

A pandemic influenza preparedness study: Use of energetic methods to decontaminate filtering facepiece respirators contaminated with H1N1 aerosols and droplets

Brian K. Heimbuch, MS,^a William H. Wallace, PhD,^a Kimberly Kinney, BS,^a April E. Lumley,^a Chang-Yu Wu, PhD,^b Myung-Heui Woo, MS,^b and Joseph D. Wander, PhD^c
Panama City and Gainesville, FL

Background: A major concern among health care experts is a projected shortage of N95 filtering facepiece respirators (FFRs) during an influenza pandemic. One option for mitigating an FFR shortage is to decontaminate and reuse the devices. Many parameters, including biocidal efficacy, filtration performance, pressure drop, fit, and residual toxicity, must be evaluated to verify the effectiveness of this strategy. The focus of this research effort was on evaluating the ability of microwave-generated steam, warm moist heat, and ultraviolet germicidal irradiation at 254 nm to decontaminate H1N1 influenza virus.

Methods: Six commercially available FFR models were contaminated with H1N1 influenza virus as aerosols or droplets that are representative of human respiratory secretions. A subset of the FFRs was treated with the aforementioned decontamination technologies, whereas the remaining FFRs were used to evaluate the H1N1 challenge applied to the devices.

Results: All 3 decontamination technologies provided >4-log reduction of viable H1N1 virus. In 93% of our experiments, the virus was reduced to levels below the limit of detection of the method used.

Conclusions: These data are encouraging and may contribute to the evolution of effective strategies for the decontamination and reuse of FFRs.

Key Words: Disinfection; reuse; infection control; microwave; respirator; steam; UVGI; virus.

Copyright © 2011 by the Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved. (*Am J Infect Control* 2011;39:e1-9.)

Pandemic influenza outbreaks historically occur every 40-50 years and have caused millions of deaths worldwide.^{1,2} After the Hong Kong flu pandemic of 1968, experts predicted that another pandemic was imminent. Their fears were realized in the spring of 2009 with the onset of the H1N1 influenza pandemic.^{3,4} On June 11, 2009, the World Health Organization (WHO) raised the pandemic alert level to Phase 6, announcing that a pandemic was underway and declaring the need

for a global response and mitigation. In their August 2010 update, the WHO reported H1N1 infections in more than 214 countries and attributed more than 18,449 deaths to H1N1 infection.⁵ Although this outbreak proved to be less severe than earlier pandemics, it was sufficiently similar to previous pandemics to merit concern. Although it is not certain that the current H1N1 strain will mutate into a more virulent strain, health care workers (HCWs) are taking the possibility very seriously.

A primary respiratory barrier used to protect HCWs from airborne infections is the National Institute for Occupational Safety and Health (NIOSH)-approved filtering facepiece respirator (FFR). Although many types of these devices are available, the present study focuses on N95 FFRs. The N95 FFR is rated to capture $\geq 95\%$ of airborne particles $\sim 0.3 \mu\text{m}$ in diameter and has been demonstrated to effectively remove infectious microorganisms from the air.^{6,7} Particles larger and smaller than $0.3 \mu\text{m}$ are captured at higher efficiencies. The modes of human transmission of influenza are a matter of active debate,^{8,9} but data exist supporting aerosol transmission.⁹ This

From Applied Research Associates, Tyndall Air Force Base, Panama City, FL^a; Environmental Engineering Sciences, University of Florida, Gainesville, FL^b; and Air Force Research Laboratory, Tyndall Air Force Base, Panama City, FL.^c

Address correspondence to Brian K. Heimbuch, MS, 104 Research Road, Bldg 9719, Tyndall AFB, Panama City, FL 32403. E-mail: bheimbuch@ara.com.

Conflict of interest: None to report.

0196-6553/\$36.00

Copyright © 2011 by the Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved.

doi:10.1016/j.ajic.2010.07.004

information led the Occupational Safety and Health Administration (OSHA) and the Centers for Disease Control and Prevention (CDC) to recommend that HCWs wear a properly fitted NIOSH-approved FFR when treating patients with influenza symptoms.^{10,11} The CDC estimates that during a pandemic lasting 42 days, HCWs will require more than 90 million FFRs.¹² These projections indicate a likely shortage of FFRs, leaving HCWs exposed and possibly aggravating the severity of the pandemic. A proposed solution to alleviate this shortage is the decontamination and reuse of FFRs.¹²

FFRs are designated as “single-use” devices and have not been approved for reuse. Consequently, little data are available on the performance of FFRs after decontamination. Many properties need to be evaluated before FFR decontamination and reuse can be recommended, including biocidal efficacy, filtration efficiency, pressure drop, fit, residual toxicity, and overall durability. Previous NIOSH studies have found that some decontamination technologies do not degrade the performance of FFRs, but that others (eg, autoclaving) make FFRs unusable.^{13,14} To expand the database on FFR decontamination, the Air Force Research Laboratory (AFRL) led a study examining the treatment of 6 commonly distributed FFRs with a diverse range of decontaminants. As part of this effort, Salter et al¹⁵ performed chemical offgas analysis of FFRs after treatment with chemical agents or ultraviolet germicidal irradiation (UVGI). The only toxic by-product detected was 2-hydroxyethyl acetate, found on the FFRs’ rubber straps after treatment with ethylene oxide. NIOSH also has performed particle performance and fit tests for the same 6 models using 3 energetic methods: microwave-generated steam (MGS), warm moist heat (WMH), and UVGI, and their data regarding particle penetration were consistent with their earlier findings of no significant effect.^{13,14,16} Fit test data are currently being evaluated, and early findings indicate that fit is not significantly affected (R.E. Shaffer, personal communication, November 16, 2009).

Enveloped viruses, such as H1N1, are less environmentally stable than other microorganisms.¹⁷ Benedictis et al,¹⁸ in a review of the disinfection of avian influenza viruses, noted that many technologies can effectively inactivate viruses. However, we could find no report on the decontamination of enveloped viruses in the presence of an FFR carrier. Carriers can impair the performance of decontamination technologies, and test methods have been developed to account for carrier-induced interference.¹⁹⁻²³ Moreover, many technologies are unsuitable for decontaminating FFRs due to the device’s fragility and operational use. The ideal FFR decontamination technology will preserve performance and fit, leave no residual toxicity, and be fast-acting, inexpensive, and readily available. Applying these criteria to a panel of

Table 1. Decontamination methods used in this study

Method	Intensity/concentration	Treatment time
MGS (with a water reservoir)	1250 W	2 min
UVGI (254 nm)	1.6-2.0 mW/cm ²	15 min
WMH	65°C ± 5°C/85% ± 5% RH	30 min

10 technologies, we identified 3 energetic methods to evaluate as candidate decontaminants against H1N1 on FFRs: WMH, UVGI, and MGS (Table 1). Our objective in the present study was to evaluate the decontamination of NIOSH-certified FFRs contaminated with H1N1 aerosols or droplets using these 3 energetic methods.

The biocidal activity of microwave energy has been well documented; however, moisture is a key factor, given that microwaves are considered by some to be non-biocidal.^{24,25} Accordingly, the FFR was positioned above an improvised water reservoir during decontamination (Fig 1A). Steam produced from microwave heating of the water is the primary means of biocidal activity. Warm temperatures are not commonly used for decontamination; most applications call for high-temperature methods. However, temperatures >100°C have been shown to destroy the performance of FFRs^{13,14} and cannot be used. Because viruses are relatively fragile microorganisms, lower-temperature applications are typically effective. Avian influenza virus was shown to be completely inactivated after a 5-minute treatment at 62°C,²⁶ but dried sample preparations displayed resistance.²⁷ To maximize the likelihood of success, a sealed chamber containing water (Fig 1B) was used to produce high humidity, based on the knowledge that moist heat is more biocidal than dry heat. UVGI has been shown to inactivate influenza viruses²⁸⁻³¹ and is endorsed by the CDC as an acceptable method for destroying microorganisms on surfaces.³² Figure 1C illustrates the treatment of FFRs using UVGI.

The process used to deposit viruses on surfaces may influence the effectiveness of the decontaminant.³³ Solution-based studies are easy to perform, but they do not mimic the airborne contamination of FFRs and are impractical for FFRs with a hydrophobic outer layer. For these reasons, we developed two aerosol-based test methods to apply H1N1 influenza virus to FFRs. The two methods mimic human respiratory secretions (aerosol and droplet), and both were approved as standards by the American Society for Testing and Materials International.^{22,23} Because these methods will be discussed in detail in a future report, we provide only brief descriptions here. The key parameters of each deposition method are droplet/particle size and composition, which profoundly influence the extent to which external factors (eg, proteins, salts, lipids) act to shield the H1N1 virus from the decontaminant and provide

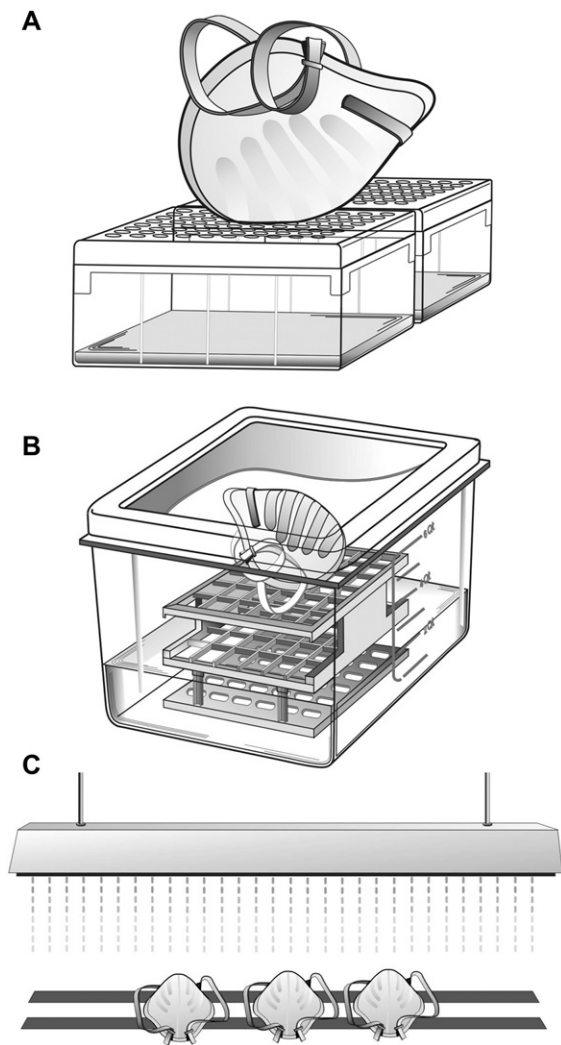


Fig 1. Devices for decontaminating FFRs. (A) MGS device for decontamination of individual FFRs. (B) Chamber for applying WMH to FFRs. (C) Decontamination of FFRs using UVGI.

conditions that allow the virus to survive in the environment.³⁴ The aerosolization medium is a mucin-based solution that simulates human saliva.³⁵ Mucin, a common component of saliva, is known to provide environmental protection to viruses.³⁶ The count median diameter (CMD) particle size was 0.8 μm for the aerosol method and 15 μm for the droplet method. The smaller particle size was verified using an Aerodynamic Particle Sizer spectrometer (TSI, Shoreview, MN), and droplet size was verified with a Spraytec particle sizer (Malvern Instruments, Westborough, MA).

MATERIALS AND METHODS

Preparation of H1N1 virus

Influenza A/PR/8/34 VR-1469 (ATCC VR-95H1N1) was propagated in embryonic chicken eggs following

standard protocols.³⁷ Virus titers were determined using a tissue culture infectious dose assay (TCID₅₀) in Madin–Darby canine kidney cells (MDCK; ATCC CCL-34) with WHO-approved cell culture techniques.³⁷

Aerosol application of H1N1 to FFRs

The laboratory-scale aerosol tunnel (LSAT; Fig 2) was used to apply H1N1 aerosols to the 6 FFR models (3 particulate, designated P1-P3, and 3 surgical, designated S1-S3). The LSAT was designed to determine the viable filtration efficiency of filtration media or energetic devices,³⁸ but it is also capable of applying viruses to FFRs. For each independent experiment, 6 replicates of a single FFR model were glue-sealed into 6 separate 15-cm-diameter sample holders. A single FFR was loaded into the LSAT and sealed using compression seal clamps. H1N1 virus was diluted in 30 mL of mucin buffer [0.04 g of MgCl₂ · 7 H₂O, 0.13 g of CaCl₂ · H₂O, 0.42 g of NaHCO₃, 7.70 mL of 0.2 M KH₂PO₄, 12.3 mL of 0.2 M K₂HPO₄, 0.11 g of NH₄Cl, 0.19 g of KSCN, 0.12 g of (NH₂)₂CO, 0.88 g of NaCl, 1.04 g of KCl, and 3.00 g of mucin (M1778; Sigma-Aldrich, St Louis, MO) in 1 L of de-ionized water (pH 7)] to a concentration of ~8 log₁₀ TCID₅₀/mL. The virus solution was added to a 6-jet Collision nebulizer (BGI, Waltham, MA) and attached to the LSAT using compression fittings. The LSAT was configured to direct the aerosol to the overflow. Compressed air (30 psi) was applied to the nebulizer, and the system was operated for 10 minutes to bring the nebulizer to steady state. The LSAT overflow valves were readjusted to direct the aerosol to the FFR for 10 minutes. After exposure, the LSAT overflow valves were reconfigured to divert the aerosol back to overflow. The exposed FFR was removed from the LSAT and replaced with a new FFR. The foregoing steps were repeated to expose 5 additional FFRs. The average flow rate in the LSAT was 18-20 L/min. The average RH and temperature conditions for all tests were 75% ± 5% and 22°C ± 2°C.

Droplet application of H1N1 to FFRs

The droplet loader (Fig 3) was used to simultaneously load 6 samples of a given FFR model. The design of the droplet loader is based on a device capable of loading large droplet nuclei onto surfaces.³⁹ Six FFRs, each 5 cm from the edge and spaced equally relative to the others, were arranged on the rotating table of the droplet loader. The door to the droplet loader was sealed, and the rotating table was adjusted to a speed of 3 rpm. H1N1 influenza was prepared as described above and loaded into a reservoir that contained a siphon tube. The tube was connected to the air-atomizing nozzle (model SA 2000; Paasche, Chicago, IL), and compressed air (3 psi) was delivered to siphon the virus into the nozzle. Liquid flow to the nozzle

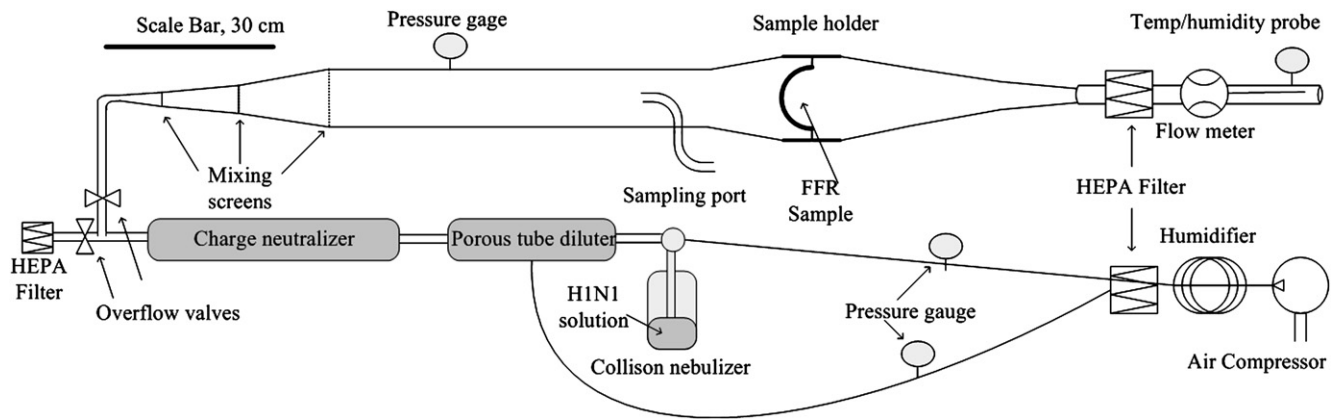


Fig 2. The LSAT device used to apply aerosols to the FFRs. The LSAT is fabricated with 10-cm-diameter stainless steel sanitary fittings and a 15-cm filter holder to accommodate the FFR. The biological aerosol is generated by a 6-jet Collision nebulizer. Dilution air, conditioned by passing the air through a humidifier, is added through the porous tube diluter, and charges created on particles are neutralized by passage through a Kr-85 sealed-source charge neutralizer. The biological aerosol travels through the overflow valves and expands in the test duct before reaching the FFR.

was adjusted to deliver 2-3 mL/min of virus. The FFRs were loaded with virus as the table revolved under the droplet stream delivered by the air-atomizing nozzle. After loading was complete, the compressed air was disconnected, and the chamber was evacuated (1.5 ft³/min) for 15 minutes to remove suspended aerosols.

Decontamination

Decontamination studies were performed on 3 of the H1N1-contaminated FFRs, with the other 3 FFRs serving as positive controls. Alternately loaded FFRs were used for decontamination studies, to reduce possible effects due to uneven loading. To minimize the loss of H1N1 viability due to normal environmental decay, decontamination studies were performed immediately after the loading of each FFR. The control FFRs were incubated at room temperature for the same duration as the FFRs treated by the decontamination technologies.

For MGS (Fig 1A), two plastic reservoirs (4.5 cm h × 12 cm w × 8 cm l) with perforated tops (192 holes of 6 mm diameter, spaced uniformly over the entire surface) were filled with 50 mL of tap water at 22°C-25°C. The reservoirs were placed together, and the H1N1-contaminated FFR was set atop the center of the assembly, with the exterior of the FFR resting on the surface of the reservoir. The reservoir assembly and FFR were loaded into the center of a 1250-watt microwave oven and irradiated at full power for 2 minutes. After treatment, the reservoir was replenished with fresh tap water (22°C-25°C), and the next FFR was processed.

For WMH (Fig 1B), a 6-L sealable container (17 cm h × 19 cm w × 19 cm l) was filled with 1 L of tap water. A plastic support rack was placed in the water to isolate the FFR from the liquid. Before the test, the container

was warmed in an oven to 65°C ± 5°C for a minimum of 3 hours. The container was removed from the oven, and an H1N1-contaminated FFR was placed on the rack. The containers were sealed and returned to the oven for 30 minutes.

For UVGI (Fig 1C), a 120-cm, 80-W UV-C (254 nm) lamp (Ultraviolet Products, Upland, CA) was adjusted to a height of 25 cm. Output from the lamp was measured using a radiometer (Ultraviolet Products). The range of UV irradiation to which the FFR was exposed varied from 1.6 mW/cm² to 2.2 mW/cm². The exterior surface of H1N1-contaminated FFRs was irradiated for 15 minutes, which provided an average dose of 18 kJ/m². The exposure varied over each FFR due to the curved shape of the device.

Virus extraction and enumeration

Four circular coupons, 38 mm in diameter, were cut from each FFR using a sterile metal punch. The coupons were placed in a 50-mL conical tube containing sfEMEM-p/s-g medium, comprised of 15 mL of serum-free Eagle's minimum essential medium (Hyclone Laboratories, Logan, UT) supplemented with 1% pen/strep (Sigma Aldrich, St. Louis, MO) and 1% L-glutamine (Lonza BioWhittaker, Walkersville, MD). The samples were mixed for 20 minutes at maximum speed using a multitube vortex mixer (VWR Scientific, West Chester, PA). Viable H1N1 in the extracts were quantified using a TCID₅₀ assay in MDCK cells as described above. To maximize sensitivity of the assay the entire extract for each decontaminated sample was analyzed. The extract for the control FFRs was serially diluted (1/10) in the sf-EMEM-p/s-g medium, and all dilutions were delivered in quadruplicate into the 24-well plates. The plates were incubated for 4 days

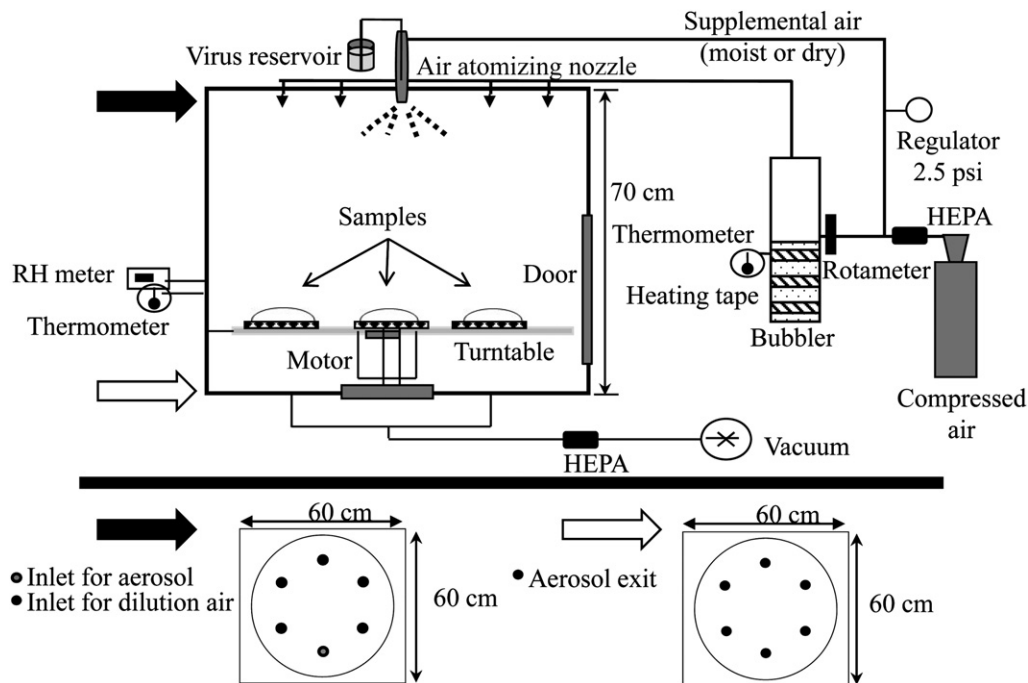


Fig 3. The droplet loader device used to apply droplets to the FFRs. The device is composed of a stainless steel shell (60 cm l × 60 cm w × 90 cm h). Droplets are created by applying compressed air to an air-atomizing nozzle that produces a droplet at the source with a CMD of ~40 μm. Uniform dispersion of the droplets onto the test specimens is achieved by rotating the samples on the turntable at 3 rpm.

at 5% CO₂/37°C before cytopathic effects were analyzed.

Data analysis

The Spearman-Kärber formula⁴⁰ was used to determine the concentration of viable virus per mL of extract (L , expressed in units of log₁₀TCID₅₀/mL). The following equation was used to determine the total amount of virus recovered from each sample (45.6 cm²):

$$\text{virus concentration/sample} = L_s = L + \log_{10}(V),$$

where V is sample volume. Log reductions were calculated by subtracting the average L_s for the decontaminated FFRs from the average L_s for the control FFRs. For decontaminated samples that yielded no detectable viable virus, we assumed the average number of live virus in the samples followed a Poisson distribution and calculated the upper 95% confidence interval.⁴¹ Because the entire extract of the treated sample was assayed, the minimum detection limit (MDL) was 1 TCID₅₀ infectious dose unit. The upper 95% CI, assuming a mean of <1 live viruses in each sample, was 3.47 (log₁₀ = 0.55) TCID₅₀ infectious dose units; this value was used as the MDL. Based on a US Environmental Protection Agency guideline,⁴² half of the MDL was used to calculate log reductions for treated

samples that had no detectable virus. The 95% CIs of the log reductions were calculated using standard equations.⁴¹

RESULTS

The average concentration of H1N1 virus recovered from the untreated FFRs for each test ranged from 4.1 to 6.1 log₁₀ TCID₅₀ per sample (Table 1). The variability is a result of day-to-day deviation in testing and does not reflect the overall consistency of the method. The average SD for the triplicate untreated samples for all 36 tests was 0.27 log₁₀TCID₅₀, similar to that reported by others.⁴³ All 3 energetic methods provided an average >4-log reduction of viable H1N1 influenza virus against both the droplet and aerosol challenges for all 6 FFRs, with the exception of the WMH treatment on the P1 FFR (Table 3). Use of a less conservative approach for calculating log reductions would have yielded higher values. In all but 8 FFRs (7.4%), the virus was reduced to levels below the detection limit. Data are not shown for individual FFRs; Tables 2 and 3 provide average values for 3 FFRs per test.

Gross physical observation of the FFRs after the WMH and UV treatments revealed no obvious signs of deterioration or deformation. MGS treatment of FFR S2 caused a slight separation of the foam nose cushion, which was also reported by Viscusi et al.¹⁴ No other

Table 2. Recovery of viable H1N1 virus from untreated and decontaminated FFRs (log₁₀ TCID₅₀ per sample)

Respirator*	UVGI	Untreated	MGS	Untreated	WMH	Untreated
Droplet application of H1N1						
S1	BDL	4.35 ± 0.29	0.39 ± 0.68	6.33 ± 0.13	BDL	5.77 ± 0.14
S2	BDL	>5.68	0.31 ± 0.53	>5.68	BDL	6.85 ± 0.14
S3	BDL	6.01 ± 0.29	BDL	5.51 ± 0.38	BDL	5.18 ± 0.25
P1	0.55 ± 0.48	5.35 ± 0.29	BDL	5.01 ± 0.38	BDL	4.10 ± 0.14
P2	1.37 ± 0.05	5.85 ± 0.29	BDL	6.10 ± 0.38	BDL	6.10 ± 0.38
P3	BDL	5.26 ± 0.14	0.26 ± 0.44	5.93 ± 0.25	BDL	5.18 ± 0.25
Aerosol application of H1N1						
S1	BDL	5.35 ± 0.14	BDL	4.51 ± 0.29	BDL	5.35 ± 0.14
S2	BDL	4.60 ± 0.76	BDL	4.68 ± 0.00	BDL	4.93 ± 0.25
S3	BDL	4.56 ± 0.18	0.62 ± 0.56	5.43 ± 0.25	BDL	4.93 ± 0.50
P1	BDL	4.93 ± 0.25	BDL	5.10 ± 0.14	BDL	4.85 ± 0.14
P2	BDL	5.26 ± 0.38	BDL	5.51 ± 0.29	BDL	4.76 ± 0.14
P3	BDL	5.10 ± 0.52	BDL	5.35 ± 0.38	BDL	5.60 ± 0.14

BDL, below detection limit (1 TCID₅₀ infectious dose unit).

*S, NIOSH- and FDA-approved N95 surgical FFR; P, NIOSH-approved N95 particulate FFR.

Table 3. Effectiveness of the decontamination methods in inactivating viable H1N1 virus on FFRs (log reduction)

Respirator*	UVGI		MGS		WMH	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Droplet application of H1N1						
S1	4.08	3.36-4.80	5.94	5.61-6.27	5.50	5.15-5.85
S2	5.41	5.41-5.41	5.37	5.37-5.37	6.58	6.22-6.94
S3	5.75	5.03-6.46	5.25	4.30-6.20	4.91	4.29-5.54
P1	4.79	4.08-5.51	4.23	3.29-5.18	3.32	2.96-3.68
P2	4.48	3.76-5.19	4.67	3.72-5.62	4.67	3.72-5.62
P3	5.00	4.64-5.36	5.67	5.05-6.29	4.91	4.29-5.54
Aerosol application of H1N1						
S1	5.08	4.72-5.44	4.25	3.53-4.96	5.08	4.72-5.44
S2	4.33	2.43-6.22	5.41	5.41-5.41	4.66	4.04-5.29
S3	4.29	2.70-5.88	4.81	4.19-5.43	4.66	3.42-5.91
P1	4.66	4.04-5.28	4.83	4.47-5.19	4.58	4.22-4.94
P2	5.00	4.05-5.95	5.25	4.53-5.96	4.50	4.14-4.86
P3	4.83	3.54-6.12	5.08	4.13-6.03	5.33	4.97-5.69

*S, NIOSH- and FDA-approved N95 surgical FFR; P, NIOSH-approved N95 particulate FFR.

FFRs showed noticeable deterioration or deformation, and no arcing in the microwave was observed during treatment.

DISCUSSION

A unique feature of the present study is the controlled contamination of FFRs with H1N1 influenza using aerosol methods, which provide a radically different challenge from solution-based tests, which require dilution of the virus in a large volume of water. As droplets form during aerosolization, they begin to dry and form droplet nuclei. As evaporation proceeds, viruses are coated with protective components from the aerosolization medium; these components can protect the virus from some decontamination technologies. In the droplet challenge, the droplets do not dry completely, but land on surfaces as small droplets

that dry eventually. Solution-based assays are performed by simply dosing a substrate with a given volume of suspended virus. These tests are easier to perform on hydrophilic surfaces, and we have not attempted to demonstrate that decontamination results will vary between liquid and aerosol deposition methods. However, given the scrutiny surrounding the overall goal of the present study, we considered aerosol and droplet contamination methods to be necessary.

No detectable viruses survived the WMH treatment in the droplet nuclei and droplet tests (Table 2). In contrast, sporadic viable viruses were detected after the UVGI and MGS treatments (Table 2). The reason for this discrepancy likely can be traced to the technologies' modes of action. The WMH technology provides a stable environment that is homogeneously distributed to the entire surface of the FFR. The MGS method

delivers steam to the FFRs from beneath, likely providing a nonuniform distribution. Moreover, the distribution of microwave energy in the oven was not mapped. Zhang et al⁴⁴ reported inconsistent disinfection of microwave-treated surfaces. Optimization and rotation of the water reservoir holder likely will minimize or eliminate this concern. Increasing steam production also might be helpful. In the present study, 20% of the water was transformed into steam. Increasing the treatment time or decreasing the amount of water in the reservoirs might increase steam production.

The UVGI treatment effectively inactivated the H1N1 virus applied to FFRs as either droplets or aerosol particles (Tables 2 and 3). Vo et al⁵¹ reported similar results using MS2 coliphage, finding inactivation of this coliphage on internal FFR layers. For the aerosol challenge, the average log reduction was 4.69, and the virus was reduced to values below the detection limit for all 6 FFR models. The average log reduction for the droplet challenge was 4.92. The larger measured log reduction is an artifact of the higher loading concentration. The two instances in which viable virus was recovered can possibly be attributed to shielding, but the method tested was not optimized, and the small viable populations found should not disqualify UVGI as an effective method for decontaminating FFRs.

Two of the 3 decontamination methods tested left trace amounts of virus on the FFRs. Optimization of treatment likely would decrease these levels, but even the possibility of trace virus may pose a risk to the wearer. For evaluation in a given situation, this risk must be factored into the operations in which the decontamination and reuse of FFRs will be implemented. The use of these methods should be considered only in the dire circumstance when no other respiratory protective device is available; that is, either wear a decontaminated FFR or wear no FFR. Another factor to consider when assessing risk is that the actual amount of agent contaminating an FFR in a pandemic setting generally will be much less than applied in these tests. We performed these decontamination tests at extreme challenge levels to ensure that we could measure the target 4-log reduction.

All 3 energetic decontamination methods evaluated in this study provide practical solutions that can be implemented in many settings. WMH is the most time-intensive method and may be useful only for home use or use by small organizations. MGS is the least time-intensive method and requires only a simple FFR holder/water reservoir. The simplicity of the technique and the ready availability of microwave ovens favor this technology for use in the home and by small organizations. The dimensions of the reservoir matter; greater volumes of water take more time to produce steam. End users also must be cognizant of the power

delivered by the microwave oven. Although UVGI is the least invasive of the 3 methods and is readily scalable to meet the needs of larger organizations, it relies on a hazardous light source, which might be prohibited for home use. However, the cost of the device could be easily absorbed by most organizations even if multiple UVGI sources are needed to meet their demand. Many types of UVGI systems are currently used in hospitals for air purification, biological safety cabinets, and surface sterilization. Adapting such systems for decontamination and reuse of FFRs could be a low-cost option for hospitals, and organizations purchasing UVGI systems for other applications might want to select designs that can be used for decontamination of FFRs as well.

All 3 decontamination technologies effectively decontaminated the H1N1 virus deposited on FFRs as either aerosols or droplets. The aerosols and droplets were designed to mimic human respiratory secretions, but it is important to note that significant data gaps exist in terms of the characteristics of droplet/particle size and composition of fluids excreted by symptomatic individuals. An increase in mucus concentration and the addition of other components due to secondary infections might increase shielding and reduce the effectiveness of some decontaminants. More data are needed on respiratory secretions produced during various states of infection. Other modes of FFR contamination, including direct contact and contamination with infectious bodily fluids, merit study as well.

Notwithstanding the findings of this H1N1 decontamination study, other factors must be considered before FFR decontamination and reuse can be recommended. Salter et al¹⁵ reported that chemical offgassing is not a concern for the 3 energetic methods that we studied, and other studies have found that none of the 3 methods significantly affects the particle filtration efficiency of the 6 FFR models that we used in this study.^{13,14,16} Fit factor is another concern. All 3 decontamination methods provided acceptable fit factors after decontamination of all 6 FFR models (R.E. Shaffer, personal communication, November 16, 2009).

The principal limitation of this study is that we evaluated only 6 out of the hundreds of FFR models available. We acknowledge this limitation and recommend evaluating additional FFRs. In addition, although this study has produced a large body of replicated data, regulatory bodies typically require many more replicate measurements to build confidence in the methods. Nonetheless, we are optimistic that our evaluation of these energetic methods may help lead to solutions to mitigate a shortage of FFRs caused by pandemic influenza.

This research was funded by Technical Support Working Group Grant CM-CM-2868. The authors thank their colleagues from NIOSH (Dr. Ron Shaffer, Dr. Kimberly Faulkner, Ed Fisher, Dennis Viscusi, and Michael Bergman) and from FDA (Dr. Marilyn Lightfoote, Dr. Michelle Chenault, Nancy Pluhowski, Dr. Vicki Hitchins, and Dr. Reza Sadaie) for their critical reviews of the manuscript.

References

- Kilbourne ED. Influenza pandemics of the 20th century. *Emerg Infect Dis* 2006;12:9-14.
- Oxford JS. Influenza A pandemics of the 20th century with special reference to 1918: virology, pathology and epidemiology. *Rev Med Virol* 2000;10:119-33.
- Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;360:2605-15.
- Naffakh N, van der Werf S. An outbreak of swine-origin influenza A (H1N1) virus with evidence for human-to-human transmission. *Microb Infect* 2009;11:725-8.
- World Health Organization. Influenza A (H1N1) update 112, August 2010. Available from: http://www.who.int/csr/don/2010_08_06/en/index.html. Accessed November 2, 2010.
- Balazy A, Toivola M, Adhikari A, Sivasubramani SK, Reponen T, Grinshpun SA. Do N95 respirators provide 95% protection level against airborne viruses, and how adequate are surgical masks? *Am J Infect Control* 2006;34:51-7.
- Heimbuch BK, Wander JD. Bioaerosol challenges to antimicrobial surface treatments: enhanced efficacy against MS2 coli phage of air filter media coated with polystyrene-4-methyltrimethylammonium triiodide. AFRL-ML-TY-TP-2006-4527. Tyndall AFB (FL): Air Force Research Laboratory; 2006.
- Bridges CB, Kuehnert MJ, Hall CB. Transmission of influenza: implications for control in healthcare settings. *Clin Infect Dis* 2003;37:1094-101.
- Tellier R. Review of aerosol transmission of influenza A virus. *Emerg Infect Dis* 2006;12:1657-62.
- Centers for Disease Control and Prevention. Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings. Available from: http://www.cdc.gov/ncidod/dhqp/gl_isolation.html. Accessed October 20, 2008.
- Occupational Safety and Health Administration. Pandemic influenza preparedness and response guidance for healthcare workers and healthcare employers. US Department of Labor Publication OSHA 3328-05:2007. Available from: <http://www.osha.gov/Publications/3328-05-2007-English.html>. Accessed November 2, 2010.
- Bailar JC, Burke DS, Brosseau LM, Cohen HJ, Gallagher EJ, Gensheimer KF, et al. Reusability of facemasks during an influenza pandemic. Washington, DC: Institute of Medicine, National Academies Press; 2006.
- Viscusi DJ, King WP, Shaffer RE. Effect of decontamination on the filtration efficiency of two filtering facepiece respirator models. *J Int Soc Resp Prot* 2007;24:93-107.
- Viscusi DJ, Bergman MS, Eimer BC, Shaffer RE. Evaluation of five decontamination methods for filtering facepiece respirators. *Ann Occup Hyg* 2009;53:815-27.
- Salter WB, Kinney K, Wallace WH, Lumley AE, Heimbuch BK, Wander JD. Analysis of residual chemicals on filtering facepiece respirators after decontamination. *J Environ Occup Health* 2010;7:437-45.
- Bergman MS, Viscusi DJ, Heimbuch BK, Wander JD, Sambol AR, Shaffer RE. Evaluation of multiple (3-cycle) decontamination processing for filtering facepiece respirators. *J Engineered Fibers Fabrics* 2010;5:33-41.
- Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 2006;6:130-7.
- Benedictis PD, Beato MS, Capua I. Inactivation of avian influenza viruses by chemical agents and physical conditions: a review. *Zoonoses Public Health* 2007;54:51-68.
- American Society for Testing and Materials International. ASTM E 2414-05: standard test method for quantitative sporicidal three-step method to determine sporicidal efficacy of liquids, liquid sprays, and vapor or gasses on contaminated carrier surfaces. Vol. E 2414-05. West Conshohocken (PA): American Society for Testing and Materials International; 2005.
- Fisher E, Rengasamy S, Viscusi DJ, Vo E, Shaffer R. Development of a test system to apply virus-containing particles to filtering facepiece respirators for the evaluation of decontamination procedures. *Appl Environ Microbiol* 2009;75:1500-7.
- Heimbuch BK, Kinney K, Nichols R, Wander JD. The dry aerosol deposition device (DADD): an instrument for depositing microbial aerosols onto surfaces. *J Microbiol Methods* 2009;78:255-9.
- American Society for Testing and Materials International. ASTM E2720-2010: standard test method for effectiveness of decontamination of air-permeable materials challenged with biological aerosols containing human pathogenic viruses. West Conshohocken (PA): American Society for Testing and Materials International; 2010.
- American Society for Testing and Materials (ASTM) International. ASTM E2721-2010: standard test method of evaluating of the effectiveness of decontamination procedures for surfaces when challenged with droplets containing human pathogenic viruses. West Conshohocken (PA): American Society for Testing and Materials International; 2010.
- Velva GR, Wu JF. Mechanism of lethal action of 2450-MHz radiation on microorganisms. *Appl Environ Microbiol* 1979;37:550-3.
- Jeng DK, Kaczmarek KA, Woodworth AG, Balasky G. Mechanism of microwave sterilization in the dry state. *Appl Environ Microbiol* 1987;53:2133-7.
- King DJ. Evaluation of different methods of inactivation of Newcastle disease virus and avian influenza virus in egg fluids and serum. *Avian Dis* 1991;35:505-14.
- Swayne DE, Beck JR. Heat inactivation of avian influenza and Newcastle disease viruses in egg products. *Avian Pathol* 2004;33:512-8.
- Deshmukh DR, Pomeroy BS. Ultraviolet inactivation and photoreactivation of avian viruses. *Avian Dis* 1969;13:596-602.
- Jakab GJ, Knight ME. Decreased influenza virus pathogenesis by infection with germicidal UV-irradiated airborne virus. *Environ Int* 1982;8:415-8.
- Jensen M. Inactivation of airborne viruses by ultraviolet irradiation. *Appl Microbiol* 1964;12:418-20.
- Vo E, Rengasamy S, Shaffer R. Development of a test system to evaluate decontamination procedures for viral droplets on respirators. *Appl Environ Microbiol* 2009;75:7303-9.
- Rutala WA, Weber DJ. Healthcare Infection Control Practices Advisory Committee. Guideline for disinfection and sterilization in healthcare facilities. Available from: www.cdc.gov/ncidod/dhqp/pdf/guidelines/Disinfection_Nov_2008.pdf. Accessed November 2, 2010.
- Lai KM, Burge HA, First MW. Size and UV germicidal irradiation susceptibility of *Serratia marcescens* when aerosolized from different suspending media. *Appl Environ Microbiol* 2004;70:2021-7.
- Boone SA, Gerba CP. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl Environ Microbiol* 2007;73:1687-96.
- Aps JKM, Martens LC. Review: the physiology of saliva and transfer of drugs into saliva. *Forensic Sci Int* 2005;150:119-31.
- Bean B, Moore BM, Sterner B. Survival of influenza viruses on environmental surfaces. *J Infect Dis* 1982;146:47-51.
- World Health Organization. Manual on animal influenza diagnosis and surveillance. Available from: http://www.who.int/vaccine_research/diseases/influenza/WHO_manual_on_animal-diagnosis_and_surveillance_2002_5.pdf. Accessed November 2, 2010.
- Foarde K, Heimbuch BK, Maxwell A, Van Osdell D. Method for evaluating air purification technologies for collective protection using

- viable microbial aerosols. Test Operating Procedure under the Army Test and Evaluation Command. Edgewood (MD): Edgewood Chemical and Biological Center; 2010.
39. Woo MH, Hsu Y-M, Wu CY, Heimbuch BK, Wander J. Method for contamination of filtering facepiece respirators by deposition of MS2 viral aerosols. *J Aerosol Sci* 2010;41:944-52.
 40. Finney DJ. *Statistical methods in biological assays*. 2nd ed. New York: Hafner; 1964.
 41. Motulsky H. *Intuitive biostatistics*. New York: Oxford University Press; 1995.
 42. Singh A, Nocerino J. Robust estimation of mean and variance using environmental data sets with below detection limit observations. *Chemometr Intell Lab Syst* 2002;60:69-86.
 43. Mo C, Yamagata R, Pan A, Reddy J, Hazari N, Duke G. Development of a high-throughput Alamar blue assay for the determination of influenza virus infectious dose, serum antiviral neutralization titer and virus ca/ts phenotype. *J Virol Methods* 2008;150:63-9.
 44. Zhang Q, Damit B, Welch J, Park H, Wu CY, Sigmund W. Microwave-assisted nanofibrous air filtration for sterilization of bioaerosols. *J Aerosol Sci* 2010;41:880-8.