was inserted into a 71°C chamber for 30 min. Measurements within the resealable bag indicated a steady-state environment of 65–70°C at 75–95% relative humidity. The benefit of humidity was observed where trials performed at the defined temperatures (using 3M 8210 and 3M 1860 mask types) without humidity yielded inactivation typically less than the desired 3 $\log_{10}$ minimum. Therefore, in order to surpass the 3 $\log_{10}$ minimum target, relative humidity ≥40% was established as a process parameter.

The washer-disinfector was only tested with *Mycobacterium* spp. and was shown to provide ≥6 $\log_{10}$ inactivation.

The mask fibre material and the exhalation valve of the 3M 8511 N95 FFR were evaluated separately for decontamination. The resulting $\log_{10}$ reductions of each were coalesced to demonstrate the effects of decontamination of the overall mask. In Table II, only the minimum $\log_{10}$ reduction observed between the two individual mask components is reported as worst case, with ≥5 $\log_{10}$ being recorded with the steam sterilizer and ≥4.5 $\log_{10}$ for the climate chamber process.

Physical evaluations of N95 FFRs

Physical performance testing of N95 FFR was performed on processed samples by the FFR manufacturer. Results were shared by the FFR manufacturer and are shown here in Table III [26].

Functionality testing of FFRs (performed by the original manufacturer) after treatment resulted in all FFRs meeting the acceptance criteria for newly produced FFRs (Table III). No significant change in filtration efficiency was observed after decontamination treatment. Visual and tactile inspections of the FFR samples showed no detectable degradation in the integrity and elastic properties of the nosefoam for any of the treatment conditions after exposure to 10–20 cycles. No reduction was observed in the average headband tensile strength test after three decontamination cycles when compared with untreated control samples, indicating no significant reduction in fit. Visual and tactile inspection of the samples also showed no detectable changes in the size, shape and texture of the innershell for any of the treatment conditions. Based on evaluations of the N95 FFR headband, nosefoam and innershell, applied treatments of FFR did not lead to a significant reduction in fit and were rated a 'pass' for fit-related evaluations, based on the acceptance criteria for new FFRs.

Dependent on the technology used, mask re-use for 10–20 cycles has been qualified. N95 masks constructed with polypropylene show excellent compatibility with VHP decontamination processes. Material evaluations of unprocessed vs 10x or 20x exposed masks showed no significant differences between unprocessed and exposed masks. While the plastic masks have excellent compatibility, masks constructed with cellulose or cellulose materials were appropriately excluded from decontamination with VHP due to known incompatibility issues between hydrogen peroxide and cellulosic materials.

Sterilant residual analysis

Evaluation for residual hydrogen peroxide in VHP-processed masks (V-PRO sterilizer non-lumen cycle or atmospheric VHP decontamination systems) found that sterilant residue was readily removed by the aeration processes associated with the decontamination cycles. 3M 8210 and 1870+ FFRs were evaluated with 7–9 mg of residual hydrogen peroxide per mask after V-PRO sterilizer non-lumen cycle processing and 3–4 mg per mask after atmospheric VHP decontamination systems, with all results meeting acceptance criteria. Note: For comparison, topical solutions of 3% hydrogen peroxide (30,000 mg/L H_2O_2) are commonly used as topical antiseptics as recommended by EU and US pharmacopeia.

Discussion

This investigation demonstrated the efficacy of numerous decontamination processes that may be applied to N95 FFRs, necessary to meet the emergency need for re-use during the COVID-19 pandemic. The solutions explored varied from very defined processes such as VHP, to novel concepts such as moist

heat in a warming cabinet. In addition to validating several decontamination methods to meet the acceptance criteria of the FDA EUA program at that time, equal investigative effort has been made into the development of novel methods that may benefit healthcare providers where specialist equipment is not readily available. Low-temperature moist heat and VHP-based decontamination processes have been demonstrated to be effective in treating N95 FFRs prior to re-use. The low-temperature, moist heat technologies included equipment such as steam sterilizers, climate chambers, warming cabinets and the drying phase in washer-disinfectors. The VHP process was tested with the V-PRO 1 Plus, maX and maX2 sterilizers (non-lumen cycle); the V-PRO 60 and s2 sterilizers (non-lumen cycle); the Victory biodecontamination unit; and the 1000ARD, 1000ED and M100X biodecontamination units. The technologies selected were tested according to recognized standard methods (ASTM) for virucidal performances, as well as for bactericidal effect. The methods used herein also included mucin and saline to simulate potential soiling during re-use, which presents a challenge to decontamination efficacy evaluations. Results obtained showed that both the moist heat and VHP technologies were demonstrated to deliver $\geq$3log$_{10}$ virus and $\geq$6 log$_{10}$ *Mycobacterium* spp. inactivation, therefore meeting the minimum FDA EUA acceptance criteria for inactivation that was provided at that time [8].

The evaluation involved assessment of processing parameters that delivered target decontamination efficacy while maintaining critical functionality of the N95 PPE. Performance evaluations by the original FFR manufacturer have demonstrated that both the moist heat and VHP decontamination process have no negative effects on processed N95 FFRs with regards to filter performance and fit, such that treated FFRs passed the same acceptance criteria as new unused FFRs. Furthermore, VHP-processed N95 FFRs have been demonstrated to be safe to use with no significant levels of residue.

It has been shown in this investigation that moist heat, when held for 30 min between 60 and 70°C and between 50 and 100% relative humidity, is effective in providing $\geq$3 log$_{10}$ reduction of the non-enveloped virus surrogate. Therefore, this would indicate that moist heat in such conditions is likely to be effective for less-resistant enveloped viruses, such as SARS-CoV-2, and may also provide possibilities for decontamination using simple containers, apparatuses, and rooms capable of delivering such parameters that warrant further investigation. This may be of particular benefit to healthcare facilities without access to specialized equipment such as steam or VHP sterilizers. For example, warming cabinets, which deliver controlled convective heating and are broadly available in hospitals and medical facilities, could be used to create a moist heat environment for FFR decontamination. As demonstrated, the necessary moist heat environment may be created by inserting an individual FFR into a sealed plastic resealable bag along with an adequate source of humidity (approximately 3 mL of sterile water). A typical warming cabinet can be loaded with 40–80 resealable bags containing FFRs. Similarly, a moist heat environment capable of decontaminating FFRs may also be achieved in climate chambers. These units deliver controlled convective heating and humidity inside an enclosed chamber. The capacity of FFRs to decontaminate will vary with the size of the chamber. The utility of simple heating chambers constructed of general purpose materials and equipment was also explored. An example of such was a specifically designed process for N95 FFP decontamination in single-chamber washer-disinfectors using a 40-min drying phase at 70°C, which allowed decontamination of 48–72 resealable bags containing FFRs, with promising results of $\geq$6 log$_{10}$ reduction of *Mycobacterium* spp.

As much as one considers the successes and possibilities for further refinement and deployment, it is equally important to reflect on the learnings of approaches that were determined to be unsuitable for decontaminating PPE: N95 masks evaluated under this study were incompatible with standard steam sterilization cycles (121°C, unreported data). Performance evaluations of masks exposed to standard steam sterilization cycles at temperatures >75°C demonstrated negative effects with regards to filter performance and fit. Additionally, for the methods developed, the challenge of process control and repeatability were considered. Consideration must be given to the traceability and recording of treatments per FFR and cycle data reporting as part of the overall management of FFR PPE provision. This is imperative given a key consideration is user acceptance: user acceptance may be improved where re-use is by the original user. N95 FFRs must be free of visible damage and soil/contamination (e.g. blood, dried sputum, make up, soil, bodily fluids). Users should discard and not process N95 FFRs that are visually soiled or damaged. Cleaning methods for N95 FFRs were not evaluated, and no cleaning practices are recommended to users as the chemistries could compromise the integrity of the FFR's barrier. As part of the review and authorization of the methods investigated, US FDA previously issued EUA authorization for the use of VHP in the non-lumen cycle of the V-PRO sterilizers (V-PRO 1 Plus, maX and maX 2, V-PRO 60 and s2) [27]. In addition, EUA was also issued for the steam decon cycle in AMSCO medium steam sterilizers which employs a process of 65°C with a 30-min exposure time (https://www.fda.gov/media/138282/download [28]).

This work demonstrates the inactivation efficacy of the methods tested and the impact of reprocessing on FFR functionality. As stated, there are additional challenges with user acceptance, traceability and stock management, and available decontamination equipment that must be considered by the user when developing a programme that may be required as a temporary solution to an urgent need, as observed during the early onset of COVID-19. Furthermore, while this work showed methods suitable for 10 and 20 decontamination cycles, users must consider all factors when determining an appropriate amount of re-use that is controllable, traceable and provides assurance to end users.

Finally, while the outcome of this investigative research resulted in FDA EUA for the temporary emergency of FFR supply during the COVID-19 pandemic, the learning may help inform future applications and needs. For example, longer term considerations may also focus on the application of such decontamination processes as part of environmentally friendly strategies for disposal, and recycling of materials used in FFR manufacture.

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