Background: Safe and effective decontamination and reuse of N95 filtering facepiece respirators (FFRs) has the potential to significantly extend FFR holdings, mitigating a potential shortage due to an influenza pandemic or other pandemic events. Ultraviolet germicidal irradiation (UVGI) has been shown to be effective for decontaminating influenza-contaminated FFRs. This study aims to build on past research by evaluating the UVGI decontamination efficiency of influenza-contaminated FFRs in the presence of soiling agents using an optimized UVGI dose.

Methods: Twelve samples each of 15 N95 FFR models were contaminated with H1N1 influenza (facepiece and strap), then covered with a soiling agent—artificial saliva or artificial skin oil. For each soiling agent, 3 contaminated FFRs were treated with 1 J/cm² UVGI for approximately 1 minute, whereas 3 other contaminated FFRs remained untreated. All contaminated surfaces were cut out and virus extracted. Viable influenza was quantified using a median tissue culture infectious dose assay.

Results: Significant reductions (≥3 log) in influenza viability for both soiling conditions were observed on facepieces from 12 of 15 FFR models and straps from 7 of 15 FFR models.

Conclusions: These data suggest that FFR decontamination and reuse using UVGI can be effective. Implementation of a UVGI method will require careful consideration of FFR model, material type, and design.

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on NIOSH-approved FFRs should consider hygiene, damage, and breathing resistance, and be replaced whenever they are damaged, soiled, or cause noticeably increased breathing resistance. Implementa-
tion of these reuse practices is up to the respiratory protection
program’s manager and is dependent on the respiratory patho-
gen’s characteristics (eg, route of transmission and severity of illness)
and local conditions (eg, number of N95 respirators available and
use rate). Among the primary concerns for implementing an FFR
extended use or limited reuse policy is the possibility of respira-
tors becoming contaminated and subsequently acting as fomites,
potentially spreading the disease. HCWs are well versed in self-
contamination incidents that occurred during the severe acute
respiratory syndrome and Ebola virus disease outbreaks and are con-
cerned that extended use of FFRs may lead to self-infection.13,14

Although guidance for limited reuse and extended use of FFRs
is currently available, implementation of FFR-DR strategies is a more
complicated process. For reprocessed single-use medical devices,
the US Food and Drug Administration (FDA) requires validation data
regarding cleaning, sterilization, and functional performance.15
Cleaning is generally performed before decontamination to ensure
soiling materials do not interfere with the decontamination process.
The common definition of a cleaned device—no visual contamination
is present—differs from the Medical Device User Fee and Moderni-
ation Act of 2002, which states that the reprocessor must establish
cleaning end points and rationale for their selection.10 Cleaning
FFRs is a difficult task because the N95 facepiece is an exposed
filter and not compatible with standard laundering techniques. Ad-
ditionally, research has been performed demonstrating that several
FFR models cannot be effectively cleaned using various cleaning
wipes.17 According to the Institute of Medicine, any method de-
contaminating a disposable N95 FFR must remove the pathogen,
be harmless to the user, and not compromise the integrity of the
various parts of the respirator.18 If the decontamination process
can eliminate viable pathogens from the medical device in the pres-
ence of other organic material, the question arises of whether
cleaning would still be required, especially during a public health
emergency.

Several studies have previously been performed evaluating the
efficacy of FFR decontamination methods. Heimbuch et al19 evalu-
ated 3 different energetic methods (microwave-generated steam
[MGS], moist heat incubation [MHI], and ultraviolet germicidal ir-
radiation [UVGI]) against H1N1 influenza-contaminated N95 FFRs.
All 3 methods demonstrated >4-log reductions in viable virus. The
results were subsequently duplicated using low-pathogenic H5N1
avian influenza by Lore et al.19 Fisher et al21 demonstrated >4-log
reductions in viable MS2 virus on FFR coupons using 0.6% sodium
hypochlorite solution and MGS treatments ≥45 seconds. Vo et al22
evaluated the disinfection efficiencies of sodium hypochlorite and
UVGI on N95 respirators contaminated with droplets containing MS2
bacteriophage, and both approaches demonstrated multi-log reduc-
tions in MS2 viability. Although there are currently no guidelines
for the level of decontamination required for contaminated FFRs,
multiple FFR-DR methods have shown significant reductions in virus
viability. Currently, there are no published data on actual influen-
cia contamination levels of FFRs in hospitals. However, Fisher et al23
validated a predictive model for estimating the level of influenza
contamination on FFRs and surgical masks resulting from aerosols
in a health care setting. The estimated contamination level for
the entire external surface of an FFR ranged from 10^1–10^5 viruses,
depending on different scenarios using airborne influenza concentra-
tions published in the literature.

The study described herein is a continuation of the UVGI-
based FFR decontamination research performed by Heimbuch et al19
in 2011. Although all 3 methods (MGS, MHI, and UVGI) demon-
strated >4-log reduction in viable virus, some methods may be better
suited for hospital use than others. The MGS method required the
longest decontamination time (30 minutes) and the use of an oven
set to 160°F. The MGS method was the shortest decontamination
time (2 minutes), but there may be concerns over wattage variabil-
ity among microwave ovens. Although the UVGI method required
a 15-minute decontamination period, this method may be most suit-
able for large-scale applications due to simplicity of use and ability
to rapidly scale. UVGI technologies for whole-room decontamina-
tion have already been developed and are commercially available.24,26
Despite showing >4-log reduction in viable influenza, some limi-
tations of the study were subsequently identified. The study authors
listed the primary limitation as being the low number of FFR models
evaluated. Also, the ultraviolet (UV) light dose (concentration × time)
could likely be optimized for hospital use by increasing the con-
centration of UV rays (ie, source and distance between the substrate
and the UV light source), and reducing the time required to achieve
decontamination, making the method more conducive to hospital
use by minimizing logistical burden. Additionally, the 2011 study19
evaluated decontamination efficiency of influenza in the absence
of soiling agents (ie, protective factors) that may shield the virus
from the decontamination source. During real-world contamin-
ation events, influenza virus could very likely be shielded by organic
soiling agents like saliva or skin oil, which can inhibit the effec-
tiveness of decontamination techniques.27-29

The objective of the current study was to evaluate the UVGI de-
contamination efficiency of an intact FFR contaminated with both
a pandemic influenza strain and a soiling agent to better simulate
real-world contamination events. Fifteen N95 FFR models were con-
taminated with viable H1N1 influenza and either artificial saliva or
artificial skin oil, then subsequently treated with UV light and evalu-
at for remaining viable virus.

MATERIALS AND METHODS

H1N1 influenza

H1N1 influenza A/PR/8/34 (VR-1469; American Type Culture Col-
lection, Manassas, VA) was propagated in embryonic chicken
eggs (Premium Specific Pathogen Free Eggs 10100326; Charles
River Laboratories, Wilmington, MA) using standard World
Health Organization protocols.20 Virus titers were determined by
a 50% tissue culture infectious dose (TCID50) assay. Madin-Darby
canine kidney cells (CCL-34; American Type Culture Collection)
were passaged and maintained using World Health Organization-
approved cell culture techniques.

Soiling agents

Mucin buffer was prepared and stored at 4°C.31 Synthetic skin
oil (Scientific Services S/D, Sparrow Bush, NY) was purchased, divided
into 2.5-mL aliquots, and stored at 37°C until use. For testing, aliquots
were heated to 70°C and poured into the base of a 100-mm Petri
dish. Continual heat was applied until the layer became even and
allowed to cool to room temperature.

Test respirators

Fifteen NIOSH-approved N95 FFR models were chosen for this
study (Table 1), with consideration given to whether the product
was cleared by FDA, its commercial availability, and its unique shapes
and materials. All of the FFR models were cleared by the FDA as sur-
gical N95 respirators, except for the EZ 22 (Moldex, Culver City, CA).
To perform statistical analyses, EZ-22 was used. The UV dose is monitored during each test to ensure consistent treatment across experiments. Inoculated control masks were held at room temperature in a class II biosafety cabinet enclosure until the treated masks completed UVGI treatment.

After UV treatment, FFRs were transferred to a class II biosafety cabinet for processing. Inoculated areas on the FFR facepiece were removed using a 1.5-in circular die and placed in 50-mL centrifuge tubes containing 15 mL serum-free Eagle’s minimum essential medium (EMEM). The FFR straps were cut at their points of attachment to the FFR, and each was placed in a 50 mL centrifuge tube containing 15 mL serum-free EMEM and vortexed for 20 minutes to extract the influenza virus. Extracts were subsequently serially diluted in serum-free EMEM and plated in quadruplicate in 24-well plates with confluent monolayers of Madin-Darby canine kidney cells, according to the World Health Organization protocol for a TCID₅₀ assay. Plates were then incubated at 37°C in 5% carbon dioxide for 7 days. After the incubation period, each well was observed under a microscope for cytopathic effects, generally demonstrated by a disruption of the cell monolayer. Plates were subsequently stained with crystal violet-glutaraldehyde to confirm the presence of cytopathic effects.

**Data analysis**

UV dose was calculated based on standard methods for mathematical modeling of UV light using Equation 1:

\[
\text{UV dose} \left( \frac{J}{cm^2} \right) = \text{Irradiance} \left( \frac{W}{cm^2} \right) \times \text{Time (s)}
\]

To determine the level of viable virus recovered from each sampled location, the Spearman-Kärber formula was used to interpret the TCID₅₀ assay data. To perform statistical analyses, Environmental Protection Agency guidance using half the detection limit (0.20 log₁₀ TCID₅₀) for below-detection limit values was followed. An unpaired, 2-tailed t test was used to compare UV-treated and control virus recoveries, as well as log reduction values for FFR facepieces and straps. Data were analyzed using statistical tools in GraphPad Prism 6 (Graph Pad Inc, La Jolla, CA).

**RESULTS**

Across all 180 FFRs tested, the mean UV dose per FFR was 1.1 ± 0.1 J/cm², the mean temperature was 21°C ± 2°C, and the mean relative humidity was 48% ± 6% within the UV device.
Mucin-soiled FFRs

For mucin-soiled FFR facepieces, the mean viable influenza recovered from control surfaces was $4.29 \pm 0.52 \log \text{TCID}_{50}$; for mucin-soiled FFR straps, the mean viable influenza recovered from control surfaces was $3.57 \pm 0.78 \log \text{TCID}_{50}$ (Fig 3).

The mean log reduction ranged from 1.42-4.84 log TCID$_{50}$ for mucin-soiled facepieces and 0.00-4.31 log TCID$_{50}$ for mucin-soiled straps. For mucin-soiled facepieces, the mean viable virus recovered from UV-treated samples was statistically significantly lower than control samples for all FFR models tested. For mucin-soiled straps, the mean viable virus recovered from UV-treated samples was significantly lower than control samples for all FFR models tested except the 3M 1860, Alpha Protech, and Moldex EZ 22. The log reduction values observed for all mucin-soiled FFR straps were statistically significantly lower than their respective FFR facepieces.

Sebum-soiled FFRs

For sebum-soiled FFR facepieces, the mean viable influenza recovered from control surfaces was $4.10 \pm 0.56 \log \text{TCID}_{50}$; for sebum-soiled FFR straps, the mean viable influenza recovered from control surfaces was $3.90 \pm 0.65 \log \text{TCID}_{50}$ (Fig 4).

The mean log reduction ranged from 1.25-4.64 log TCID$_{50}$ for sebum-soiled facepieces and 0.08-4.40 log TCID$_{50}$ for sebum-soiled straps. For sebum-soiled facepieces, the mean viable virus recovered from UV-treated samples was significantly lower than control samples for all FFR models tested. For sebum-soiled straps, the mean viable virus recovered from UV-treated samples was significantly lower than control samples for all FFR models tested except the 3M 1860, Alpha Protech, and Moldex EZ 22. The log reduction values observed for the sebum-soiled FFR straps were significantly lower than the respective FFR facepieces.

DISCUSSION

In this study, evaluation of the UVGI decontamination method focused on log reduction rather than total absence of viable virus because the viral challenge was selected to far exceed what would may occur during a real-world contamination event. Based on the Fisher et al.$^{23}$ model predicting influenza contamination levels of FFRs in hospitals from aerosol sources, the highest estimated contamination level ($10^5$ virus/FFR) would result in a loading concentration of $10^3$ virus/cm$^2$ for a 200-cm$^2$ FFR, requiring a 3-log reduction to fully decontaminate. The virus loading concentration used in the current study is approximately 100-times higher than the loading concentration resulting from the highest contamination level estimated by Fisher et al.$^{23}$ Maximizing the loading concentration is important for laboratory studies in order to generate a measurable log reduction and overcome the virus loss resulting from variable extraction efficiencies of different materials, potential loss of viability due to environmental exposure, and the detection limit of the log$_e$-based viable assay ($\approx 0.50$ TCID$_{50}$).

Compared with the Heimbuch et al.$^{19}$ 2011 study that evaluated 6 FFR models, the current study provides a broader view of the diversity among FFR models currently available on the market. Across the 15 FFR models evaluated for this study there are considerable differences in FFR facepiece design, varying in overall shape (eg, cup, flat-fold, or pouch), material composition, and other design

*Fig 1. Ultraviolet germicidal irradiation device. (A) Top view: a, ultraviolet light bulb; b, heat exchanger; c, fan; d, hinged door; e, power supply; f, sliding mesh wire shelf; g, power switch; h, filtering facepiece respirator (example); i, radiometer; j, temperature/humidity probe; k, ethylene glycol supply line. (B) Side view.

*Fig 2. Locations of influenza droplets applied to filtering facepiece respirators.

*Droplets not drawn to scale
features (e.g., pleats and flaps). Variability in design between FFR models has the potential to influence the effectiveness of FFR-DR strategies, making it important to understand which design attributes may be less or more advantageous for a given decontamination method. Reductions in virus viability ≥3 log were considered significant because a 3-log reduction would be required to fully disinfect an FFR contaminated with the highest level of influenza contamination predicted by the Fisher et al.²³ model. Significant reductions in influenza viability were observed on both mucin- and sebum-soiled facepieces for 12 of 15 FFR models post-UV treatment. The remaining 3 FFR models—Gerson 1730 (Louis M. Gerson Co, Inc, Middleboro, MA), Sperian HC-NB095 (Honeywell Safety Products USA, Smithfield, RI), and U.S. Safety AD2N95A—still demonstrated statistically significant reductions in virus viability. The facepieces from these 3 FFR models are relatively similar in appearance—white, cup-shaped, with slightly rough texture. The Gerson 1730 and U.S. Safety AD2N95A facepieces both appear to be hydrophilic, as indicated by the immediate absorption of the liquid inocula, whereas the Sperian HC-NB095 appears to be hydrophobic. Absorption of the viral inoculum away from the surface could potentially limit the UVGI decontamination efficiency because UV light is primarily effective for surface decontamination. Although the Sperian HC-NB095 facepieces did not appear hydrophilic, the relatively low log reduction may be attributed to the presence of horizontal ridges across the front of the facepieces, which may have created small shadowing effects while the mask was exposed to UV light. In general, the presence of shadows indicates the blocking of UV light, thus inhibiting UVGI efficiency. Although the data demonstrate UVGI can be effective, additional research would be required to define how specific FFR design attributes may influence UVGI efficiency, either individually or in combination.

Similar to the variability observed with FFR facepieces, FFR straps also vary in design (e.g., material type, thickness, width, and elasticity), which may influence the effectiveness of FFR decontamination methods. Significant reductions in virus viability were observed for both mucin- and sebum-soiled straps from 7 of 15 FFR models post-UV treatment, whereas 5 FFR models demonstrated <3-log reductions for both soiling agents. Of these 5 FFR models, 4 models—3M 1860, U.S. Safety AD2N95A, Moldex EZ-22, and Alpha Protech 695—appear to have hydrophilic straps and thus absorption of the virus away from the surface could potentially limit UVGI effectiveness. As with FFR facepieces, shadowing during UVGI treatment could also influence the UVGI effectiveness on FFR straps. The presence of shadows are likely a greater concern for FFR straps than FFR facepieces due to their propensity to twist and orientation based on how much slack is available. Ultimately, FFR straps pose a logistic challenge for UVGI decontamination strategies. This is a significant finding because proper doffing techniques for FFRs require handling of the straps, increasing the likelihood of fomite transfer. Thus, FFRs with straps that are amenable to UVGI disinfection would be preferred. Unlike the facepiece component, straps could potentially be disinfected using a disinfecting wipe or similar approach, but determining the effectiveness of these methods would require additional research outside the scope of this study.

Addressing a limitation of the 2011 Heimbuch et al. study, soiling agents were used to shield the virus inocula, acting as protective factors. UVGI effectiveness has been shown to correlate with soil load, decreasing in disinfection efficiency as the soil load increases.²⁹

### Fig 3. Viable virus recovered from mucin-soiled N95 respirators. Respirator manufacturers were 3M Company, Minneapolis, MN; Alpha Protech, Markham, Canada; Louis M. Gerson Co, Inc, Middleboro, MA; Halyard Health Inc., Alpharetta, GA; Moldex, Culver City, CA; Precept Medical Products, Inc, Arden, NC; Prestige Ameritech, North Richland Hills, TX; Honeywell Safety Products USA, Smithfield, RI; and Dentech Safety Specialists, Lenexa, KS.

- **Facemask Control**
- **Facemask Treated**
- **Strap Control**
- **Strap Treated**
As previously mentioned, the levels of sebum and mucin buffer used were intentionally high to act as a worst-case scenario. Pochi and Strauss\(^35\) measured casual sebum levels of 51 male subjects with and without acne to determine a cause-and-effect relationship. They found that subjects with severe acne had a mean density of \(0.18 \pm 0.08 \text{mg/cm}^2\) sebum on their face based on the samples taken. Compared with the sebum level of subjects with acne, the sebum challenge used in this study is approximately a 7-fold increase. For mucin buffer, 5 loadings were used for each influenza droplet to provide multilayered shielding against UVGI. Although the level of soiling agents used in this study may be considered excessive and thus a limitation, significant reductions in viable influenza were still observed for both soiling agents, indicating UVGI decontamination of influenza could be performed in the absence of cleaning.

Other potential limitations of the current research effort were identified. For the purposes of this study, only the exterior surface of the facemask was evaluated for UVGI effectiveness. If implemented in a hospital setting, a UVGI application would likely need to treat both the interior and exterior FFR surfaces, accounting for potential contamination resulting from either the environment or the user. Additionally, the soiling agents used were artificial and thus a difference in UVGI effectiveness between artificial and natural soiling agents could potentially occur. There was also some loss in viable virus between the inoculation and extraction of control samples. The source of this reduced fraction of viable virus could be attributed to natural decay of the virus or influenced by the material’s extraction efficiency; the effect of either factor is unclear.

The study described herein addresses a significant concern for HCWs during an influenza pandemic—the unavailability of N95 FFRs. Although FFR-DR is a possible mitigation strategy for a potential N95 shortage, the research related to FFR-DR methodology is limited. If implemented, an FFR-DR strategy should not only be effective against the pathogen of concern while maintaining the respirator’s performance specifications, but also must be compatible with HCW operations and logistics to be successful. This study demonstrates significant reductions in viable influenza under substantial soiling conditions after being exposed to ~1-minute UVGI treatment. UVGI-based FFR-DR would allow hospitals to treat FFRs in a quick and efficient manner, benefiting HCWs during a potential influenza pandemic. Follow-up research to better understand the effect of multiple UVGI cycles on N95 respirator durability and performance using the current study’s conditions has also been performed and will be submitted for publication. Additionally, future work evaluating the effectiveness of UVGI on contaminated respirators under conditions that more closely resemble real-world contamination events would be beneficial.

**CONCLUSIONS**

The results of this study indicate FFR-DR can be effective. Building on the Heimbuch et al 2011 study,\(^19\) this study evaluated the decontamination efficiency of an optimized UVGI dose (1 J/cm\(^2\)) delivered to an intact FFR contaminated with both H1N1 influenza and a soiling agent. Significant reductions in influenza viability (\(\geq 3 \text{log}\)) were observed for both soiling conditions (artificial saliva and artificial skin oil) on UVGI-treated facepieces from 12 of 15 FFR models and UVGI-treated straps from 7 of 15 FFR models. Log reductions were considered significant based on the decontamination efficiency required to fully disinfect the highest level of influenza contamination on FFRs predicted by Fisher et al.\(^23\) For FFR-DR, FFR

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**Fig 4.** Viable virus recovered from sebum-soiled N95 respirators. Respirator manufacturers were 3M Company, Minneapolis, MN; Alpha Protech, Markham, Canada; Louis M. Gerson Co, Inc, Middleboro, MA; Kimberly-Clark Corp, Irving, TX; Moldex, Culver City, CA; Precept Medical Products, Inc, Arden, NC; Prestige Ameritech, North Richland Hills, TX; Honeywell Safety Products USA, Smithfield, RI; and Dentech Safety Specialists, Lenexa, KS.
facepieces pose the greatest challenge for disinfection, whereas FFR straps can likely be disinfected through alternative means (eg, disinfecting wipes). Implementation of a UVGI method will likely require careful consideration of FFR material type and design. These data are critically important for regulators and hospitals to understand whether UVGI-based FFR-DR technologies are being considered for deployment in the event of an influenza pandemic. They also provide the basis for future design of new FFR models that are highly amenable to FFR-DR.

Acknowledgments

The authors thank the National Institute of Occupational Safety and Health’s National Personal Protective Technology Laboratory for its involvement in the study design and Chris Church and Bob McDonald of Applied Research Associates for preparing the ultraviolet germicidal irradiation device.

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