


Original Article

Hydrogen peroxide vapor decontamination of N95 respirators for reuse

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Abstract

Objective: The coronavirus disease 2019 (COVID-19) pandemic has led to global shortages of N95 respirators. Reprocessing of used N95 respirators may provide a higher filtration crisis alternative, but whether effective sterilization can be achieved for a virus without impairing respirator function remains unknown. We evaluated the viricidal efficacy of Bioquell vaporized hydrogen peroxide (VHP) on contaminated N95 respirators and tested the particulate particle penetration and inhalation and exhalation resistance of respirators after multiple cycles of VHP.

Methods: For this study, 3M 1870 N95 respirators were contaminated with 3 aerosolized bacteriophages: T1, T7, and *Pseudomonas* phage phi-6 followed by 1 cycle of VHP decontamination using a BQ-50 system. Additionally, new and unused respirators were sent to an independent laboratory for particulate filter penetration testing and inhalation and exhalation resistance after 3 and 5 cycles of VHP.

Results: A single VHP cycle resulted in complete eradication of bacteriophage from respirators (limit of detection 10 PFU). Respirators showed acceptable limits for inhalation/exhalation resistance after 3 and 5 cycles of VHP. Respirators demonstrated a filtration efficiency >99 % after 3 cycles, but filtration efficiency fell below 95% after 5 cycles of HPV.

Conclusion: Bioquell VHP demonstrated high viricidal activity for N95 respirators inoculated with aerosolized bacteriophages. Bioquell technology can be scaled for simultaneous decontamination of a large number of used but otherwise intact respirators. Reprocessing should be limited to 3 cycles due to concerns both about impact of clinical wear and tear on fit, and to decrement in filtration after 3 cycles.

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The coronavirus disease 2019 (COVID-19) pandemic has led to unprecedented changes in the utilization of healthcare resources, including global shortages of personal protective equipment (PPE), including N95 respirators.¹ N95 respirators are highly regulated by the Centers for Disease Control and Prevention (CDC), National Institute for Occupational Safety and Health (NIOSH), and Occupational Safety and Health Administration (OSHA), and they are considered class II devices by the US Food and Drug Administration (FDA).² N95 respirators are intended for use in a healthcare setting as a single-use, disposable respiratory protective device to be worn by healthcare personnel to protect both the patient and healthcare personnel from the transfer of microorganisms, body

fluids, and particulate material.² NIOSH has exclusive authority for testing and certification of respirators to ensure that the product filters at least 95% of airborne particles and that it demonstrates the ability to resist penetration by fluids, such as blood and body fluids, at a velocity consistent with the intended use of the device.² The CDC has recently modified PPE recommendations and has provided “crisis alternate strategies” if the respirator supply is exhausted, including use of respirators that are not approved by the NIOSH, and homemade masks as a last resort.^{3,4} Improvised fabric masks provide only marginal protection and are inferior to N95 respirators, which is of particular concern during the COVID-19 pandemic because of the high rate of healthcare personnel infection.^{5–7} Reused respirators may become a reservoir for pathogens, presenting a potential risk.^{8,9} In addition, the CDC has stated that decontamination and reuse of N95 respirators may need to be considered as a crisis capacity strategy to ensure continued availability.¹⁰ Reprocessing of used N95 respirators may ameliorate supply-chain constraints and provide a higher filtration

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crisis alternative, but whether effective sterilization can be achieved for a virus without impairing respirator function remains unknown.

To adequately disinfect or sterilize N95 respirators, the methodology must meet 4 criteria: (1) effectiveness against the target organism, (2) preservation of filtration, (3) conservation of fit, and (4) safety for the person wearing the respirator. A variety of processes have been evaluated, and each has limitations.¹¹ Thermal reprocessing deforms respirators and alters fit.¹² Ultraviolet germicidal irradiation of respirators reduces virus viability but efficiency is hampered by shadowing.¹³ Vaporized hydrogen peroxide (VHP) is viricidal on hard surfaces, and it has been shown not to impair respirator performance.^{14,15} The FDA Medical Countermeasures Initiative funded a study of VHP decontamination of respirators using a Clarus C system (Bioquell, Horsham, PA) which normally is used to fumigate hospital rooms.¹⁶ This system uses a generator to convert 30% liquid hydrogen peroxide into VHP, until $\sim 1 \mu\text{m}$ hydrogen peroxide is present on all surfaces before being converted to oxygen and water vapor by catalytic converters. This system achieves a 6 log reduction in environmental bioburden. The respirator function was excellent, with no impairment of aerosol collection efficiency or air flow resistance after 50 cycles. Although there was complete inactivation of aerosolized *Geobacillus stearothermophilus*, it is unknown if VHP would be viricidal because respirators have porous fabric that may harbor virus. VHP is not compatible with respirators that contain cellulose.

Methods

We evaluated the viricidal activity of VHP using a BQ-50 system (Bioquell, Horsham, PA), a more automated model of the Clarus C, after inoculating each 3M 1870 N95 respirator (3M, St Paul, MN) with 1 of 3 aerosolized bacteriophages: T1, T7, or *Pseudomonas* phage phi-6. These bacteriophages are a reasonable proxy for severe acute respiratory coronavirus virus 2 (SARS-CoV-2) and are an ideal system to study because they potentially encompass the spectrum of viral diversity due to notable stability to desiccation (T1), ease of use (T7), and possession of a viral envelope (phi-6).¹⁷

A second experiment was performed to evaluate the particulate filter penetration and inhalation and exhalation resistance (breathability) of respirators after reprocessing. Moreover, 40 new and unused respirators were sent to an independent laboratory, Nelson Labs (Salt Lake City, UT), for particulate filter penetration testing using NIOSH procedure TEB-APR-STP-0058 after 3 cycles ($n = 20$) and 5 cycles ($n = 20$) of VHP. Also, 6 new and unused respirators were sent for testing of resistance to inhalation and exhalation using NIOSH procedures TEB-APR-STP-0007 and TEB-APR-STP-0003, after 3 cycles ($n = 3$) and 5 cycles ($n = 3$) of VHP. Testing was performed in compliance with the US FDA good manufacturing practice (GMP) regulations.

Inoculation

The bacteriophage solution was prepared by diluting a concentrated bacteriophage stock (1×10^{10} PFU/ML) in phosphate buffered saline (PBS) with 10 mM MgSO_4 , to stabilize the phage particles. Aerosols containing 1 of 3 bacteriophages were generated using a fine-mist spray bottle aimed directly at an inverted N95 respirator that was placed in a 1-L beaker and was allowed to soak into the material for 30 minutes (until dry) in a biosafety cabinet.

Inoculation resulted in contamination of the respirator with 9.87×10^4 plaque-forming units (PFU) of bacteriophage phi-6, 4.17×10^7

PFU of phage T7, and 1.35×10^7 PFU of phage T1 per respirator. Concentrations were selected to approximate viral titers necessary for 50% tissue-culture infectious dose (TCID₅₀) of SARS-CoV-2.¹⁸

Decontamination

Respirators were suspended by their elastic using paper clips on racks in a 33-m^3 room. They were decontaminated with a BQ-50 using a 4-minute conditioning phase, a 30–40-minute gassing phase (varies with humidity and room size) at 16 grams per minute, a 25-minute dwell phase, and a 120–150-minute aeration phase (varies with number of respirators and room size). This long duration was intended to reduce residual hydrogen peroxide.^{13,15} As a comparison, half of the respirators had subsequent steam sterilization at 135°C (275°F) for 5 minutes.

Bacteriophage recovery

Bacteriophage were recovered by submerging respirators in a sterile 1-L flask containing 500 mL PBS with 10 mM MgSO_4 for 30 minutes then plated for PFU using a standard double-overlay agar method.¹⁹ Actual counts were determined using the double-overlay agar method and by counting visible plaques of serially diluted PBS solution. All experiments were performed in triplicate.

Results

Bacteriophages from the untreated respirators were recovered with a range of 1×10^5 to 1×10^7 PFU/mL (average, 1.85×10^7 PFU/mL), confirming successful inoculation. A single VHP cycle resulted in complete eradication of bacteriophage from the respirators (limit of detection, 10 PFU, lower than the infectious dose of most respiratory viral pathogens).²⁰ After 5 VHP cycles, the respirators appeared similar to new with no apparent deformity by visual inspection. The addition of steam sterilization led to visible degradation of the respirators, and given complete eradication of the bacteriophage contamination by VHP alone, offered no additional detectable viricidal activity.

Testing of respirators that underwent either 3 ($n = 3$) or 5 cycles ($n = 3$) of VHP showed acceptable limits for inhalation and exhalation resistance parameters. Respirators that underwent 3 cycles of VHP ($n = 20$) all demonstrated a filtration efficiency $>99\%$. For the respirators that underwent 5 cycles of VHP ($n = 20$), only 3 respirators were tested due to the fact that the third respirator tested failed with $<95\%$ filtration efficiency (94.99%). Therefore, the remaining 17 respirators were not tested, consistent with NIOSH requirements.

Discussion

With N95 respirators in critically short supply during the COVID-19 pandemic, alternative crisis capacity strategies have been adopted by many institutions to ensure availability. In line with a CDC statement that decontamination and reuse of N95 respirators is an option, several healthcare institutions have evaluated VHP decontamination.¹⁰ The FDA has issued several emergency use authorizations (EUA) for decontamination of N95 respirators using hydrogen peroxide, along with revised policy on the types of respirators that can be decontaminated for reuse.^{10,21}

Antimicrobial efficacy of VHP was demonstrated against *Geobacillus stearothermophilus* spores in a comprehensive study by Battelle.¹⁶ Our results demonstrate that VHP is highly viricidal, with complete eradication of bacteriophages from inoculated respirators after a single VHP cycle. However, the Battelle study reported no

impact from Bioquell VHP on the structural and functional integrity of selected unused N95 respirators after 50 decontamination cycles; a recent letter in *JAMA* reported a correlation with fit failures and extended use and reuse of N95 respirators.²² Thus, the VHP process itself may not impair fit, but that the wear and tear from clinical use may result in respirator deformity and loss of fit. The fit of N95s should be closely evaluated during use and reuse.²¹

In our study, independent laboratory testing demonstrated intact filtration after 3 cycles (99% efficiency) but decrement in filtration after 5 cycles, with the third respirator falling below the 95% threshold by 0.01%. Whether this is a clinically meaningful decline remains unknown, but it suggests that repeated reprocessing may impact filtration. We were unable to validate the results published by the Battelle group. Based on these results, our institution now limits reprocessing to 3 cycles as long as the respirator remains intact and clean following clinical use.

In our program, respirators were collected and delivered to a central location where they were visually inspected for debris, structural defects, fabric imperfections, and damaged elastic straps prior to decontamination. Respirators not meeting predefined tolerances were discarded. A typical VHP cycle was able to reprocess ~2,700 respirators and required ~12 hours of time to sort, hang, process, aerate, and package and label them for distribution. We have successfully reprocessed 26,000 respirators, and ~58% of those collected were deemed unsuitable for reprocessing, and most of those were rejected due to makeup and lipstick stains.

Our study has several limitations. First, the study was small; however, the experiments were performed in triplicate with no evidence of viable bacteriophage after VHP decontamination. Second, bacteriophages were used as surrogates for the coronavirus. We did use 3 bacteriophages with differing characteristics, and VHP treatment eliminated all viable virus. We believe that it is reasonable to expect that VHP would likewise be highly effective against SARS-CoV-2. Finally, in our study, air pressure through the respirator was not used to simulate normal respiratory effects during respirator use. However, the high titer of bacteriophage that was inoculated on to the respirators as well as the elution process used to recover remaining viable bacteriophage was expected to be a sufficient challenge and model.

In summary, Bioquell VHP has high viricidal activity for N95 respirators inoculated with aerosolized viruses. Bioquell technology can be scaled to permit simultaneous decontamination of a large number of used but otherwise intact respirators. VHP reprocessing may ease shortages and provide a higher filtration alternative to other crisis strategies. Reprocessing should be limited to 3 cycles due to concerns both about impact of clinical wear and tear on fit and due to decrement in filtration after 3 cycles.

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References

1. Mahase E. Novel coronavirus: Australian GPs raise concerns about shortage of face masks. *BMJ* 2020;368:m477.
2. N95 respirators, surgical masks, and face masks. US Food and Drug Administration website. <https://www.fda.gov/medical-devices/personal-protective-equipment-infection-control/n95-respirators-surgical-masks-and-face-masks>. Published 2020. Accessed on March 20, 2020.
3. Interim infection prevention and control recommendations for patients with suspected or confirmed coronavirus disease 2019 (COVID-19) in healthcare settings. Centers for Disease Control and Prevention website. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/infection-control-recommendations.html>. Published 2020. Accessed March 20, 2020.
4. Strategies for optimizing the supply of N95 respirators. Centers for Disease Control and Prevention website. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/respirators-strategy/index.html>. Accessed March 20, 2020.
5. Rengasamy S, Eimer B, Shaffer RE. Simple respiratory protection—evaluation of the filtration performance of cloth masks and common fabric materials against 20–1000 nm size particles. *Ann Occup Hyg* 2010;54:789–798.
6. Remuzzi A. COVID-19 and Italy: what next? *Lancet* 2020;395:1225–1228.
7. Chu DK, Elie AA, Solo K, Yaacoub S, Shinemann HJ. Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. *Lancet* 2020. doi: 10.1016/S0140-6736(20)31142-9.
8. Coulliette AD, Perry KA, Edwards JR, Noble-Wang JA. Persistence of the 2009 pandemic influenza A (H1N1) virus on N95 respirators. *Appl Environ Microbiol* 2013;79:2148–2155.
9. APIC position paper: Extending the use and/or reusing respiratory protection in healthcare settings during disasters. Association for Professionals in Infection Control and Epidemiology website. http://www.apic.org/Resource_/TinyMceFileManager/Advocacy-PDFs/APIC_Position_Ext_the_Use_and_or_Reus_Resp_Prot_in_Hlthcare_Settings12091.pdf. Published 2009. Accessed March 20, 2020.
10. Decontamination and reuse of filtering facepiece respirators. Centers for Disease Control website. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/ppe-strategy/decontamination-reuse-respirators.html>. Published 2020. Accessed March 20, 2020.
11. Seresirikachorn K, Phoophiboon V, Chobarporn T, et al. Decontamination and reuse of surgical masks and N95 filtering facepiece respirators during the COVID-19 pandemic: a systematic review. *Infect Control Hosp Epidemiol* 2021;42:25–30.
12. Viscusi DJ, Bergman MS, Novak DA, et al. Impact of three biological decontamination methods on filtering facepiece respirator fit, odor, comfort, and donning ease. *J Occup Environ Hyg* 2011;8:426–436.
13. Mills D, Harnish DA, Lawrence C, Sandoval-Powers M, Heimbuch BK. Ultraviolet germicidal irradiation of influenza-contaminated N95 filtering facepiece respirators. *Am J Infect Control* 2018;46:e49–e55.
14. Viscusi DJ, Bergman MS, Eimer BC, Shaffer RE. Evaluation of five decontamination methods for filtering facepiece respirators. *Ann Occup Hyg* 2009;53:815–827.
15. Goyal SM, Chander Y, Yezli S, Otter JA. Evaluating the viricidal efficacy of hydrogen peroxide vapour. *J Hosp Infect* 2014;86:255–259.
16. Battelle. Final report for the Bioquell hydrogen peroxide vapor (HPV) decontamination for reuse of N95 respirators. US Food and Drug Administration website. <https://www.fda.gov/emergency-preparedness-and-response/mcm-regulatory-science/investigating-decontamination-and-reuse-respirators-public-health-emergencies>. Published 2016. Accessed March 20, 2020.
17. Drexler H. Bacteriophage T1. In: Calendar R (editor), *The Bacteriophages*. New York: Plenum;1988:235–258.
18. van Doremalen N. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 2020;382:1564–1567.
19. Kropinski, AM, Mazzocco, A, Waddell, TE, Lingohr, E, Johnson, RP. Enumeration of bacteriophages by double agar overlay plaque assay. *Methods Mol Biol* 2009;501:69–76.
20. Yezli S, Otter JA. Minimum infective dose of the major human respiratory and enteric viruses transmitted through food and the environment. *Food Environ Virol* 2011;3:1–30.
21. Coronavirus (COVID-19) update: FDA reissues emergency use authorizations revising which types of respirators can be decontaminated for reuse. US Food and Drug Administration website. <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-reissues-emergency-use-authorizations-revising-which-types>. Published 2020. Accessed June 9, 2020.