Coronavirus transmission and the use of masks

This article examines: 1) how long the coronavirus remains infectious on various surfaces; 2) what kind of mask should be used to stop this virus; and 3) whether or not a single-use mask can be reused.

What kind of mask should be used and whether or not a single-use mask can be reused are important questions. Using the best mask available helps protect the wearer, and because there are never enough masks during a major epidemic, masks are going to be reused. The wearer therefore has to know how long the virus will survive on the mask and how the mask can be sterilized so that it is safe to use more than once.

This article therefore examines what international scientists have written about these topics in 16 peer-reviewed papers published in major scientific journals before the current coronavirus outbreak occurred. In the analysis section below, each statement about this virus includes a reference to one of these 16 papers, and the complete texts of all papers are found at the accompanying hyperlinks. Each article also contains an extensive bibliography: Hundreds of the best scientific articles written on this topic have therefore been identified for readers who wish to examine the full complexity of these questions.

Disclaimer: 1) This is not medical advice: It is a description of scientific literature from reputable international sources, but it is not guaranteed to be accurate. If you have a medical question, ask your doctor. 2) As novel coronavirus has just been isolated, the scientific papers cited in this article refer to other viruses that are thought to be similar, like SARS, MERS, and two avian respiratory viruses. It is not yet known how similar the novel coronavirus is to these other respiratory viruses.

Questions:

1. How long does the coronavirus remain infectious on various surfaces?
2. Can a mask stop coronavirus?
3. Can a single-use mask be reused?

Short answers:

1. The coronavirus remains infectious for different periods of time on different surfaces.
    a) Hard surfaces like stainless steel may remain infectious for up to 6 days, but typically become less infectious after 24-48 hours.
    b) Porous surfaces like clothing and masks typically remain infectious for 8-12 hours.

2. Can a mask prevent the inhalation of coronavirus?

    a) Surgical masks are designed to prevent bacteria and other particles from contaminating a sterile field, as when a surgeon is performing an operation. Surgical masks are not designed to prevent the wearer from inhaling viruses: 20-85% of viruses 0.1 microns in diameter can penetrate these masks. They are therefore not the best way to prevent the inhalation of viruses. However, if the wearer already has already contracted a virus, a surgical mask can help prevent the transmission of the virus to others.

    b) N95 respirators are designed to reduce the wearer's exposure to airborne particles like viruses. When worn properly, only 5% of particles 0.1 microns in size can penetrate these masks. The Centers for Disease Control recommend the use of N95 respirators for SARS and flu pandemics.

    c) P100 respirators are also designed to reduce the wearer's exposure to airborne particles like viruses. They are also resistant to oil, and they tend to be more durable than N95 respirators, so P100 respirators typically last longer. P100 respirators also do a better job of stopping airborne viruses. When worn properly, only 0.1% of particles 0.1 microns in size can penetrate these masks. P100 respirators are the best, and they are much more expensive than N95 respirators.

3. Can a single-use mask be reused?

    a) Yes, single use N95 and P100 respirators can be reused if they have not been damaged, have not been heavily contaminated, and have been properly sterilized. Manufacturers sometimes make claims regarding how long a respirator will last: An N95 mask might be expected to last for 8 hours, while a P100 mask might be expected to last for 40 hours. The Centers for Disease Control say that disposable respirators might "remain functional for weeks to months" if proper precautions have been taken.

    b) As noted in 1(b) above, coronavirus can remain infectious on soft surfaces like masks for many hours, so the wearer must be very careful when removing the mask: Touching an infected portion of the mask could transmit the virus. The wearer must also sterilize both sides of the mask before reuse. UV light can sterilize a mask without damaging it; other sterilization methods are effective as well.

Analysis:

1. Viruses remain infectious for different periods of time on different surfaces.

    a) On common surface materials, human coronavirus 229E remains infectious for several days. It survives for at least 5 days on Teflon, PVC, ceramic tiles,

glass, and stainless steel; it survives for at least 3 days on silicon rubber. (Warnes, “Human Coronavirus 229E Remains Infectious,” 2.)

b) On N95 respirators, the influenza virus H1N1 remains infectious for 6 days, although other studies concluded that this virus became less infectious after 24-48 hours. (Coulliette, “Persistence of the 2009 Pandemic Influenza,” 5.)

c) On nonporous surfaces like steel, latex, ceramic tiles, and plastic, two avian respiratory viruses survived for 24-48 hours. (Tiwari, “Survival of Two Avian Respiratory Viruses,” 286.)

d) On porous surfaces like cotton and polyester fabrics, wood, paper, and tissue, two avian respiratory viruses survived for 8-12 hours. The viruses survived on some surfaces like bird feathers for up to 6 days, but they did not survive for 9 days. (Tiwari, “Survival of Two Avian Respiratory Viruses,” 286.)

e) After drying, human coronaviruses survive for only a few hours. (Chan, “The Effects of Temperature,” 1.)

f) At a higher temperature and humidity (38°C and relative humidity above 95%) SARS virus viability rapidly decreased. The virus is killed by heat at 56°C for 15 minutes. (Chan, “The Effects of Temperature,” 1, 3.)

g) In an air-conditioned environment, the SARS virus can survive at least two weeks. During the SARS epidemic, in Singapore and Hong Kong the transmission of SARS largely occurred in well air-conditioned environments like hospitals and hotels. But during the SARS outbreak in Guangzhou Province, the windows in patient rooms were kept open and the rooms were well ventilated; this is thought to have reduced virus survival. (Chan, “The Effects of Temperature,” 1, 3.)

2. Can a mask prevent the inhalation of coronavirus?

a) Surgical masks are primarily designed to protect the environment from the wearer. Surgical masks are not designed to prevent the wearer from inhaling viruses: 20-85% of viruses from 0.1 to 0.8 microns in diameter can penetrate these masks. (Bałazy, “Do N95 Respirators Provide 95% Protection,” 51.)

b) Surgical masks are intended to prevent bacteria and other particles exhaled by the wearer from contaminating a sterile field like a patient’s wound. Because there are no requirements for small particle filtration efficiency or fit, surgical masks should not be expected to provide respiratory protection. The infection rate of those who wore surgical masks was about twice as high as for those who wore N95 masks. Surgical masks should not be used to protect people from viruses like H1N1. (Janssen, “The Use of Respirators,” 1, 4, 5.)

c) Surgical masks are not recommended to protect the wearer from the inhalation of airborne viruses. (Harnish et al., "Challenge of N95 Filtering Facepiece Respirators," 2.)

d) The bare influenza virus is approximately 0.1 micron, but the influenza virus expelled from humans via respiratory secretions is typically much larger at 0.8 micron. (Harnish et al., "Challenge of N95 Filtering Facepiece Respirators," 5.)

e) N95 respirators, when worn properly, effectively filter over 95% of particles between 0.1 and 0.3 microns. (Coulliette, "Persistence of the 2009 Pandemic Influenza," 1.)

f) N95 respirators are designed to reduce the wearer's exposure to airborne particles like viruses. N95 respirators should be used to protect people from viruses like H1N1. (Janssen, "The Use of Respirators," 1, 4, 5.)

g) N95 respirators were penetrated by approximately 5% of 0.1 micron particles at a constant air flow rate of 85 liters per minute. (Eshbaugh, "N95 and P100 Respirator Filter Efficiency," 58, figure 2.)

h) N95 respirators that fit properly reduce toxic inhalation exposure by a factor of 10 or more. (Harnish et al., "Challenge of N95 Filtering Facepiece Respirators," 7.)

i) P100 respirators are also designed to reduce the wearer's exposure to airborne particles like viruses. P100 respirators also do a better job of stopping airborne viruses. When worn properly, fewer than 0.1% of 0.1 micron particles can penetrate these masks at a constant air flow rate of 85 liters per minute. (Eshbaugh, "N95 and P100 Respirator Filter Efficiency," 58, figure 2.)

3. Can a single-use mask be reused?

a) Disposable respirators can be used by the same healthcare worker to protect against tuberculosis as long as the functional and structural integrity of the respirator is maintained. (Fisher, "Commentary Considerations for Recommending Extended Use," 14.)

b) The Centers for Disease Control published guidance that supported the extended use and limited reuse of respirators for public health emergencies like SARS and H1N1 flu pandemics. (Fisher, "Commentary Considerations for Recommending Extended Use," 16.)

c) N95 respirator filter efficiency fell below their original 95% standard after the respirators were used for 9 to 13 weeks. (Fisher, "Commentary Considerations for Recommending Extended Use," 6.)

d) Disposable respirators can be reused for weeks to months. (Fisher, "Commentary Considerations for Recommending Extended Use," 8.)

e) Decontamination: Viruses may remain on a respirator after use and pose a risk of virus transfer to the wearer. Respirators should therefore be decontaminated before reuse. (Casanova, “Survival of a Surrogate Virus,” 1335.)

f) Ultraviolet light of 15 watts at a distance of 25 cm for 15 minutes completely removed detectable H5N1 virus from a respirator. (Lore, “Effectiveness of Three Decontamination Treatments,” 95, 99.)

g) Five decontamination methods were studied for N95 and P100 respirators, including ultraviolet irradiation, ethylene oxide, vaporized hydrogen peroxide, microwave oven irradiation, and bleach. Ultraviolet irradiation, ethylene oxide, and vaporized hydrogen peroxide were the most promising decontamination methods, as the original characteristics of the respirators remained unchanged. The other decontamination methods were not recommended. (Viscusi, “Evaluation of Five Decontamination Methods,” 824-25.)

Conclusions:

1. Human coronavirus can remain infectious on various surfaces for days: When a mask is removed, you should assume that it is infected and can continue to transmit the virus for days.
2. Wear the best mask you can find: P100 respirators stop the virus better than N95 respirators, and both are recommended by the Centers for Disease Control for SARS and flu epidemics. Surgical masks do not stop the virus as well, but they can still help prevent transmission of the virus.
3. A respirator can be reused: However, you must first decontaminate the respirator and make sure it not too dirty or damaged. Ultraviolet light decontaminates respirators well and does not damage them.

References

1. Anna Bałazy, Mika Toivola, Atin Adhikari, Satheesh K. Sivasubramani, Tiina Reponen, and Sergey A. Grinshpun, "Do N95 Respirators Provide 95% Protection Level Against Airborne Viruses, and How Adequate are Surgical Masks?," *American Journal of Infection Control* 34, no. 2 (March 2006): 51, https://www.ajicjournal.org/article/S0196-6553(05)00911-9/pdf.

2. Lisa M. Casanova and Belehem Waka, "Survival of a Surrogate Virus on N95 Respirator Material," *Infection Control and Hospital Epidemiology* 34, no. 12 (December 2013): 1334, https://www.cambridge.org/core/services/aop-cambridge-core/content/view/4E8B5B4CC1E116453C3E0F241C83636C/S0195941700034597a.pdf/survival_of_a_surrogate_virus_on_n95_respirator_material.pdf

3. Lisa Casanova, Edie Alfano-Sobsey, William A. Rutala, David J. Weber, and Mark Sobsey, "Virus Transfer from Personal Protective Equipment to Healthcare Employees' Skin and Clothing," *Emerging Infectious Diseases* 14, no. 8 (August 2008): 1291, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2600382/pdf/08-0085_finalD.pdf.

4. K. H. Chan, J. S. Malik Peiris, S. Y. Lam, L. L. M. Poon, K. Y. Yuen, and W. H. Seto, "The Effects of Temperature and Relative Humidity on the Viability of the SARS Coronavirus," *Advances in Virology* 2011, article ID 734690, http://downloads.hindawi.com/journals/av/2011/734690.pdf.

5. A. D. Coulliette, K. A. Perry, J. R. Edwards, and J. A. Noble-Wang, "Persistence of the 2009 Pandemic Influenza A (H1N1) Virus on N95 Respirators," *Applied Environmental Microbiology* 79, no. 7 (April 2013): 2148, https://aem.asm.org/content/aem/79/7/2148.full.pdf.

6. J. P. Eshbaugh, P. D. Gardner, A. W. Richardson, and K.C. Hofacre, "N95 and P100 Respirator Filter Efficiency Under High Constant and Cyclic Flow," *Journal of Occupational and Environmental Hygiene* 6, no. 1 (2008): 52, https://www.ncbi.nlm.nih.gov/pubmed/19012163.

7. Edward M. Fisher and Ronald E. Shaffer, "Commentary Considerations for Recommending Extended Use and Limited Reuse of Filtering Facepiece Respirators in Health Care Settings," *Journal of Occupational and Environmental Hygiene* 11, no. 8 (2014): D115, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4610368/pdf/nihms728417.pdf.

8. Delbert A. Harnish, Brian K. Heimbuch, Michael Husband, April E. Lumley, Kimberly Kinney, Ronald E. Shaffer, and Joseph D. Wander, "Challenge of N95 Filtering Facepiece Respirators with Viable H1N1 Influenza Aerosols," *Infection Control & Hospital Epidemiology* 34, no. 5 (May 2013): 494, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4646082/pdf/nihms730655.pdf.

9. B. K. Heimbuch, W. H. Wallace, K. Kinney, A. E. Lumley, C. Y. Wu, M. H. Woo, and J. D. Wander, "A Pandemic Influenza Preparedness Study: Use of Energetic Methods to Decontaminate Filtering Facepiece Respirators Contaminated with H1N1 Aerosols and Droplets," *American Journal of Infection Control* 39 (2011): e1, https://www.ncbi.nlm.nih.gov/pubmed/21145624.

10. Larry Janssen, Harry Ettinger, Stephan Graham, Ronald Shaffer, and Ziqing Zhuang, "The Use of Respirators to Reduce Inhalation of Airborne Biological Agents," *Journal of Occupational and Environmental Hygiene* 10, no. 8 (2013): D97, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4670234/pdf/nihms738710.pdf.

11. Michael B. Lore, Brian K. Heimbuch, Teanne L. Brown, Joseph D. Wander, and Steven H. Hinrichs, "Effectiveness of Three Decontamination Treatments against Influenza Virus Applied to Filtering Facepiece Respirators," *Annals of Occupational Hygiene* 56, no. 1 (2012): 92, https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1101&context=usafresearch.

12. C. Raina MacIntyre, Quanyi Wang, Bayzidur Rahman, Holly Seale, Iman Ridda, Zhanhai Gao, Peng Yang, Weixian Shi, Xinghuo Pang, Yi Zhang, Aye Moa, and Dominic E. Dwyer, "Efficacy of Face Masks and Respirators in Preventing Upper Respiratory Tract Bacterial Colonization and Co-infection in Hospital Healthcare Workers," *Preventive Medicine* 62 (2014): 1, https://www.sciencedirect.com/science/article/pii/S0091743514000322?via%3Dihub.

13. S. Rengasamy, B. C. Eimer, and R. E. Shaffer, "Comparison of Nanoparticle Filtration Performance of NIOSH-approved and CE-marked Particulate Filtering Facepiece Respirators," *Annals of Occupational Hygiene* 53, no. 2 (2009): 117, https://www.ncbi.nlm.nih.gov/pubmed/19261695.

14. A. Tiwari, Devi P. Patnayak, Yogesh Chander, Minakshi Parsad, and Sagar M. Goyal, "Survival of Two Avian Respiratory Viruses on Porous and Nonporous Surfaces," *Avian Diseases* 50 (2006): 284, https://www.researchgate.net/profile/Minakshi_Prasad/publication/6923195_Survival_of_Two_Avian_Respiratory_Viruses_on_Porous_and_Nonporous_Surfaces/links/54a809d20cf256bf8bb7de1c/Survival-of-Two-Avian-Respiratory-Viruses-on-Porous-and-Nonporous-Surfaces.pdf?origin=publication_detail.

15. Dennis J. Viscusi, Michael S. Bergman, Benjamin C. Eimer, and Ronald E. Shaffer, "Evaluation of Five Decontamination Methods for Filtering Facepiece Respirators," *Annals of Occupational Hygiene* 53, no. 8 (2009): 815, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2781738/pdf/mep070.pdf.

16. Sarah L. Warnes, Zoë R. Little, and C. William Keevil, "Human Coronavirus 229E Remains Infectious on Common Touch Surface Materials," *mBio* 6, no. 6 (November/December 2015): e01697-15, https://mbio.asm.org/content/mbio/6/6/e01697-15.full.pdf.

12. C. Raina MacIntyre, Quanyi Wang, Bayzidur Rahman, Holly Seale, Iman Ridda, Zhanhai Gao, Peng Yang, Weixian Shi, Xinghuo Pang, Yi Zhang, Aye Moa, and Dominic E. Dwyer, "Efficacy of Face Masks and Respirators in Preventing Upper Respiratory Tract Bacterial Colonization and Co-infection in Hospital Healthcare Workers," *Preventive Medicine* 62 (2014): 1, https://www.sciencedirect.com/science/article/pii/S0091743514000322?via%3Dihub.

13. S. Rengasamy, B. C. Eimer, and R. E. Shaffer, "Comparison of Nanoparticle Filtration Performance of NIOSH-approved and CE-marked Particulate Filtering Facepiece Respirators," *Annals of Occupational Hygiene* 53, no. 2 (2009): 117, https://www.ncbi.nlm.nih.gov/pubmed/19261695.

14. A. Tiwari, Devi P. Patnayak, Yogesh Chander, Minakshi Parsad, and Sagar M. Goyal, "Survival of Two Avian Respiratory Viruses on Porous and Nonporous Surfaces," *Avian Diseases* 50 (2006): 284, https://www.researchgate.net/profile/Minakshi_Prasad/publication/6923195_Survival_of_Two_Avian_Respiratory_Viruses_on_Porous_and_Nonporous_Surfaces/links/54a809d20cf256bf8bb7de1c/Survival-of-Two-Avian-Respiratory-Viruses-on-Porous-and-Nonporous-Surfaces.pdf?origin=publication_detail.

15. Dennis J. Viscusi, Michael S. Bergman, Benjamin C. Eimer, and Ronald E. Shaffer, "Evaluation of Five Decontamination Methods for Filtering Facepiece Respirators," *Annals of Occupational Hygiene* 53, no. 8 (2009): 815, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2781738/pdf/mep070.pdf.

16. Sarah L. Warnes, Zoë R. Little, and C. William Keevil, "Human Coronavirus 229E Remains Infectious on Common Touch Surface Materials," *mBio* 6, no. 6 (November/December 2015): e01697-15, https://mbio.asm.org/content/mbio/6/6/e01697-15.full.pdf.

1

Do N95 respirators provide 95% protection level against airborne viruses, and how adequate are surgical masks?

Anna Bałazy, MSc,[a,b] Mika Toivola, PhD,[a] Atin Adhikari, PhD,[a] Satheesh K. Sivasubramani, PhD,[a] Tiina Reponen, PhD,[a] and Sergey A. Grinshpun, PhD[a]
Cincinnati, Ohio, and Warsaw, Poland

Background: Respiratory protection devices are used to protect the wearers from inhaling particles suspended in the air. Filtering face piece respirators are usually tested utilizing nonbiologic particles, whereas their use often aims at reducing exposure to biologic aerosols, including infectious agents such as viruses and bacteria.
Methods: The performance of 2 types of N95 half-mask, filtering face piece respirators and 2 types of surgical masks were determined. The collection efficiency of these respiratory protection devices was investigated using MS2 virus (a nonharmful simulant of several pathogens). The virions were detected in the particle size range of 10 to 80 nm.
Results: The results indicate that the penetration of virions through the National Institute for Occupational Safety and Health (NIOSH)-certified N95 respirators can exceed an expected level of 5%. As anticipated, the tested surgical masks showed a much higher particle penetration because they are known to be less efficient than the N95 respirators. The 2 surgical masks, which originated from the same manufacturer, showed tremendously different penetration levels of the MS2 virions: 20.5% and 84.5%, respectively, at an inhalation flow rate of 85 L/min.
Conclusion: The N95 filtering face piece respirators may not provide the expected protection level against small virions. Some surgical masks may let a significant fraction of airborne viruses penetrate through their filters, providing very low protection against aerosolized infectious agents in the size range of 10 to 80 nm. It should be noted that the surgical masks are primarily designed to protect the environment from the wearer, whereas the respirators are supposed to protect the wearer from the environment. (Am J Infect Control 2006;34:51-7.)

N95 filtering face piece respirators and surgical masks are commonly used to protect the human respiratory system against fine airborne particles that are known to be associated with various respiratory and heart diseases.[1] The aerosol particles of biologic origin, eg, viruses, bacterial cells, bacterial and fungal spores, fragments, and pollen grains, may cause major health effects, including infectious diseases. The adverse health effects of the biologic particles, particularly pathogenicity, depend not on the mass of the inhaled particles but on the number of particles. Viral particles, or virions, are one of the smallest known bioaerosol agents, with a particle diameter ranging from 20 to 300 nm.[2] Because of their small size, virions can easily penetrate through the human respiratory system and may cause diseases, such as colds, flu, measles, mumps, pneumonia, rubella, or chickenpox. The respiratory protection devices are usually tested using nonbiologic particles as the challenge aerosol, although their use often aims at reducing exposure to biologic particles. The results on the protection of filtering face piece respirators against submicron and supermicron particles have been widely reported in the literature.[3-9] The data on the penetration of nanosize sodium chloride particles through the N95 respirators have been recently reported by our research team.[10] It is acknowledged that the penetration of biologic particles through respirator filters may differ from that of their corresponding nonbiologic simulants. The attempts to

From the Center for Health-Related Aerosol Studies, Department of Environmental Health, University of Cincinnati, Cincinnati, OH,[a] and the Warsaw University of Technology, Department of Chemical and Process Engineering,[b] Warsaw, Poland.

Reprint requests: Sergey A. Grinshpun, PhD, Center for Health-Related Aerosol Studies, Department of Environmental Health, POB 670056, 3223 Eden Ave, University of Cincinnati Medical Center, Cincinnati, OH 45267-0056. E-mail: sergey.grinshpun@uc.edu.

Supported in part by the Kosciuszko Foundation (American Center for Polish Culture).

0196-6553/$32.00

doi:10.1016/j.ajic.2005.08.018

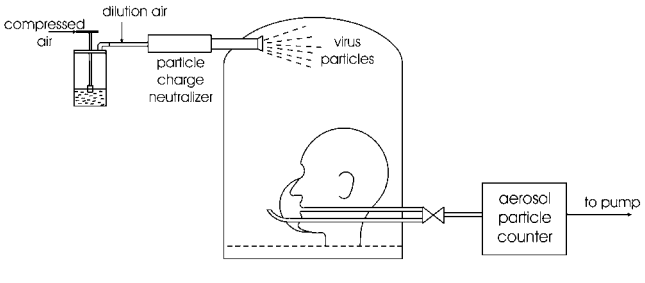


Fig 1. Experimental setup.

evaluate the respirator performance directly with biologic particles have been primarily focused on airborne bacteria.[7,11-16]

N95 filtering face piece respirators are certified under NIOSH 42 CFR 84 regulations.[17] Uncharged sodium chloride (NaCl) particles of 300 nm in diameter are utilized as the tested aerosol. The penetration, P, of such particles through a certified N95 respirator cannot exceed 5%; thus, the efficiency, E, of the respirator, which is calculated as $E = 100\% - P$, must be at least 95%. Surgical masks are not NIOSH certified. The performance of some masks has been evaluated when they were challenged either with latex sphere particles or aerosolized bacteria. The particulate filtration efficiency, PFE, is defined as the percentage of monodispersed nonneutralized latex particles that do not pass through the face mask at a specific inhalation flow rate. The F 2299 test method utilizes a light-scattering particle counted in the size range from 100 to 5000 nm and airflow test velocities from 0.5 to 25 cm/s.[18] The bacterial filtration efficiency, BFE, can be determined by 2 methods: in vitro using a biologic aerosol of *Staphylococcus aureus* or in vivo (modified Greene and Vesley test) when the masks are worn by a subjects while he/she enunciates the word ''chew'' 120 times over a 2-minute period, and viable aerosol particles are collected onto agar plates of the Andersen sampler. The filtration efficiency is calculated by comparing the concentration levels determined when the subject does and does not wear the mask, respectively.[19,20]

The studies on the respiratory protection against airborne biologic agents have been recently reviewed by Rengasamy et al.[21] From this and other reviews, it is clearly seen that there is a lack of direct measurement data on the efficiency of respirators and health care masks against aerosolized viral particles.

METHODS

Experimental setup

Figure 1 depicts the schematic diagram of the experimental setup. The challenge aerosol was generated using a 6-jet Collison nebulizer (BGI Inc., Waltham, MA), which was supplied by a compressed air system. Before entering the nebulizer, the air was purified by passing through a high-efficiency particulate air (HEPA) filter. Generated aerosol was diluted by clean air, which was also derived from the compressed air system, and then passed through an ^{85}Kr source charge neutralizer (Model 3054; TSI Inc., Minneapolis, MN). Charge-neutralized aerosol was supplied to the top part of the test chamber. The tested respirators and surgical masks were sealed by silicon sealant to the face of a manikin, which was placed inside the chamber. A bubble-producing liquid was used to assure that there were no leaks between the tested devices and the manikin's surface. The sealant surface was covered by this liquid, and the compressed air flowing through the respirator or surgical mask caused bubbles formation in case of a leak. The places at which the leakages were detected were additionally sealed and checked for leaks again. This leak-detection method allows identifying microleaks; however, it may not be sufficient to identify the leaks below 100 nm.

The experiments were carried out at 2 different constant flow rates: 30 L/min (which simulates inhalation at light workload) and 85 L/min (which simulates inhalation at heavy workload). These specific flow rates were controlled by a rotameter adjacent to an air supply pump. The aerosol generation system and the test chamber were located inside a class II biosafety cabinet (Sterilchem GARD; Baker Co., Sanford, ME). The particle concentrations and size distributions outside and inside the tested respiratory protection device were determined using a wide-range particle spectrometer (WPS; model 1000 XP, configuration A; MSP Corp., Shoreview, MN). The WPS is a device that combines 3 different instruments, namely the differential mobility diameter (DMA), the condensation particle counter (CPC) and the laser particle spectrometer (LPS). The combination of the 2 first instruments allows counting the particles of 10 to 500 nm, whereas the LPS covers the particle diameter range between 350 and 10,000 nm. The electrical mobility analysis utilized in the DMA is the most efficient and commonly used technique for measuring the aerosol particle size distribution in the nanometer size range (suitable for MS2 virions used in this study).

MS2 viruses

MS2 is a bacteriophage that contains single-stranded RNA, consisting of 3569 nucleotides.[22] Single MS2 virion with a referred physical diameter of approximately 27.5 nm contains 180 copies of the coat protein, which form a near spherical icosahedral shell. This small RNA virus infects only male *Escherichia coli* bacteria by injection of its RNA and A-protein.[23,24]

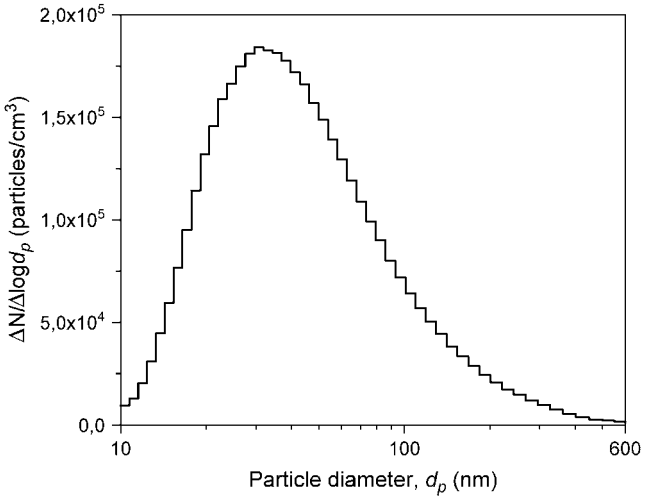


Fig 2. The particle size distribution of the aerosolized MS2 virions measured by the WPS.

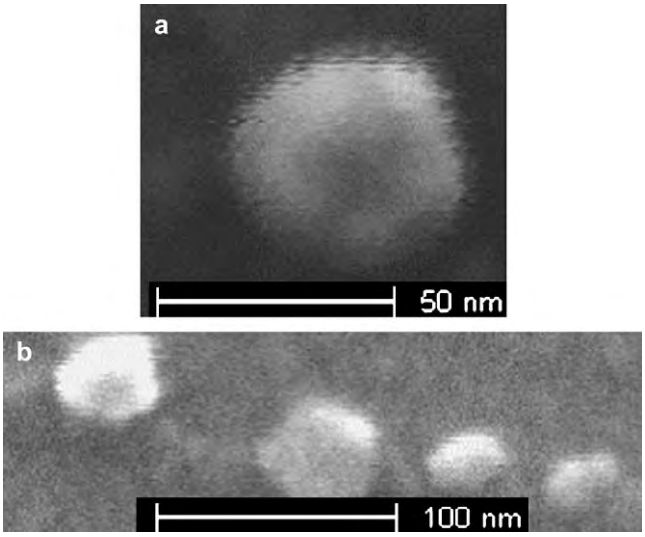


Fig 3. Scanning electron micrographs of the dried MS2 viral suspension (10 times concentrated) used for aerosolization: (a) a single MS2 virus particle (Magnification = ×400,000), (b) dispersed single virus particles (Magnification = ×200,000).

Stock suspension of MS2 virus was prepared by adding 9 mL Luria-Bertani broth (prepared using ultrafiltered deionized water) to freeze-dried phage vial (ATCC 15597-B1). This suspension was serially diluted, and the final suspension used for the aerosolization experiments had 10^8 to 10^9 plaque-forming units (pfu/mL) of MS2 virus. In some experiments, the suspension was prepared by plate lysis and elution (using the host *Escherichia coli;* ATCC 15597, strain C3000). MS2 phage titer was determined by using a modified plaque assay protocol of Adams.[25]

The size distribution of the aerosolized virus particles, measured by WPS, is presented in Fig 2. It is seen that the peak is for the particles of approximately 30 nm, which is in a good agreement with the referred size of a single MS2 virion (27.5 nm). However, in addition to these particles, the population of the WPS-detected aerosol particles includes smaller as well as much larger particles. We assume that some of the larger ones can be agglomerated virions. The contribution of large agglomerates is expected to be relatively low because the scanning electron microscope analysis revealed very few of the large agglomerates in the suspension prepared for this study. Digital micrographs of MS2 virus particles taken by using a scanning electron microscope (SEM) (Phillips XL-30 ESEM; FEI Co., Hillsboro, OR) are presented in Fig 3. The stock suspension used for aerosolization experiments was also utilized for the electron microscopy. On the other hand, large MS2 agglomerates have been observed in the MS2 viral suspension by other investigators. For instance, Hogan et al referred to the agglomerates larger than 200 nm seen on the SEM images obtained from a liquid viral suspension.[26] It should be acknowledged, however, that the above study utilized a much more concentrated suspension (0.0749 g/L of MS2) compared with the one we used for our SEM analyses. Nevertheless, the agglomerates in the suspension can be broken during aerosolization. In this study, we did not use a dryer, so the water content of the particles aerosolized by the Collison nebulizer could not fully evaporate, thus increasing the number of larger particles that carry single viruses or viral agglomerates. Similarly, in the field, the viruses are usually carried by droplets nuclei or other larger airborne particles.[27] As to the particles smaller than a single virion, which were detected by the aerosol particle counter, we speculate that these can be the fragments of some virions formed during the freezing-drying process.

We anticipate that some nonvirus-containing particles could be generated in addition to the virions. To reduce the influence of these particles in our aerosol count and concentrate on the specific particle size range that is primarily populated by virions, we limited our analysis to 50% of the total population of the WPS-detected particles, among which there were particles larger and smaller than the peak size. It is seen that the particle size distribution curve is rather steep below approximately 30 nm so that the size range from 10 to 30 nm covers only 12.5% of the total particle count. To make the postulated 50% of the total count, another 37.5% of particles were taken from the range above 30 nm. As a result, the particle diameter range of 10 to 80 nm was considered as the MS2 virions in this study.

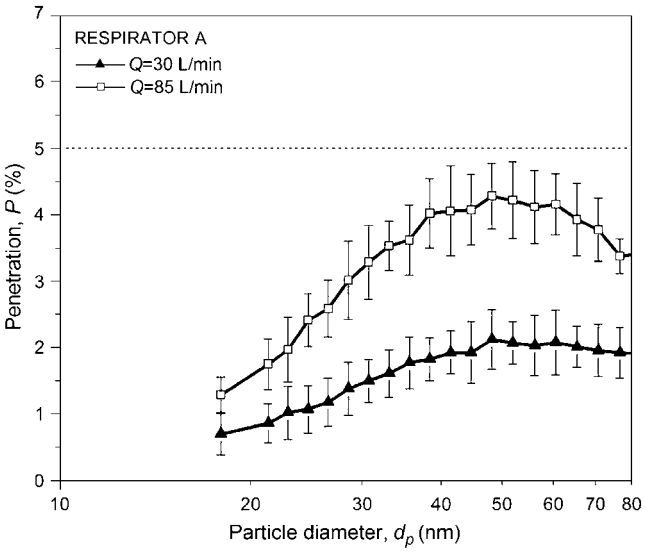


Fig 4. Effect of the inhalation flow rate on the fractional penetration of MS2 virus through respirator A (n = 5). Each point on the graphs represents the mean value of the particle penetration, and the error bars represent the standard deviations for respirators.

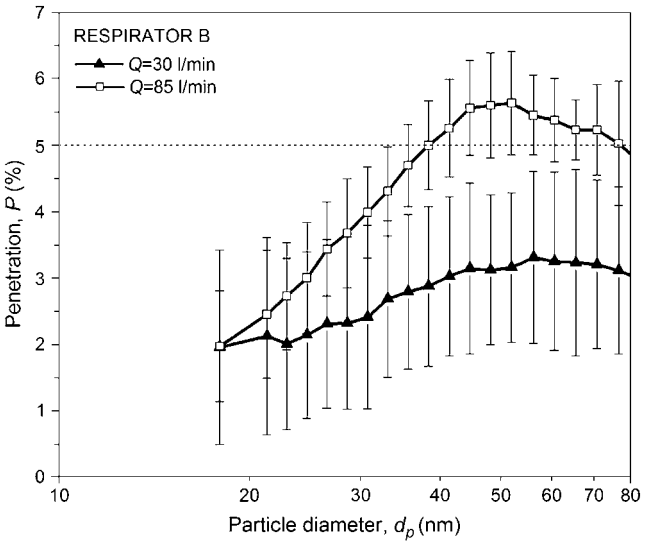


Fig 5. Effect of the inhalation flow rate on the fractional penetration of MS2 virus through respirator B (n = 5). Similar to Fig 4, the points and error bars represent the mean values and the standard deviations, respectively.

N95 respirators and surgical masks

Two different models of N95 filtering face piece respirators and 2 different models of surgical masks were evaluated in this study. The N95 respirators were obtained from 2 different manufacturers. Both respirators had multilayer structure, and the main layers of filters were composed of polypropylene fibers with electrical charge. The N95 respirators were chosen using the performance data presented by Coffey et al.[28] One of the respirators is characterized by relatively high fit-factor value (respirator A) and the other one by lower fit factor (respirator B).[28]

Two types of the surgical masks, SM1 and SM2, used in this study were made by the same manufacturer and were both fluid resistant. According to their manufacturer, BFE (determined by the modified Green and Vesley method) of SM1 was above 96%, whereas BFE of SM2 exceeded 99% and its collection efficiency for 200-nm latex spheres was at least 95% at a flow rate of 28.3 L/min.

The N95 filtering face piece respirators and the surgical masks used in this study were sealed to the face of the manikin, so their efficiency determined during experiments is defined as the efficiency of the filter material. The actual field-measured efficiency may be lower if there are some leakages between the wearer's face and the material of the respirator or surgical mask.

Penetration

The concentration of the particles was measured outside [$c_{out}(d_p)$] and inside [$c_{in}(d_p)$] of each tested N95 filtering face piece respirator or the surgical masks by the WPS. Based on the results obtained for each channel of the aerosol measurement instrument, the penetration of the particles with given diameter (which is the fraction of the particles that pass through the filter) was determined as:

$$P(d_p) = \frac{c_{in}(d_p)}{c_{out}(d_p)} \cdot 100\% \quad (1)$$

Because some channels of the WPS detected very few particles, the results obtained for 2 or more channels were combined to achieve representative data. This was done in cases in which there were fewer than 50 particles per channel. This approach allowed us to eliminate accidental deviations of the penetration data in case very few particles per channel are detected. Generally, according to the WPS manufacturer, the particle fractional concentration can be accurately determined within a range of <1 particle/cm^3 to 10,000 particles/cm^3.[29]

RESULTS

The penetrations of MS2 virions through respirator A at flow rates of 30 and 85 L/min are presented in Fig 4. Each point represents the penetration mean

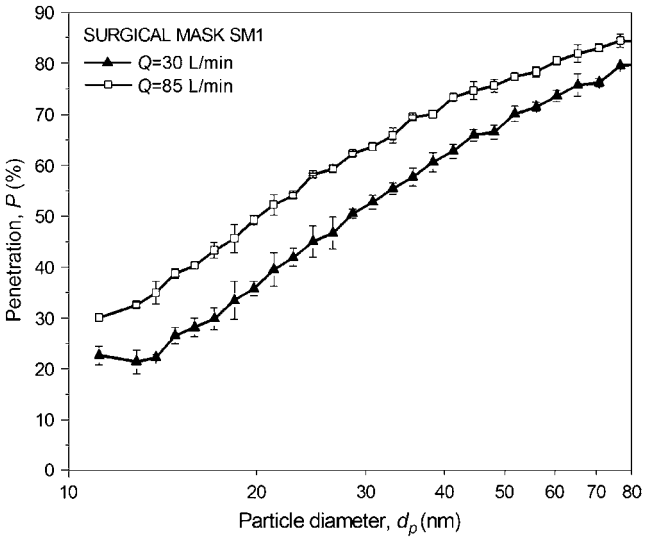


Fig 6. Effect of the inhalation flow rate on the fractional penetration of MS2 virus through the surgical mask SM1 (n = 2). Each point represents the mean penetration value, and the error bars represent the standard deviation.

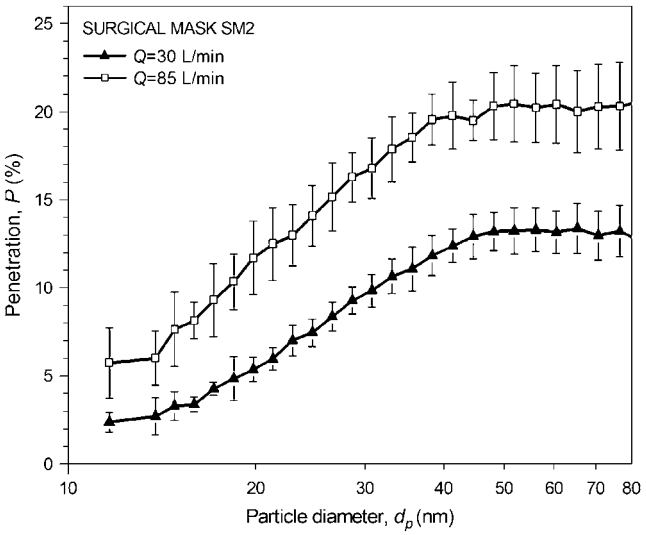


Fig 7. Effect of the inhalation flow rate on the fractional penetration of MS2 virus through the surgical mask SM2 (n = 3). Each point represents the mean penetration value, and the error bars represent the standard deviation.

value determined for 5 identical respirators, and the corresponding error bars represent the standard deviation. Figure 5 depicts the results of similar experiments carried out for respirator B. All values of the virion penetration through respirator A are below 5% as anticipated because this is a certified N95 respirator. However, for respirator B, the penetration exceeds the 5% threshold at the higher inhalation flow rate with the mean value of 5.6%. We found that, in the size range of 10 to 80 nm, the maximum penetration occurred at the particle diameter of approximately 50 nm. Our previous study conducted with NaCl particles[10] revealed that 300 nm is not the most penetrating particle size through N95 respirators at a flow rate of 85 L/min as is conventionally believed and postulated in the respirator evaluation standard. Instead, the maximum penetration was observed for particles of 40 to 50 nm.[10] We have shown that, for a mechanical filter (when the particle deposition on fibers occurs because of diffusion, direct interception, and inertial impaction), the particle diameter of approximately 300 nm is rightfully believed to be the most penetrating particle size, although it may slightly vary depending on the filter's structure and other factors. However, the N95 filtering face piece respirators are composed of charged fibers. This property leads to a considerable shift of the maximum penetration toward smaller particles because the additional polarization force has a great importance in the process of the particle deposition on fibers. Similar results were reported by Martin and Moyer, who found that the maximum penetration of particles through the fiber-charged N95 respirators occurred in the 50- to 100-nm size range.[9] Thus, it should be emphasized that the certified N95 respirators will protect their wearers properly against the particles of 300 nm and larger, but their performance may be below the threshold for aerosol particles of the nanosize range. The penetration values of the nanoparticles through N95 respirators depend on their filter media characteristics.

Figure 6 and Fig 7 present the evaluation data obtained with 2 types of surgical masks, SM1 and SM2, which are widely used to control human inhalation exposure to airborne infectious agents in health care environments (although the surgical masks were designed to protect the environment from the wearer). Two identical SM1 masks and 3 identical SM2 masks were tested. Similarly to the experiments conducted with the N95 respirators, 2 inhalation flow rates, 30 and 85 L/min, were tested. These data show that the penetration of MS2 virions through the surgical masks is much higher than that observed for N95 filtering face piece respirators. For example, at 85 L/min, the particle penetration curve for SM2 reaches a plateau at 20.5%, whereas, for SM1, the penetration increases with increasing particle size to 84.5% for particles of 80 nm in diameter. The fibers of the surgical masks are not electrically pretreated, and these devices act like poor mechanical filters. In the absence of electrostatic effects, based on theoretic calculations, the diameter of approximately 300 nm is anticipated to be the maximum penetrating particle size for these masks.

Table 1. Paired *t* test comparison of the penetration values obtained for MS2 viruses and sodium chloride particles

Type of respiratory protection device	Inhalation flow rate, Q (L/min)	P value
Respirator A	30	.004*
	85	.220
Respirator B	30	.156
	85	.532
SM1†	30	.997
	85	.962
SM2†	30	.716
	85	.608

*$P_{MS2} > P_{NaCl}$.
†Type of surgical mask.

The data presented in this paper resulted from the experiments carried out with the clean unloaded respiratory protection devices. In this light, the penetration values presented in Fig 4 to Fig 7 represent the initial penetrations of virions through the N95 respirators and surgical masks. Because the fibers of N95 filtering face piece respirators are charged, the penetration through these respirators increases with the time because of the reduction in fiber charges, which was proven experimentally by Martin and Moyer.[9] Martin and Bergman showed that the filter degradation resulting from its exposure to aerosol depends not only on the amount of the deposited particles but also on the time over which the aerosol deposition occurred.[30] However, after achieving a certain level of the filter loading, the pretreated respirator filter (''electret'') starts behaving like a mechanical filter, and the penetration decreases. This means that the initial virion penetration through the N95 respirators obtained in our experiment may somewhat differ from that found in the field during a long-term use of the respirator in bioaerosol-contaminated environments. The penetration through the surgical masks should decrease during the filtration process because of loading because they act as mechanical filters from the very beginning.

For all filtering face piece respiratory protection devices, the penetration increases with increasing inhalation flow. Although the flow rate of 85 L/min used in this study simulates a heavy workload and is utilized in the respirator certification tests, some studies refer to even higher inhalation flow rates.[21] At those rates, the penetration is anticipated to be even greater compared with the values reported in this paper.

The data on the virion penetration obtained in this study were compared with the results of our earlier experiments in which the same respiratory protection devices were challenged with nonbiological (sodium chloride) particles.[10] The comparison was performed using paired *t* tests (utilizing program Origin 6.0, OriginLab Corp., Northampton, MA). The data sets obtained for sodium chloride and MS2 virus were combined in the same manner into 10 size fractions for N95 respirators and into 11 channels for surgical masks in the size range from 10 to 80 nm. The results presented in Table 1 indicate that, generally, the penetrations of MS2 virions and sodium chloride particles through the tested respiratory protection devices were not significantly different. Thus, nonbiological particle simulants can be used for assessing the performance of these devices against virions of similar shape and the same size. The only exception is respirator A operated at 30 L/min, in which case the *t* test revealed significant difference between the penetration of sodium chloride and MS2 virions. However, even in this case, the difference between the penetration of biologic and nonbiologic particles did not exceed 1%.

The penetration data presented in this paper were obtained using manikin-based tests. Thus, the respirators and surgical masks were sealed to the manikin's face. Such procedure eliminated the leakages, which can occur when a subject wears the personal respiratory protection devices. In real life, the leaks may lead to considerably increased particles penetration. Coffey et al indicated that, without proper fit testing, the wearer of a respirator cannot achieve the desired protection level.[31] Therefore, it seems critical to perform a proper fit test before wearing a N95 filtering face piece respirator.

CONCLUSIONS

Two types of N95 half-mask respirators and 2 types of surgical masks were challenged with aerosolized MS2 virus. The experiments were carried out following a manikin-based protocol. The results indicate that N95-certified respirators may not necessarily provide a proper protection against virus, which is considerably smaller than the accepted most penetrating particle size of 300 nm used in the certification tests. Thus, the protection against the airborne viral agents provided by some N95 respirators may fall below 95%, especially at higher inhalation flow rates. The efficiency of the surgical masks is much lower than that of the N95 respirators so that the MS2 virions penetrate readily through the surgical masks. The performance tests conducted with surgical masks challenged with latex spheres of ~300 nm or bacterial particles may underestimate the penetration of nanosize virions.

The authors thank the NIOSH for providing equipment for aerosol measurements and the Kosciuszko Foundation for support.

References

1. World Health Organization (WHO). Hazard prevention and control in the work environment: airborne dust. 1999. Available at: http://www.who.int/occupational_health/publications/en/oehairbornedust3.pdf. Accessed April 21, 2005.
2. Reponen T, Willeke K, Grinshpun SA, Nevalainen A. Biological particle sampling. In: Baron PA, Willeke K, editors. Aerosol measurement. principles, techniques and applications. New York: Wiley-Interscience; 2001. p. 751-79.
3. Chen CC, Willeke K. Characteristics of face seal leakage in filtering facepieces. Am Ind Hyg Assoc J 1992;53:533-9.
4. Barrett LW, Rousseau AD. Aerosol loading performance of electret filter media. Am Ind Hyg Assoc J 1998;59:532-9.
5. Huang C, Willeke K, Qian Y, Grinshpun SA, Ulevicius V. Method for measuring the spatial variability of aerosol penetration through respirator filters. Am Ind Hyg Assoc J 1998;59:461-5.
6. Qian Y, Willeke K, Ulevicius V, Grinshpun SA. Particle reentrainment from fibrous filters. Aerosol Sci Tech 1997;27:394-404.
7. Qian Y, Willeke K, Grinshpun SA, Donnelly J, Coffey CC. Performance of N95 respirators: filtration efficiency for airborne microbial and inert particles. Am Ind Hyg Assoc J 1998;59:128-32.
8. Han DH. Performance of respirator filters using quality factor in Korea. Ind Health 2000;38:380-4.
9. Martin SB Jr, Moyer ES. Electrostatic respirator filter media: filter efficiency and most penetrating particle size effects. App Occup Environ Hyg 2000;15:609-17.
10. Bałazy A, Toivola M, Reponen T, Podgórski A, Zimmer A, Grinshpun SA. Manikin-based performance evaluation of N95 filtering-facepiece respirators challenged with nanoparticles. Ann Occup Hyg 2006; in press.
11. Brosseau LM, McCullough NV, Vesley D. Mycobacterial aerosol collection efficiency of respirator and surgical mask filters under varying conditions of flow and humidity. Appl Occup Environ Hyg 1997;12:435-45.
12. McCullough NV, Brosseau LM, Vesley D. Collection of three bacterial aerosols by respirator and surgical mask filters under varying conditions of flow and relative humidity. Ann Occup Hyg 1997;41:677-90.
13. Qian Y, Willeke K, Grinshpun SA, Donnelly J. Performance of N95 respirators: reaerosolization of bacteria and solid particles. Am Ind Hyg Assoc J 1997;58:876-80.
14. Reponen T, Wang Z, Willeke K, Grinshpun SA. Survival of Mycobacteria on N95 personal respirators. Infect Control Hosp Epidemiol 1999;20:237-41.
15. Wang Z, Reponen T, Willeke K, Grinshpun SA. Survival of bacteria on respirator filters. Aerosol Sci Tech 1999;30:300-8.
16. Lee K, Slavcev A, Nicas M. Respiratory protection against Mycobacterium tuberculosis: quantitative fit test outcomes for five types N95 filtering-facepiece respirators. J Occup Environ Hyg 2004;1:22-8.
17. National Institute for Occupational Safety and Health (NIOSH). 42 CFR 84 Respiratory protective devices: final rules and notice. Federal Register 60:110. US Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health; 1997.
18. American Society for Testing and Materials (ASTM) F2299-03. Test method for determining the initial efficiency of materials used in medical face masks to penetration by particulates using latex spheres.
19. American Society for Testing and Materials (ASTM) F2100-04. Standard specification for performance of materials used in medical face masks.
20. American Society for Testing and Materials (ASTM) F2101-01. Standard test method for evaluating the bacterial filtration efficiency (BFE) of medical face mask materials, using a biological aerosol of *Staphylococcus aureus*.
21. Rengasamy A, Zhuang Z, BerryAnn R. Respiratory protection against bioaerosols: literature review and research needs. Am J Infect Control 2004;32:345-54.
22. Fiers W. Studies on the bacteriophage MS2. III. Sedimentation heterogeneity of viral DNA preparations. Virology 1967;33:413-24.
23. Valegard K, Liljas L, Fridborg K, Unge T. The three-dimensional structure of the bacterial virus MS2. Nature 1990;345:36-41.
24. Golmohammadi R, Valegard K, Fridbord K, Liljas L. The refined structure of bacteriophage MS2 at 28 Å resolution. J Mol Biol 1993;234:620-39.
25. Adams MH. Bacteriophages. New York: Interscience Publishers, Inc; 1959.
26. Hogan CJ Jr, Lee MH, Biswas P. Capture of viral particles in soft X-ray-enhanced corona systems: charge distribution and transport characteristics. Aerosol Sci Tech 2004;38:475-86.
27. Hinds WC. Aerosol technology. New York: John Wiley & Sons; 1999.
28. Coffey CC, Campbell DL, Zhuang Z. Simulated workplace performance of N95 respirators. Am Ind Hyg Assoc J 1999;60:613-24.
29. MSP Corporation. Model 1000XP wide-range particle spectrometer: configuration A. User guide; 2004.
30. Moyer ES, Bergman MS. Electrostatic N-95 respirator filter media efficiency degradation resulting from intermittent sodium chloride aerosol exposure. App Occup Environ Hyg 2000;15:600-8.
31. Coffey CC, Lawrence RB, Campbell DL, Zhuang Z, Calvert CA, Jensen PA. Fitting characteristics of eighteen N95 filtering-facepiece respirators. J Occup Environ Hyg 2004;1:262-71.

2

Survival of a Surrogate Virus on N95 Respirator Material

To the Editor—Protecting healthcare providers from occupational respiratory disease is crucial for public health preparedness; outbreaks of severe acute respiratory syndrome and influenza have shown that transmission from patient to healthcare worker is an occupational hazard.[1,2] While N95 respirators are vital for protection against occupational respiratory infection, potential shortages in outbreak situations are a serious preparedness issue.[3] Reuse of respirators is a potential solution; however, contaminated respirators are potential vehicles for pathogen spread during handling and reuse. Methods for respirator decontamination have been explored, but developing effective decontamination protocols requires data on virus survival on respirator surfaces to determine the frequency and efficacy of decontamination required to reduce the risks of reuse. The goal of this research is to determine the inactivation rates of virus on the surface of N95 respirators at ambient temperature and humidity levels using bacteriophage Φ6, an enveloped virus and potential surrogate for human respiratory viruses.

Bacteriophage and host were kindly provided by Leonard Mindich, University of Medicine and Dentistry, New Jersey. Virus was propagated in host *Pseudomonas syringae* using the soft agar propagation method. Thirty milliliters of host bacterial culture were grown for 24 hours with shaking (100 rpm, 25°C). Virus stock (2 mL) was added and incubated with shaking for another 24 hours. This virus culture (0.5 mL) and fresh host culture (0.5 mL) were added to 30 mL of soft agar (0.7% agar), dispensed into tryptic soy bottom agar plates, and incubated at 25°C for 24 hours. The top layer was then harvested, pooled, purified by centrifugation (5,900 *g*, 30 minutes, 4°C), and stored as stock in tryptic soy broth with 20% glycerol at −80°C.

Virus stock was diluted in phosphate buffered saline (PBS) to target a concentration of 10^5 plaque-forming units (PFUs) in 10 μL. Ten microliters were placed onto six 1-cm^2 coupons of N95 respirator material (model 1860, 3M). Time 0 carriers were sampled immediately. For sampling, coupons were placed in tubes using sterile forceps. Two milliliters of 1.5% beef extract (pH 7.5) were added into each tube and agitated on a shaker at 60 rpm for 20 minutes. Samples were assayed using the double agar layer plaque assay on tryptic soy agar and incubated at 25°C for 24 hours. For the other time points, carriers were placed into controlled humidity environments at 22°C and either 40% (±2%) or 60% (±2%) relative humidity (RH), created by placing saturated salt solutions in sealed glass containers. Virus survival at each time point was expressed as $\log_{10}(N_t/N_0)$, where N_t is the virus concentration (PFU/mL) at time t and N_0 is the initial virus concentration (PFU/mL) in the control sample at time 0. Data were analyzed with Excel 2007 (Microsoft) and GraphPad Prism 5 (GraphPad).

Over 24 hours, there was an ~1 $\log_{10}$ reduction in infectious virus at 22°C and 40% RH, while there was an ~4 $\log_{10}$ reduction at 22°C and 60% RH (Figure 1). The rate of virus inactivation is significantly less at 40% RH (slope = −0.046 ± 0.007) than at 60% RH (slope = −0.20 ± 0.006;

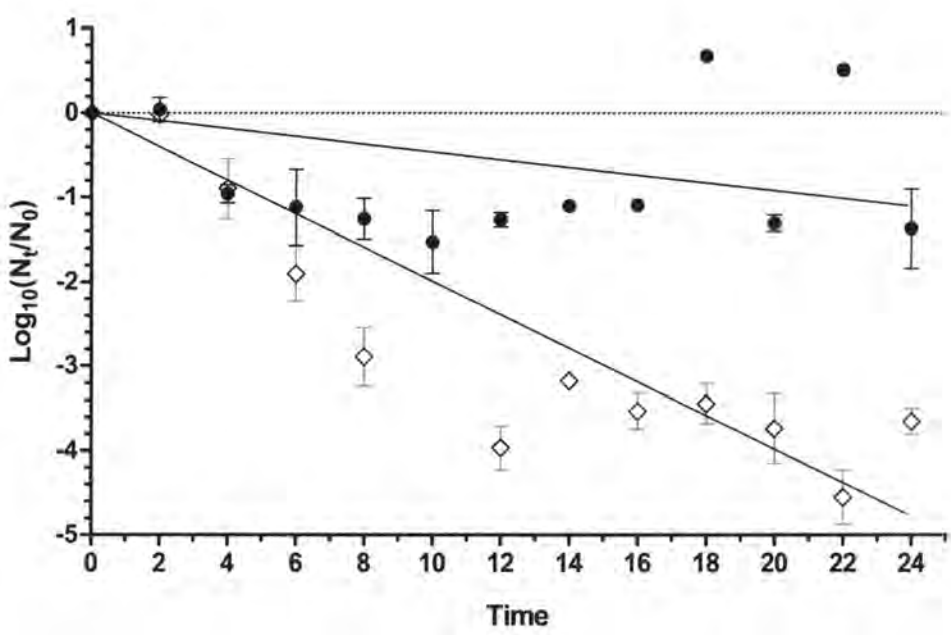


FIGURE 1. Survival of bacteriophage Φ6 over 24 hours at 22°C at 40% and 60% relative humidity (6 replicates per point). Circles, 40%; diamonds, 60%. Regression lines: solid lines, 40%; dashed line, 60%. Bars, 95% confidence interval.

$P < .0001$). Within the time frame of a typical patient care encounter (approximately 30 minutes), there was a <0.02 $\log_{10}$ reduction in virus at 40% RH, while there was a <0.1 $\log_{10}$ reduction at 60% RH. Achieving a 4 log reduction of infectious virus on a mask surface would take 87 hours at 40% RH and 20 hours at 60% RH.

Enveloped bacteriophage Φ6 can survive on the surface of an N95 respirator longer than a single patient care encounter. High levels of virus remaining on a respirator may pose a risk of virus transfer to the wearer during handling and reuse.[4] The use of a bacteriophage provides a simple, low-cost method for evaluating survival and transfer risks; bacteriophages are already used as surrogates in studies of respirator decontamination.[5] Bacteriophage Φ6 was inactivated somewhat more rapidly than H1N1 influenza on N95 surfaces at 60% RH (possibly as a result of the matrix used), and a similar trend of greater inactivation was observed at higher humidity levels.[6] The results are similar to those found for transmissible gastroenteritis virus, a member of the coronavirus family, on respirator surfaces.[7] This suggests that bacteriophage Φ6 is a potential surrogate for studies of human respiratory viruses on personal protective equipment.

The inactivation observed demonstrates that residual virus on a respirator surface is an important factor when reuse is considered. If a respirator is used over an 8- or 12-hour shift, even 90% inactivation during that time raises the possibility that that reuse over multiple patient encounters may add additional viral load to an already contaminated respirator. Therefore, decontamination of respirators is an important consideration in any reuse scenario.[8] Studies of infectious virus reduction[9] suggest that decontamination may be a viable option if pandemic situations or shortages make respirator reuse an alternative that needs to be considered. The design of effective respirator decontamination protocols should include the intervals at which a respirator needs to be decontaminated between uses, as well as how long a respirator should be used before discarding. Virus survival data is needed to model inactivation, decontamination, and recontamination to determine safe and effective reuse protocols. Long-term survival of respiratory viruses on the surface of N95 respirators needs to be taken into account when evaluating decontamination protocols and weighing the risks and benefits of respirator reuse for outbreak and pandemic preparedness.

ACKNOWLEDGMENTS

Financial support. This project was supported by a Georgia State University Research Initiation Grant.

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article. All authors submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and the conflicts that the editors consider relevant to this article are disclosed here.

Lisa M. Casanova, PhD;[1] Behelhem Waka, MPH[1]

Affiliation: 1. School of Public Health, Georgia State University, Atlanta, Georgia.

Address correspondence to Lisa M. Casanova, PhD, School of Public Health, PO Box 3995, Atlanta, GA 30302 (lcasanova@gsu.edu).

Presented in part: General Meeting of the American Society for Microbiology; San Francisco, California; 2012.

Infect Control Hosp Epidemiol 2013;34(12):1334-1335

 0899-823X/2013/3412-0022$15.00. DOI: 10.1086/673994

REFERENCES

1. Wise ME, De Perio M, Halpin J, et al. Transmission of pandemic (H1N1) 2009 influenza to healthcare personnel in the United States. *Clin Infect Dis*2011;52(suppl 1):S198–S204.
2. Ho AS, Sung JJ, Chan-Yeung M. An outbreak of severe acute respiratory syndrome among hospital workers in a community hospital in Hong Kong. *Ann Intern Med* 2003;139(7):564–567.
3. Institute of Medicine of the National Academies. *Reusability of Facemasks during an Influenza Pandemic: Facing the Flu.* Washington, DC: National Academies Press, 2006.
4. Fisher EM, Richardson AW, Harpest SD, Hofacre KC, Shaffer RE. Reaerosolization of MS2 bacteriophage from an N95 filtering facepiece respirator by simulated coughing. *Ann Occup Hyg* 2012; 56(3):315–325.
5. Vo E, Rengasamy S, Shaffer R. Development of a test system to evaluate procedures for decontamination of respirators containing viral droplets. *Appl Environ Microbiol* 2009;75:7303–7309.
6. Coulliette A, Perry K, Edwards J, Noble-Wang J. Persistence of the 2009 pandemic influenza A (H1N1) virus on N95 respirators. *Appl Environ Microbiol* 2013;79(7):2148–2155.
7. Casanova L, Rutala WA, Weber DJ, Sobsey MD. Coronavirus survival on healthcare personal protective equipment. *Infect Control Hosp Epidemiol* 2010;31(5):560–561.
8. Viscusi DJ, Bergman MS, Eimer BC, Shaffer RE. Evaluation of five decontamination methods for filtering facepiece respirators. *Ann Occup Hyg* 2009;53(8):815–827.
9. Heimbuch BK, Wallace WH, Kinney K, et al. A pandemic influenza preparedness study: use of energetic methods to decontaminate filtering facepiece respirators contaminated with H1N1 aerosols and droplets. *Am J Infect Control* 2011;39(1):e1–e9.

Evaluation of Universal Methicillin-Resistant *Staphylococcus aureus* Screening Using Nasal Polymerase Chain Reaction Compared with Nasal, Axilla, and Groin and Throat and Perianal Cultures in a Hospital Setting

To the Editor—Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage by polymerase chain reaction (PCR) methods and early patient isolation could reduce the chances of nosocomial transmission between patients.[1] However, the cost of PCR and MRSA prevalence could influence choice of testing method in a hospital screen-

3

Virus Transfer from Personal Protective Equipment to Healthcare Employees' Skin and Clothing

Lisa Casanova,* Edie Alfano-Sobsey,† William A. Rutala,* David J. Weber,* and Mark Sobsey*

We evaluated a personal protective equipment removal protocol designed to minimize wearer contamination with pathogens. Following this protocol often resulted in virus transfer to hands and clothing. An altered protocol or other measures are needed to prevent healthcare worker contamination.

Caring for patients with communicable diseases places healthcare workers (HCWs) at risk. Infected HCWs may not only incur serious illness or death themselves but may spread infection to others. Methods to prevent HCW infections include vaccination (*1*), hand hygiene (*2*), and isolation of patients with communicable diseases (*3*).

A key aspect of patient isolation is proper use of personal protective equipment (PPE) to protect HCWs from pathogen exposure during patient care. PPE includes use of barriers (gowns, gloves, eye shields) and respiratory protection (masks, respirators) to protect mucous membranes, airways, skin, and clothing from contact with infectious agents (*3*). The importance of PPE was underscored in the recent outbreak of severe acute respiratory syndrome (SARS). HCWs accounted for ≈20% of cases (*4*); failure to properly use PPE was a risk factor for HCW infection (*5*).

This outbreak raised concern that HCWs could contaminate their skin or clothes with pathogens during PPE removal, resulting in accidental self-inoculation and virus spread to patients, other HCWs, or fomites. The Centers for Disease Control and Prevention (CDC) addressed this concern by designing a protocol to minimize contamination to the wearer during PPE removal (Figure 1) (*6*). However, the effectiveness of this protocol in preventing self-contamination has not been validated. To determine if removing PPE according to the CDC protocol prevents viral contamination of the wearer, a human challenge study was undertaken using a nonpathogenic virus.

*University of North Carolina Chapel Hill, Chapel Hill, North Carolina, USA; and †Wake County Human Services, Raleigh, North Carolina, USA

DOI: 10.3201/eid1408.080085

The Study

PPE (gowns, gloves, respirators, and goggles) donned by volunteers was contaminated with bacteriophage MS2, a nonenveloped, nonpathogenic RNA virus suspended in 0.01 mol/L phosphate-buffered saline and GloGerm (GloGerm, Moab, UT, USA), synthetic beads that uoresce under UV light (for visual tracking of virus). Sites of contamination were as follows: front shoulder of gown, back shoulder of gown, right side of N95 respirator, upper right front of goggles, and palm of dominant hand. Each site was contaminated with a total of 10^4 PFU of MS2 in 5 drops of 5 μL each. Participants performed a healthcare task (measuring blood pressure on a mannequin) and then removed PPE according to CDC protocol. Hands, items of PPE, and scrubs worn underneath were sampled for virus. Hands were sampled by using the glove juice method (*7*). Each hand was placed inside a bag containing 75 mL stripping solution (0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, 1.0 mL Triton-X/L) and massaged for 60 seconds to cover all hand surfaces with solution. PPE items were immersed in 1.5% beef extract, pH 7.5, and agitated on a shaker for 20 minutes. Eluent from hands and PPE was assayed by the most probable number (MPN) enrichment infectivity assay (*8*). To prevent cross-contamination, samples from only 1 volunteer were processed at a time, and individual eluent samples were processed separately in a biological safety cabinet, with decontamination in between.

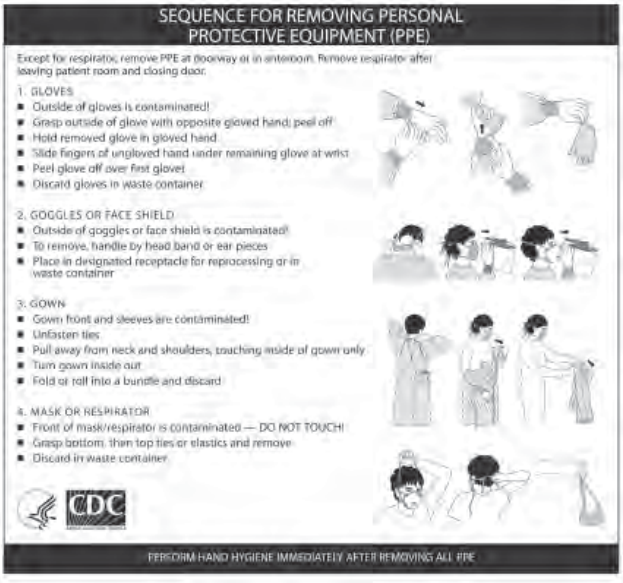


Figure 1. Centers for Disease Control and Prevention protocol for removing healthcare worker PPE.

When an a priori value of 25% was used for the 95% upper con dence limit when p (transfer) = 0, the sample size was N = 10. Protocols were approved by the University of North Carolina (UNC) Biomedical Institutional Review Board, and written informed consent was obtained. Enrolled participants met the following inclusion criteria: >18 years of age, nonpregnant, nonallergic to latex, no active skin disorders, and medical evaluation approval for N95 respirator t testing and use (*9*). Experiments took place in a patient care room in the UNC Hospitals' General Clinical Research Center. The experimental protocol is shown in Figure 2. Participants were shown the poster distributed by CDC (Figure 1) and given an opportunity to read it and ask questions. The poster was placed in front of the participants for reference while they donned and removed PPE.

Ten study participants were enrolled in this study: 9 women and 1 man. Nine participants were right-handed, and 1 was left-handed. Transfer of virus to both hands, the initially uncontaminated glove on the nondominant hand, and the scrub shirt and pants worn underneath the PPE was observed in most volunteers (Table). Because of the dif- culty of sampling large facial areas, visible uorescent tracer was used as the criterion to determine whether the face would be sampled. No tracer was observed on the facial areas of any volunteer. The uorescent tracer was not a consistent indicator of virus contamination; virus was recovered both from sites where tracer was visible and where it was not detected.

The amount of virus recovered was 1–3 $\log_{10}$ MPN for hands and 1–4 $\log_{10}$ MPN for scrubs. The mean amount of virus recovered from the right hand (the dominant hand of 90% of volunteers) was greater than that recovered from the left hand. While removal of gloves and gowns required 2 hands, mask and goggle removal was one-handed, which could have resulted in larger quantities of virus being transferred to the dominant hand during removal. In the single left-handed study participant, recovery of virus was greater from the left hand than the right (1.82 $\log_{10}$ vs. 0.98 $\log_{10}$ MPN). The mean amount of virus recovered from scrub shirts was signi cantly greater than that recovered from pants (p = 0.01), possibly because of contact with hands when the gown is pulled away from the shoulder during removal.

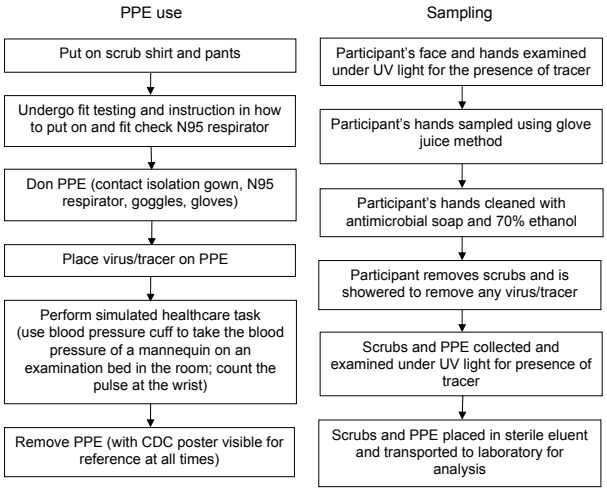


Figure 2. Protocol for human challenge experiments. PPE, personal protective equipment; CDC, Centers for Disease Control and Prevention.

Conclusions

PPE is vital for protecting HCWs from occupationally acquired infection during patient care, particularly from droplet- or airborne-transmitted diseases. However, removing PPE after patient care without contaminating skin or clothes is important. Although PPE is usually worn only for short periods, viruses such as in uenza (*10*) and SARS coronavirus (*11*) can survive for hours on surfaces, and viral infection can be spread by surface-to-hand (*12*) and hand-to-hand contact (*13*).

Developing and validating an algorithm for removing PPE that prevents contamination of the skin and clothes of HCWs are key to interrupting nosocomial transmission of infectious agents. These experiments demonstrate that the current CDC algorithm is insuf cient to protect HCWs from contamination during PPE removal. However, options that might prevent such contamination do exist, including double gloving, use of surgical protocols for PPE removal, and PPE impregnated with an antimicrobial agent.

A double-glove removal sequence would begin with removal of the outer glove, followed by removal of goggles or face shield, gown, and respirator/mask, and n- ishing with removal of the inner glove followed by hand

Table. Frequency and levels of viral contamination of selected sites, virus transfer study, 2007*

Site	% Volunteers who transferred virus to site (N = 10)	Mean viral titer recovered from site ($\log_{10}$ MPN)	% Contaminated sites with visible tracer (N = 10)
Nondominant glove	80	2.2	10
Right hand (skin)	90	2.4	20
Left hand (skin)	70	1.8	0
Scrub shirt	100	3.2	10
Scrub pants	75†	2.1	0
Face	0	–	–

*MPN, most probable number; –, not measured.
†N = 8.

hygiene; handling of PPE with ungloved hands is avoided. Borrowing PPE protocols from surgery, in which the ends of gown sleeves are tucked underneath gloves during wear, might also reduce contamination. When the HCW is n-ished, goggles and respirator are removed rst, and gown and gloves are then removed together by peeling off both at the same time, again avoiding handling PPE with ungloved hands. Finally, the use of PPE impregnated with antimicrobial agents might also reduce or eliminate contamination of skin and clothes.

This study also indicates the need for continued emphasis on hand hygiene. A barrier to improving hand hygiene compliance rates is the belief that gloves make hand hygiene unnecessary (*14*). This is contradicted by our study and others showing that organisms can spread from gloves to hands after glove removal (*15*). Even if double gloving is incorporated into protocols for PPE use, it is not a substitute for proper hand hygiene. Before these or other candidate methods are introduced into clinical practice, their impact on the safety of HCWs should be validated by testing with methods such as we have described.

Acknowledgment

We thank Maria Gergen for technical assistance.

This research was supported by the Centers for Disease Control and Prevention and by a grant (RR00046) from the General Clinical Research Centers program of the Division of Research Resources, National Institutes of Health.

Ms Casanova is a doctoral student in environmental health sciences at the University of North Carolina, Chapel Hill. Her research interests include the detection and fate of viruses in natural and human-built environments, and low-cost drinking water treatment technologies for developing countries.

References

1. Centers for Disease Control and Prevention. Immunization of health-care workers: recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Hospital Infection Control Practices Advisory Committee (HICPAC). MMWR Morb Mortal Wkly Rep. 1997;46(RR-18):1–42.
2. Boyce JM, Pittet D. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Infect Control Hosp Epidemiol. 2002;23(Suppl):S3–40. DOI: 10.1086/503164
3. Siegel J, Rhinehart E, Jackson M, Chiarello L; Healthcare Infection Control Practices Advisory Committee. Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings 2007 [cited 2007 Dec 31]. Available from http://www.cdc.gov/ncidod/dhqp/pdf/guidelines/Isolation2007.pdf
4. Chan-Yeung M. Severe acute respiratory syndrome (SARS) and healthcare workers. Int J Occup Environ Health. 2004;10:421–7.
5. Lau JTF, Fung KS, Wong TW, Kim JH, Wong E, Chung S, et al. SARS transmission among hospital workers in Hong Kong. Emerg Infect Dis [serial online] 2003 Feb [cited 2007 Dec]. Available from http://www.cdc.gov/ncidod/EID/vol10no2/03-0534.htm
6. Centers for Disease Control and Prevention. Sequence for donning and removing personal protective equipment (ppe). 2004 [cited 2007 Jan 21]. Available from http://www.cdc.gov/ncidod/sars/ic.htm
7. American Society for Testing and Materials. Standard test method for evaluation of health care professional handwash formulation (method e 1174–94). West Conshohocken (PA): The Society; 1994.
8. Environmental Protection Agency. Method 1601: male-speci c (F+) and somatic coliphage in water by two-step enrichment procedure. 2001 [cited 2008 Jan 17]. Available from http://www.epa.gov/nerlcwww/1601ap01.pdf
9. Respiratory Protection. 29 C.ER § 1910.134 (1998) [cited 2008 Jan 6]. Available from http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=12716
10. Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH Jr. Survival of in uenza viruses on environmental surfaces. J Infect Dis. 1982;146:47–51.
11. Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS coronavirus. Med Microbiol Immunol. 2005;194:1–6. DOI: 10.1007/s00430-004-0219-0
12. Gwaltney JM Jr, Hendley JO. Transmission of experimental rhinovirus infection by contaminated surfaces. Am J Epidemiol. 1982;116:828–33.
13. Gwaltney JM, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. Ann Intern Med. 1978;88:463–7.
14. Pittet D. Improving adherence to hand hygiene practice: a multidisciplinary approach. Emerg Infect Dis. 2001;7:234–40.
15. Doebbeling BN, Pfaller MA, Houston AK, Wenzel RP. Removal of nosocomial pathogens from the contaminated glove. Implications for glove reuse and handwashing. Ann Intern Med. 1988;109:394–8.

Address for correspondence: Mark Sobsey, Environmental Sciences and Engineering; CB# 7431 McGavran-Greenberg, University of North Carolina, Chapel Hill, NC 27599, USA; email: sobsey@email.unc.edu

4

Hindawi Publishing Corporation
Advances in Virology
Volume 2011, Article ID 734690, 7 pages
doi:10.1155/2011/734690

Research Article

The Effects of Temperature and Relative Humidity on the Viability of the SARS Coronavirus

K. H. Chan, J. S. Malik Peiris, S. Y. Lam, L. L. M. Poon, K. Y. Yuen, and W. H. Seto

Department of Microbiology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong

Correspondence should be addressed to K. H. Chan, chankh2@hkucc.hku.hk

Received 25 November 2010; Revised 31 July 2011; Accepted 31 July 2011

Academic Editor: Alain Kohl

The main route of transmission of SARS CoV infection is presumed to be respiratory droplets. However the virus is also detectable in other body fluids and excreta. The stability of the virus at different temperatures and relative humidity on smooth surfaces were studied. The dried virus on smooth surfaces retained its viability for over 5 days at temperatures of 22–25°C and relative humidity of 40–50%, that is, typical air-conditioned environments. However, virus viability was rapidly lost (>3 $\log_{10}$) at higher temperatures and higher relative humidity (e.g., 38°C, and relative humidity of >95%). The better stability of SARS coronavirus at low temperature and low humidity environment may facilitate its transmission in community in subtropical area (such as Hong Kong) during the spring and in air-conditioned environments. It may also explain why some Asian countries in tropical area (such as Malaysia, Indonesia or Thailand) with high temperature and high relative humidity environment did not have major community outbreaks of SARS.

1. Introduction

Severe acute respiratory syndrome (SARS), was a new emerging disease associated with severe pneumonia and spread to involve over 30 countries in 5 continents in 2003. A novel coronavirus was identified as its cause [1–3]. SARS had a dramatic impact on health care services and economies of affected countries, and the overall mortality rate was estimated to be 9%, but rising to 50% in those aged 60 or above [4]. A notable feature of this disease was its predilection for transmission in the health care setting and to close family and social contacts. The disease is presumed to be spread by droplets, close direct or indirect contact, but the relative importance of these routes of transmission is presently unclear. A study showed that viral aerosol generation by a patient with SARS was possible and therefore airborne droplet transmission was a possible means of transmission [5]. However, the role of fomites and environmental contamination in transmission of infection is presently still unclear. An outbreak of disease affecting over 300 residents in high-rise apartment block (Amoy Gardens) in Hong Kong could not be explained by respiratory droplet transmission from infected patients [6]. Infectious virus is detectable in the faeces [7], and aerosolization of virus in contaminated faeces is believed to be the mode of transmission of this outbreak [8].

We and others have reported that infectivity of SARS CoV (SARS coronavirus) was lost after heating at 56°C for 15 minutes but that it was stable for at least 2 days following drying on plastic. It was completely inactivated by common fixatives used in laboratory [9, 10]. Another study showed that it was inactivated by ultraviolet light, alkaline (pH > 12), or acidic (pH < 3) conditions [11]. Human coronaviruses have been shown to survive in PBS or culture medium with 5–10% FCS for several days [12–14] but they only survive a few hours after drying [13, 14]. There have been some studies reporting an association between the SARS outbreak, metrological factors, and air pollution [15–17]. Thus, information on the survival of the SARS coronavirus (SCoV) in the environment at different temperature and humidity conditions is of significant interest to understanding virus transmission. A recent study using surrogate coronaviruses (transmissible gastroenteritis virus (TGEV) and mouse hepatitis virus (MHC)) has investigated the effect of air

Table 1: WHO SARS report—based on data as of the 31st December 2003.

Areas	Total	Medan age	Deaths	Case fatality Ratio (%)	No. of imported Cases (%)	No. of HCW (%)	First case	Last case
China	5327	NKn	349	7	NA	1002 (19)	Nov-02	Jun-03
Hong Kong	1755	40	299	17	NA	386 (22)	Feb-03	May-03
Taiwan	346	42	37	11	21 (6)	68 (20)	Feb-03	Jun-03
Singapore	238	35	33	14	8 (3)	97 (41)	Feb-03	May-03
Viet Nam	63	43	5	8	1 (2)	36 (57)	Feb-03	Apr-03
Indonesia	2	56	0	0	2 (100)	0 (0)	Apr-03	Apr-03
Malaysia	5	30	2	40	5 (100)	0 (0)	Mar-03	Apr-03
Thailand	9	42	2	22	9 (100)	1 (11)	Mar-03	May-03
Philippines	14	41	2	14	7 (50)	4 (29)	Feb-03	May-03
Total	8096		774	9.6	142	1706 (21)		

temperature and relative humidity on coronavirus survival on surface [18]. The survival effects of these environmental factors on SARS coronavirus remain unclear. In the present study, we report the stability of the SARS coronavirus at different temperatures and relative humidity.

2. Material and Methods

2.1. Virus Strain and Cell Line. The SARS CoV strain used in this study is HKU39849. Foetal monkey kidney cells (FRhK-4) were cultured in minimal essential medium (MEM, Gibco, USA) with 10% foetal calf serum and penicillin streptomycin (Gibco, USA) at 37°C in 5% CO_2 and were used for growing stock virus and for titration of viral infectivity [1, 2].

2.2. Preparation of Stock Virus. Stock virus was harvested when infection approximately 75% of the cell monolayer of a virus infected flask manifested cytopathic effect (CPE). Infected cells were subjected to one cycle of freeze and thaw centrifuged at 2000 rpm for 20 minutes to remove cell debris and the culture supernatant was aliquoted and stored at −80°C until use.

2.3. Determination of Tissue Culture Infectious Dose (50%) ($TCID_{50}$). 96-well microtitre plates containing 100 μL of confluent FRhK-4 were infected with 100 μL of serial 10-fold of dilutions of stock virus in minimal essential medium with 1% FCS (maintenance medium) starting from 10^{-1} to 10^{8}. Titrations were done in quadruplicate. Infected cells were incubated for 4 days at 37°C. Appearance of CPE was recorded daily. $TCID_{50}$ was determined according to Reed and the Muench method [19].

2.4. Effect of Drying, Heat, and Relative Humidity. Ten microlitre of maintenance medium containing 10^7 $TCID_{50}$ per mL of virus was placed in individual wells of a 24-well plastic plates and allowed to dry at room temperature (22∼25°C) and relative humidity of 40–50% (i.e., conditions prevailing in a typical air-conditioned room). One hundred microlitre of MM was used to resuspend the virus at 0 hr, 3 hr, 7 hr, 11 hr, 13 hr, 24 hr, and up to 4 weeks and the residual virus infectivity was titrated. Controls in closed screw cap eppendorf tube were included each time and treated similarly but without drying.

The experiment was repeated at different temperatures (38°C, 33°C, 28°C) and relative humidities (>95%, 80∼89%) for 3 hr, 7 hr, 11 hr, 13 hr, and 24 hr. A nebulizer under a controlled condition was used to generate high and relative low humidity environment. All the experiments above were conducted in duplicate and the residual viral infectivity was titrated.

2.5. Infectivity Assay. The infectivity of residual virus was titrated in quadruplicate on 96-well microtitre plates containing 100 μL of confluent FRhK-4 cells. 100 μL of serial 10-fold of dilutions of virus in maintenance medium starting from 10^{-1} to 10^{8} was added into FRhK-4 cells. The infected cells were incubated at 37°C for 4 days. Appearance of CPE was recorded daily. $TCID_{50}$ was determined according to the Reed and Muench method [19].

3. Results

Ten microlitre of 10^7 $TCID_{50}$ per mL of virus was placed in individual wells of a 24-well plastic plate (representing a nonporous surface) and dried. The dried virus was then incubated at different temperatures (38°C, 33°C, 28°C) at different relative humidity (>95%, 80∼89%) for 3 hr, 7 hr, 11 hr, 13 hr, and 24 hr and the residual viral infectivity was titrated. A similar experiment was conducted at room temperature and relative humidity of about 40–50% (air-conditioned room) for up to 4 weeks. Virus dried on plastic retained viability for up to 5 days at 22∼25°C at relative humidity of 40∼50% with only 1 $\log_{10}$ loss of titre (Figure 1). After that virus infectivity is gradually lost ever time. Loss of virus infectivity in solution was generally similar to dried virus under these environmental conditions. This indicates that SARS CoV is a stable virus that may potentially be transmitted by indirect contact or fomites, especially in air-conditioned environments.

High relative humidity (>95%) at comparatively low temperature (28°C and 33°C) did not affect the virus infectivity significantly (Figure 2(a)). High temperature (38°C) at 80–90% relative humidity led to a 0.25∼2 $\log_{10}$ loss of titre at 24 hr (Figure 2(b)). However, if the dried virus was stored at

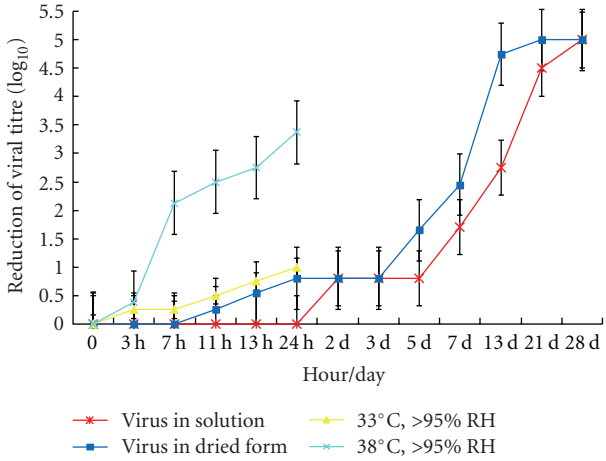


FIGURE 1: Residual virus infectivity at 22–25°C with relative humidity 40–50% (starting titre $10^5/10\,\mu$L) and at 33°C or 38°C with relative humidity >95%.

high temperature (38°C) and high relative humidity (>95%), there was a further ~1.5 log loss of titre for each time point up to 24 hr (0.38~3.38 $\log_{10}$) when compared with high temperature (38°C) at a lower relative humidity 80–90% (Figures 3(a)–3(c)).

4. Discussion

Viruses do not replicate outside living cell but infectious virus may persist on contaminated environmental surfaces and the duration of persistence of viable virus is affected markedly by temperature and humidity. Contaminated surfaces are known to be significant vectors in the transmission of infections in the hospital setting as well as the community. The role of fomites in the transmission of RSV has been clearly demonstrated [20]. Survival of viruses on a variety of fomites has been studied for influenza viruses, paramyxoviruses, poxviruses, and retroviruses [21]. The human coronavirus associated with the common cold was reported to remain viable only for 3 hours on environmental surfaces after drying, although it remains viable for many days in liquid suspension [13]. Parainfluenza and RSV viruses were viable after drying on surfaces for 2 and 6 hours, respectively [20, 22]. In aerosolised form, human coronavirus 229E is generally less stable in high humidity [12]. The environmental stability of SCoV was previously unknown and this information is clearly important for understanding the mechanisms of transmission of this virus in a hospital and community setting.

In the present study, we have demonstrated that SARS CoV can survive at least two weeks after drying at temperature and humidity conditions found in an air-conditioned environment. The virus is stable for 3 weeks at room temperature in a liquid environment but it is easily killed by heat at 56°C for 15 minutes [9]. This indicates that SARS CoV is a stable virus that may potentially be transmitted by indirect contact or fomites. These results may indicate that contaminated surfaces may play a major role in transmission of infection in the hospital and the community.

Our studies indicate that SCoV is relatively more stable than the human coronaviruses 229E or OC43 and some other viral respiratory pathogens such as respiratory syncytial virus. These findings suggest that, while direct droplet transmission is an important route of transmission [23], the role of fomites and environmental contamination in virus transmission may play a significant role in virus transmission. In particular, fomites may contribute to the continued transmission of infection in the nosocomial setting that continues to occur in spite of the great attention and stringent precautions taken to prevent droplet spread. In addition to droplet precautions, reenforcing contact precautions and hand washing is called for.

Faecal contamination of SCoV coronavirus may thus be an effective route of transmission of the disease. The outbreak in Amoy Garden in Hong Kong which affected over 300 residents in a single-apartment block with thought to have been transmitted by contaminated sewage. The stability of the virus on environmental surfaces and its presence in faeces indicates the potential that fecal contamination of fresh-food production may pose a threat for virus transmission; especially in countries with poor sanitation and sewage disposal systems and that studies to address this possibility are needed.

In this study, we showed that high temperature at high relative humidity has a synergistic effect on inactivation of SARS CoV viability while lower temperatures and low humidity support prolonged survival of virus on contaminated surfaces. The environmental conditions of countries such as Malaysia, Indonesia, and Thailand are thus not conducive to the prolonged survival of the virus. In countries such as Singapore and Hong Kong where there is a intensive use of air-conditioning, transmission largely occurred in well-air-conditioned environments such as hospitals or hotels. Further, a separate study has shown that during the epidemic, the risk of increased daily incidence of SARS was 18.18-fold higher in days with a lower air temperature than in days with a higher temperature in Hong Kong [24] and other regions [15–17]. Taken together, these observations may explain why some Asian countries in tropical area (with high temperature at high relative humidity) such as Malaysia, Indonesia, and Thailand did not have nosocomial outbreaks of SARS (Tables 1 and 2(a)–2(c)). It may also explain why Singapore, which is also in tropical area (Table 2(d)), had most of its SARS outbreaks in hospitals (air-conditioned environment). Interestingly, during the outbreak of SARS in Guangzhou, clinicians kept the windows of patient rooms open and well ventilated and these may well have reduced virus survival and this reduced nosocomial transmission. SARS CoV can retain its infectivity up to 2 weeks at low temperature and low humidity environment, which might facilitate the virus transmission in community as in Hong Kong which locates in subtropical area (Table 2(e)). Other environmental factors including wind velocity, daily sunlight, and air pressure, had shown to be associated with SARS epidemic, should also be considered [16, 17]. The dynamics of SARS epidemic involves multiple factors

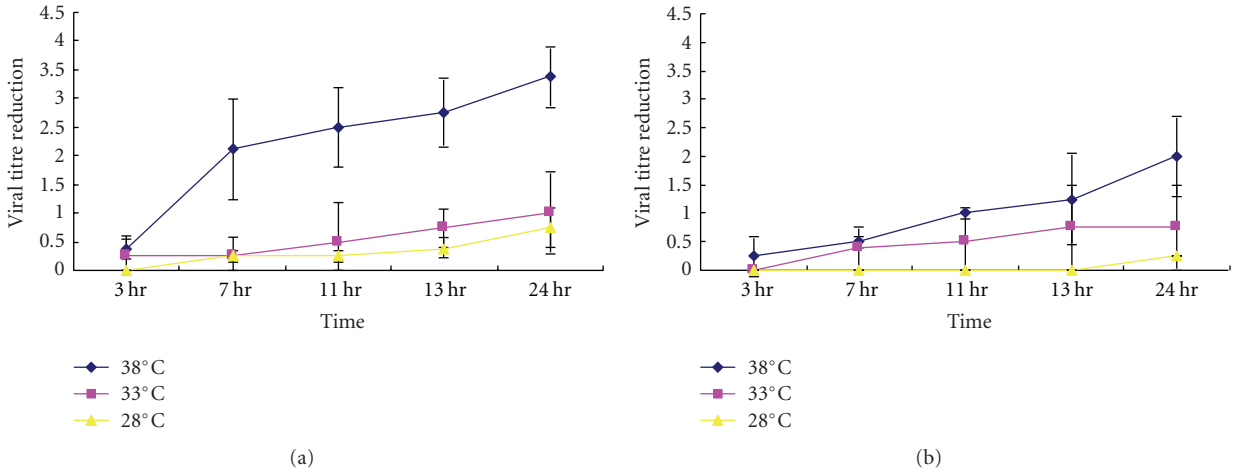


Figure 2: Infectivity of SARS Coronavirus ($10^5/10\,\mu$L) to different temperatures at (a) >95% relative humidity, (b) >80–89%.

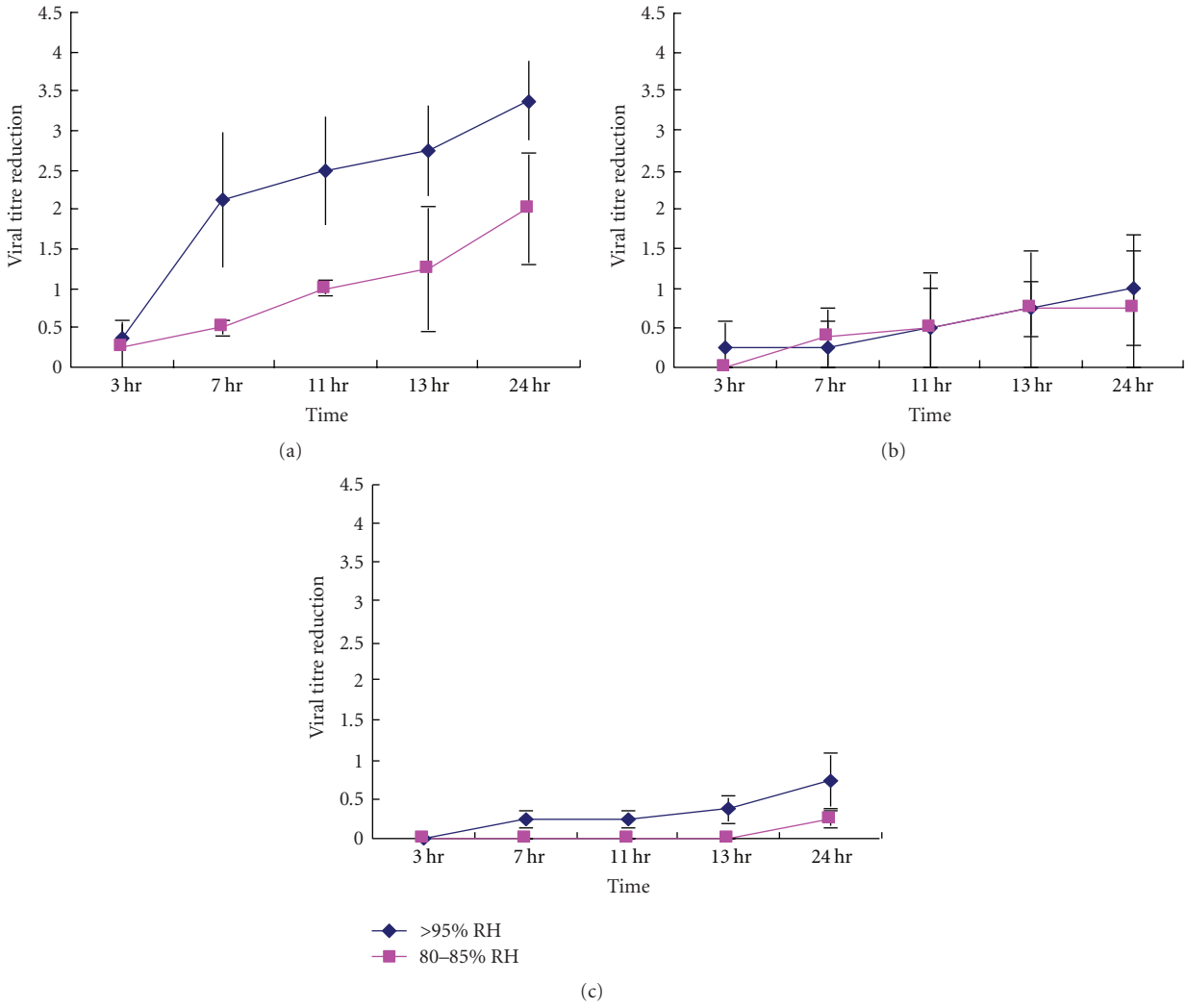


Figure 3: Infectivity of SARS Coronavirus (starting titre $10^5/10\,\mu$L) at different relative humidity at (a) 38°C, (b) 33°C, and (c) 28°C.

Table 2: A summary of the meteorological data of 2005 in average weather conditions*.

Month	Average sunlight (hours)	Temperature		Discomfort from heat and humidity	Relative humidity	
		Min	Max		am	pm
(a) Kuala Lumpur, Malaysia						
Jan	6	22	32	High	97	60
Feb	7	22	33	High	97	60
March	7	23	33	High	97	58
April	6	23	33	High	97	63
May	6	23	33	High	97	66
June	7	22	33	High	96	63
July	7	23	32	High	95	63
Aug	6	23	32	High	96	62
Sept	6	23	32	High	96	64
Oct	5	23	32	High	96	65
Nov	5	23	32	High	97	66
Dec	5	22	32	High	97	61
(b) Jakarta, Indonesia						
Jan	5	23	29	High	95	75
Feb	5	23	29	High	95	75
March	6	23	30	High	94	73
April	7	24	31	High	94	71
May	7	24	31	High	94	69
June	7	23	31	High	93	67
July	7	23	31	High	92	64
Aug	8	23	31	High	90	61
Sept	8	23	31	High	90	62
Oct	7	23	31	High	90	64
Nov	6	23	30	High	92	68
Dec	5	23	29	High	92	71
(c) Bangkok, Thailand						
Jan	9	20	32	High	91	53
Feb	8	22	33	High	92	55
March	9	24	34	High	92	56
April	8	25	35	Extreme	90	58
May	8	25	34	Extreme	91	64
June	6	24	33	Extreme	90	67
July	5	24	32	High	91	66
Aug	5	24	32	High	92	66
Sept	5	24	32	High	94	70
Oct	6	24	31	High	93	70
Nov	8	22	31	High	92	65
Dec	9	20	31	High	91	56
(d) Singapore						
Jan	5	23	30	High	82	78
Feb	7	23	31	High	77	71
March	6	24	31	High	76	70
April	6	24	31	High	77	74
May	6	24	32	Extreme	79	73
June	6	24	31	High	79	73
July	6	24	31	High	79	72
Aug	6	24	31	High	78	72
Sept	5	24	31	High	79	72
Oct	5	23	31	High	78	72
Nov	5	23	31	High	79	75
Dec	4	23	31	High	82	78

Table 2: Continued.

Month	Average sunlight (hours)	Temperature		Discomfort from heat and humidity	Relative humidity	
		Min	Max		am	pm
(e) Hong Kong						
Jan	5	13	18	—	77	66
Feb	4	13	17	—	82	73
March	3	16	19	—	84	74
April	4	19	24	Medium	87	77
May	5	23	28	Medium	87	78
June	5	26	29	High	86	77
July	8	26	31	High	87	77
Aug	6	26	31	High	87	77
Sept	6	25	29	High	83	72
Oct	7	23	27	Medium	75	63
Nov	7	18	23	Moderate	73	60
Dec	6	15	20	—	74	63

* Data is available at BBC weather website (http://www.bbc.co.uk/weather/world/city_guides/results).

including physical property of virus, outdoor and indoor environments, hygiene, space, and genetic predispositions [10, 15–17, 24, 25]. Understanding the stability of viruses in different temperature and humidity conditions is important in understanding transmission of novel infectious agent including that of the recent influenza Apandemic H1N12009.

Conflict of Interests

The authors declare that there is no conflict of interest.

References

[1] J. S. M. Peiris, S. T. Lai, L. L. M. Poon et al., "Coronavirus as a possible cause of severe acute respiratory syndrome," *The Lancet*, vol. 361, no. 9366, pp. 1319–1325, 2003.

[2] T. G. Ksiazek, D. Erdman, C. S. Goldsmith et al., "A novel coronavirus associated with severe acute respiratory syndrome," *New England Journal of Medicine*, vol. 348, no. 20, pp. 1953–1966, 2003.

[3] C. Drosten, S. Günther, W. Preiser et al., "Identification of a novel coronavirus in patients with severe acute respiratory syndrome," *New England Journal of Medicine*, vol. 348, no. 20, pp. 1967–1976, 2003.

[4] C. A. Donnelly, A. C. Ghani, G. M. Leung et al., "Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong," *The Lancet*, vol. 361, no. 9371, pp. 1761–1766, 2003.

[5] T. F. Booth, B. Kournikakis, N. Bastien et al., "Detection of airborne severe acute respiratory syndrome (SARS) coronavirus and environmental contamination in SARS outbreak units," *Journal of Infectious Diseases*, vol. 191, no. 9, pp. 1472–1477, 2005.

[6] Department of Health, "Outbreak of sever acute respiratory syndrome (SARS) at Amoy Gardens, Kowloon Bay, Hong Kong main findings of the investigation," 2003.

[7] J. S. M. Peiris, C. M. Chu, V. C. C. Cheng et al., "Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study," *The Lancet*, vol. 361, no. 9371, pp. 1767–1772, 2003.

[8] I. T. S. Yu, Y. Li, T. W. Wong et al., "Evidence of airborne transmission of the severe acute respiratory syndrome virus," *New England Journal of Medicine*, vol. 350, no. 17, pp. 1731–1739, 2004.

[9] WHO Report, "First data on stability and resistance of SARS coronavirus compiled by members of WHO laboratory network," http:www.who.int/csr/sars/survival_2003_05_04/en/#.

[10] M. Y. Lai, P. K. Cheng, and W. W. Lim, "Survival of severe acute respiratory syndrome coronavirus," *Clinical Infectious Diseases*, vol. 41, no. 7, pp. e67–e71, 2005.

[11] M. E. R. Darnell, K. Subbarao, S. M. Feinstone, and D. R. Taylor, "Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV," *Journal of Virological Methods*, vol. 121, no. 1, pp. 85–91, 2004.

[12] M. K. Ijaz, A. H. Brunner, and S. A. Sattar, "Survival characteristics of airborne human coronavirus 229E," *Journal of General Virology*, vol. 66, no. 12, pp. 2743–2748, 1985.

[13] J. Sizun, M. W. N. Yu, and P. J. Talbot, "Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections," *Journal of Hospital Infection*, vol. 46, no. 1, pp. 55–60, 2000.

[14] H. F. Rabenau, J. Cinatl, B. Morgenstern, G. Bauer, W. Preiser, and H. W. Doerr, "Stability and inactivation of SARS coronavirus," *Medical Microbiology and Immunology*, vol. 194, no. 1-2, pp. 1–6, 2005.

[15] J. Tan, L. Mu, J. Huang, S. Yu, B. Chen, and J. Yin, "An initial investigation of the association between the SARS outbreak and weather: with the view of the environmental temperature and its variation," *Journal of Epidemiology and Community Health*, vol. 59, no. 3, pp. 186–192, 2005.

[16] J. Yuan, H. Yun, W. Lan et al., "A climatologic investigation of the SARS-CoV outbreak in Beijing, China," *American Journal of Infection Control*, vol. 34, no. 4, pp. 234–236, 2006.

[17] Q. C. Cai, J. Lu, Q. F. Xu et al., "Influence of meteorological factors and air pollution on the outbreak of severe acute respiratory syndrome," *Public Health*, vol. 121, no. 4, pp. 258–265, 2007.

[18] L. M. Casanova, S. Jeon, W. A. Rutala, D. J. Weber, and M. D. Sobsey, "Effects of air temperature and relative humidity on coronavirus survival on surfaces," *Applied and Environmental Microbiology*, vol. 76, no. 9, pp. 2712–2717, 2010.

[19] L. J. Reed and H. Muench, "A simple method of estimating fifty per cent endpoints," *American Journal of Epidemiology*, vol. 27, no. 3, pp. 493–497, 1938.

[20] C. B. Hall, R. G. Douglas, and J. M. Geiman, "Possible transmission by fomites of respiratory syncytial virus," *Journal of Infectious Diseases*, vol. 141, no. 1, pp. 98–102, 1980.

[21] E. C. Pirtle and G. W. Beran, "Virus survival in the environment," *OIE Revue Scientifique et Technique*, vol. 10, no. 3, pp. 733–748, 1991.

[22] M. T. Brady, J. Evans, and J. Cuartas, "Survival and disinfection of parainfluenza viruses on environmental surfaces," *American Journal of Infection Control*, vol. 18, no. 1, pp. 18–23, 1990.

[23] W. H. Seto, D. Tsang, R. W. H. Yung et al., "Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS)," *The Lancet*, vol. 361, no. 9368, pp. 1519–1520, 2003.

[24] K. Lin, D. Y. K. Fong, B. Zhu, and J. Karlberg, "Environmental factors on the SARS epidemic: air temperature, passage of time and multiplicative effect of hospital infection," *Epidemiology and Infection*, vol. 134, no. 2, pp. 223–230, 2006.

[25] V. S. F. Chan, K. Y. K. Chan, Y. Chen et al., "Homozygous L-SIGN (CLEC4M) plays a protective role in SARS coronavirus infection," *Nature Genetics*, vol. 38, no. 1, pp. 38–46, 2006.

5

Persistence of the 2009 Pandemic Influenza A (H1N1) Virus on N95 Respirators

A. D. Coulliette, K. A. Perry, [...], and J. A. Noble-Wang

ABSTRACT

In the United States, the 2009 pandemic influenza A (H1N1) virus (pH1N1) infected almost 20% of the population and caused >200,000 hospitalizations and >10,000 deaths from April 2009 to April 2010. On 24 April 2009, the CDC posted interim guidance on infection control measures in health care settings explicitly for pH1N1 and recommended using filtering face respirators (FFRs) when in close contact with a suspected- or confirmed-to-be-infected individual, particularly when performing aerosol-generating procedures. The persistence and infectivity of pH1N1 were evaluated on FFRs, specifically N95 respirators, under various conditions of absolute humidity (AH) (4.1×10^5 mPa, 6.5×10^5 mPa, and 14.6×10^5 mPa), sample matrices (2% fetal bovine serum [FBS], 5 mg/ml mucin, and viral medium), and times (4, 12, 24, 48, 72, and 144 h). pH1N1 was distributed onto N95 coupons (3.8 to 4.2 cm^2) and extracted by a vortex-centrifugation-filtration process, and the ability of the remaining virus to replicate was quantified using an enzyme-linked immunosorbent assay (ELISA) to determine the $\log_{10}$ concentration of the infectious virus per coupon. Overall, pH1N1 remained infectious for 6 days, with an approximately 1-$\log_{10}$ loss of virus concentrations over this time period. Time and AH both affected virus survival. We found significantly higher ($P \leq 0.01$) reductions in virus concentrations at time points beyond 24 to 72 h (−0.52-$\log_{10}$ reduction) and 144 h (−0.74) at AHs of 6.5×10^5 mPa (−0.53) and 14.6×10^5 mPa (−0.47). This research supports discarding respirators after close contact with a person with suspected or confirmed influenza infection due to the virus's demonstrated ability to persist and remain infectious.

INTRODUCTION

The 2009 H1N1 pandemic influenza A (H1N1) virus (pH1N1) outbreak affected >214 countries and caused at least 18,449 deaths worldwide (WHO, 6 August 2010). The estimated impact, as extrapolated from laboratory-confirmed hospitalizations in the United States from April 2009 to April 2010, was 60.8 million cases (range, 43.3 to 89.3 million), 274,304 hospitalizations (range, 195,086 to 402,719), and 12,469 deaths (range, 8,868 to 18,306) (1). The current Centers for Disease Control and Prevention (CDC) *Prevention Strategies for Seasonal Influenza in Healthcare Settings: Guidelines and Recommendations* states that face masks are a sufficient form of personal protective equipment (PPE) for hospital staff, associated workers, patients, and visitors when a person is suspected or known to be infected (3). During the pandemic, the first CDC interim guidance statement was posted on 24 April 2009 regarding infection control measures in health care settings specifically for pH1N1. Filtering facepiece respirators (FFRs) (i.e., N95) were recommended (in addition to standard precautions) in this guidance document as a conservative measure to protect health care personnel when patients are in isolation, particularly during aerosol-generating procedures, and for those in close contact with patients with suspected or confirmed pH1N1 infections (2, 4).

The number of N95 FFRs used during the 2009 pandemic period is unclear, and supply shortages were acknowledged in the CDC 2009 H1N1 Influenza Interim Guidance document (2). A study by the Institute of Medicine stated that 90 million respirators would be needed for a 42-day influenza pandemic (5). Meanwhile, Murray et al. (6) found that facial protective equipment (e.g., masks, respirators, and disposable eyewear) use more than doubled in the Vancouver Coastal Health service region during the 2009 pandemic. Specifically for respirators, the rate of use during the pandemic was 51% higher than the historical baseline; to estimate the supplies needed in the event of a pandemic, the authors suggested a 1:1 ratio of respirators to masks in acute care facilities where aerosol-generating medical procedures are performed (6). The numbers of FFRs used during influenza virus outbreaks are daunting due to the protocols (i.e., “donning and doffing” for every room), while minimal direct evidence on the exclusion of influenza A virus during FFR use and survival after deposition remains elusive.

N95s provide 99.5% filtration efficiency for particles >0.75 μm and ≥95% for particles between 0.1 to 0.3 μm (7). Influenza A virus is approximately 120 nm in diameter (8). Thus, with a proper seal, N95s deliver protection from infectious particles ranging from large droplets (>100 μm) to inhalable droplets (10 to 100 μm) and to nuclear aerosols (<10 μm) (9, 10). However, the main transmission route of influenza virus infection continues to be a topic of debate (9–12). Some contend that airborne transmission via small-particle aerosols is a feasible pathway that has not been given the appropriate attention (10, 11), while others cite evidence for close contact and large droplets as the cause of influenza infection (9, 12). Fomite transmission, particularly within the hospital setting, is another area for which data are limited. Regardless of deposition and transmission routes, knowledge about the survival and persistence of influenza A virus on the exterior of the facepiece is needed because of the repeated donning and doffing of FFRs and subsequent hand hygiene considerations.

Influenza A virus is an enveloped virus, and its lipid bilayer is a main determinant of survival, as viruses with higher lipid contents persist better under lower-humidity conditions (13). Research regarding influenza virus survival on surfaces has mostly focused on stainless steel (14–16). For the study of survival and interactions on respirators, MS2 coliphage, a single-stranded RNA [ss(+)RNA] virus that infects *Escherichia coli*, has been used as the

surrogate (17–19). Previous studies provided insight into mostly older strains of influenza A virus, such as A/Brazil/11/78-like (16) and A/PR/8/34 (20), various materials (pajamas, tissue, soft toys, surgical masks, and hospital gowns) (16, 21), and a single absolute humidity (AH) (16, 20, 21). We used a robust design to evaluate the persistence and infectivity, as defined by the ability to infect tissue culture, of the pH1N1 virus deposited on N95 FFR materials under different conditions of AH, tested in various sample matrices (component of the sample besides pH1N1, such as mucus), and measured at time periods up to 6 days.

MATERIALS AND METHODS

The study evaluated the survival and infectivity of the pH1N1 virus within three matrices: viral medium (Dulbecco's modified Eagle medium [DMEM]) (Gibco, Grand Island, NY), 2% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA), and 5 mg/ml mucin (MP Biomedicals, Soloni, OH) on coupons of N95 respirators (model no. 8210; 3M, St. Paul, MN). We studied survival under AH conditions of 4.1×10^5 mPa (18°C and 20% relative humidity [RH]), 6.5×10^5 mPa (25°C and 20% RH), and 14.6×10^5 mPa (21°C and 58.5% RH) for 0, 4, 12, 24, 48, 72, and 144 h time points. The experiments were performed three times for all conditions, with the exception of the 144-h time point at the 4.1×10^5 mPa AH, which was performed twice. All sample sizes were 9, with the following exceptions: FBS, 4.1×10^5 mPa for 12 h ($n = 6$) and 144 h ($n = 3$); viral medium, 14.6×10^5 mPa for 24 h ($n = 8$) and 144 h ($n = 6$); mucin, 4.1×10^5 mPa for 12 to 72 h ($n = 6$); mucin, 6.5×10^5 mPa for 72 and 144 h ($n = 6$); and mucin, 14.6×10^5 mPa for 24 and 48 h ($n = 8$).

Experiment parameters. (i) Influenza pH1N1 virus and propagation. Influenza virus A/California/04/2009 H1N1 (influenza virus A [H1N1] pdm; CDC identification no. 2009712047; lot no. 08/13/2009) was obtained from the Influenza Division, CDC, and propagated in Madin-Darby canine kidney (MDCK) cells as described by Szretter et al. (22). The method is briefly detailed here. Confluent MDCK cells were washed twice with room temperature phosphate-buffered saline (PBS) (Gibco, Grand Island, NY) and once with complete DMEM (cDMEM)–7.5% bovine serum albumin (BSA) (Fisher, Fair Lawn, NJ). The virus, thawed in cool water, was diluted to obtain a multiplicity of infection (ratio of influenza virus to MDCK cells) of 1:100 with viral growth medium (diethyl maleate [DEM], 7.5% BSA), 2% penicillin-streptomycin (stock concentration, 10,000 units/ml penicillin G sodium and 10,000 μg/ml streptomycin sulfate) (Life Technologies, Carlsbad, CA), HEPES buffer (Gibco, Grand Island, NY), and tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-treated trypsin (ThermoScientific, Rockford, IL). One milliliter of the diluted virus suspension was added to the MDCK cell monolayer. The suspension was rotated to thoroughly cover the entire monolayer and was incubated at 37°C for 45 min. Viral growth medium (20 ml) was added to the monolayer, and the flask (75 cm^2) was not harvested until cytopathic effects (CPE) were detected in 75% of the monolayer. The monolayer supernatant was centrifuged for 15 min at 300 × *g*, and the supernatant was then divided into cryovials and stored at −80°C until the experiment. In an effort to prepare sufficient pH1N1 for the entire experiment, several flasks were prepared to propagate the virus at the same time. Once all the flasks showed the proper percentages of CPE, the virus was isolated from all the flasks and combined into one large population, and a stock concentration was rendered, averaging a 4.3×10^5 tissue culture infectious dose of 50% ($TCID_{50}$) per ml. Infectious pH1N1 was quantified as $TCID_{50}$, which refers to the number of pH1N1 that produced CPE in 50% of the cells inoculated.

(ii) Test matrices. Viral medium, 2% FBS, and mucin (5 mg/ml) were used as the test matrices. Viral medium (detailed above) was used as a control matrix, while 2% FBS and mucin were proxies for sputum and mucus-like material generated during sneezing and coughing. The stock matrices of 4% FBS and mucin (10 mg/ml) were prepared and stored at −20°C, which were later combined during the experiment with equal volumes of virus suspension to achieve the desired 2% FBS and 5-mg/ml mucin concentrations. Viral medium was stored at 4°C until the experiment and also was combined with equal volumes of the virus suspension for the experiments.

(iii) N95 respirator coupons. The 3M model no. 8210 N95 was chosen for evaluation because that respirator was listed in the Strategic National Stockpile (CDC, Atlanta, GA), approved for infection control in health care settings, and readily available. Additional details regarding the respirator can be found in a report by Fisher et al. (23). Circular coupons (3.8 to 4.2 cm^2) were punched from N95 respirators using a grommet and hammer, placed in six-well plates with the exterior of the mask facing upwards for the outer shell to be exposed (Costar, Corning, NY), and UV sterilized for ≥15 min prior to the experiment.

(iv) Absolute humidity. AH was defined by Shaman and Kohn as the "actual water vapor content of air irrespective of temperature" (24). This parameter reflects the relationship between percent relative humidity (% RH) and temperature, both of which are documented to influence the survival of influenza virus (24). Absolute humidity (AH) was calculated from measured temperature (°C) and % RH conditions. The vapor pressure (VP) of water used in the measurement for AH was VPw = % RH × ([SVP/100]%), where VPw is the vapor pressure of water vapor, % RH is percent relative humidity, and SVP is the saturated vapor pressure (in mPa), defined as $SVP = (6.11 \times 10^5 \text{ mPa}) \times e^{(0.067 \times T)}$, where T is the temperature in degrees Celsius. The three AH conditions as measured via vapor pressure (VP), 4.1×10^5 mPa (18°C and 20% RH), 6.5×10^5 mPa (25°C and 20% RH), and 14.6×10^5 mPa (21°C and 58.5% RH), were maintained within an environmental chamber (model no. 6030; Caron, Marietta, OH) that was monitored with a temperature- and % RH-traceable sensor (Control Company, Friendswood, TX). The temperature (°C) and % RH were checked at least twice a day during the experimental time periods to ensure that the correct predetermined AH was attained within the environmental chamber.

(v) Time points. Previous research on the survival of influenza A virus when suspended in viral medium on porous surfaces showed compelling reductions in viable viruses within approximately 24 to 48 h (16, 20). We studied additional time points within this 24- to 48-h period (4, 12, and 24 h) and also extended testing to 72 h. In our initial experiments, 72 h was the final time point at which we measured survival, similar to the procedure followed by Bean et al. (16). However, testing at 144 h (6 days) was added after the first two experiments at 4.1×10^5 mPa VP AH to detect if complete die-off occurred. In summary, triplicate coupons were processed for each VP value, matrix, and time point (0, 4, 12, 24, 48, 72, and 144 h), with the exception of the 144-h time point for the first two experiments at 4.1×10^5 mPa.

Sample processing. (i) Cell culture. MDCK cells (CCL-3; ATCC, Manassas, VA) were maintained in tissue culture flasks (Corning, Corning, NY) until passage 90, at which time new cells were started. A modified procedure, as described by Szretter et al., was followed (22). The flasks (150 cm^2) were seeded with 4×10^4 to 2.0×10^5 cells per ml, and cultures were grown to approximately 90 to 95% confluence under a 5% CO_2 atmosphere at 37°C for 24 to 72 h. The medium for cell growth consisted of DMEM, containing fetal bovine serum (10% for growth and 2% for maintenance) and 2% penicillin-streptomycin.

(ii) N95 respirator and pH1N1 processing. The UV-sterilized N95 respirator coupons and required sterilized supplies (forceps, cell spreaders, pipettes, pipette tips, etc.) were placed in a biosafety cabinet, in addition to the H1N1 stock inoculum and sample matrices (viral medium, 2% FBS, and 5 mg/ml mucin). The virus and sample matrices were prepared in equal parts and mixed. The virus-matrix suspension was inoculated (100 µl) onto individual respirator coupons in triplicate for each time point (see Fig. 1). The inoculated coupons dried in the biosafety cabinet for 1 h.

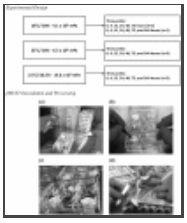


Fig 1

Experimental design and photographs of the procedure for inoculating pH1N1 virus onto N95 respirators, where pH1N1 in the sample matrix was inoculated onto the exterior layer of three N95 coupons (a) and spread evenly for homogenous distribution and to ...

Once the virus dried on the coupon, the inoculated coupon was placed inside a 15-ml conical tube (BD Falcon, Franklin Lakes, NJ) and 5 ml of 2% BSA–1× PBS (pH 8.5) was added. To separate pH1N1 from cell debris, the sample was vortexed for 20 min and centrifuged for 5 min at 3,000 × *g* to pellet the cell debris. To further purify the sample, the supernatant was removed and filtered through a premoistened (2% BSA–1× PBS) 0.22-µm syringe filter (Fisherbrand, Pittsburgh, PA; Millex-GS, Billerica, MA) into 1.5-ml Safe-Lock tubes (Eppendorf, Hauppauge, NY). The samples were labeled and stored at −80°C until processing.

(iii) ELISA. MDCK cells at approximately 95% confluence were washed with 1× PBS, separated from the flask using trypsin-EDTA, concentrated by centrifugation at 500 × *g* for 10 min, and resuspended in viral medium (as described above). The sample (150 µl) underwent a 1:3 dilution (50 µl) in 96-well plates (Costar, Corning, NY) with 100 µl of viral culture medium (DMEM–1% BSA), for a total of 10 dilutions. MDCK cells (100 µl) were then pipetted into each well in the 96-well plates with the diluted samples. The plates were incubated under a 5% CO_2 atmosphere at 37°C overnight. The range of detection for the experiments was 1.44×10^1 to 3.40×10^5 $TCID_{50}$ per ml.

Using a BioTek ELx405 Select CW plate washer (BioTek Instruments, Winooski, VT), the plates were rinsed with 1× PBS. Manually, 80% acetone–1× PBS (cold) was added to every well and incubated for 8 min at room temperature. The acetone mixture was removed and the plates dried for 20 min. The BioTek plate washer was used for the remainder of the wash steps during immunostaining. Mouse anti-influenza A virus monoclonal antibody (Millipore, Temecula, CA) diluted 1:1,000 in 1× PBS–Tween 20–1% BSA was added (100 µl) to each well, incubated for an hour, and washed three times with 1× PBS–Tween 20 (200 µl). The secondary antibody, peroxidase-labeled affinity-purified goat anti-mouse IgG (KPL, Gaithersburg, MD), diluted 1:1,000 in 1× PBS–Tween 20–1% BSA, was then added to each well (100 µl), incubated at room temperature for 1 h, and washed three times with 1× PBS–Tween 20 (200 µl). A substrate development solution consisting of phosphate-citrate buffer with sodium (Sigma-Aldrich, St. Louis, MO), *o*-phenylenediamine dihydrochloride (ODP) tablets (10 mg) (Sigma-Aldrich, St. Louis, MO), and hydrogen peroxide (Sigma-Aldrich, St. Louis, MO) was added to each well and incubated at room temperature for 10 min, followed by the addition of 33 µl of sulfuric acid (1 N). The samples in the 96-well plates were read by a Synergy II plate reader (BioTek Instruments) with the Gen5 (v1.11 and 2.00) program set for reading a 96-well full plate at 490 nm absorbance. Data output was transferred to Microsoft Excel v14 (Redmond, WA) and the $TCID_{50}$ for each sample was calculated using the method of Reed and Muench (25).

(iv) Data analysis. Microsoft Excel v14 (Redmond, WA) was used for data formatting, $\log_{10}$ transformation, and averaging, while IBM SPSS v19 (Somers, NY) was used for descriptive statistics (median, mean, minimum, and maximum) and the box plot graphic. The virus concentration for each coupon was $\log_{10}$ transformed. The triplicate coupons for each time point were averaged, and the $\log_{10}$ change was calculated by subtracting the $\log_{10}$ virus per coupon from the sample's respective time zero $\log_{10}$ per coupon. The $\log_{10}$ change relative to the zero time point was used for statistical

analysis.

SAS v9.2 (Cary, NC) was used to create general linear models that assessed the potential relationships between the mean $\log_{10}$ change of virus concentration and the three independent parameters under study: viral medium, absolute humidity, and die-off time. For these analyses, the data points were used individually and not averaged. Maximum likelihood estimates (MLEs) and standard errors were analyzed to determine the statistical differences within the levels of each parameter (i.e., sample matrix, absolute humidity, and time points). To account for the correlation of the mean $\log_{10}$ differences due to clustering of replicates over time, the method of generalized estimating equations (GEE) with a compound symmetrical correlation structure was implemented. GEE parameter estimates and robust empirical standard errors were obtained using a P value of 0.01 as the significance level for staying in the model.

RESULTS

The average starting inoculum was 4.3×10^5 pH1N1 $TCID_{50}$ per ml ($n = 27$), or 4.3×10^4 pH1N1 $TCID_{50}$ per coupon (100 µl), and the average recovery concentration was 1×10^3 pH1N1 $TCID_{50}$ per ml, or 1×10^2 pH1N1 $TCID_{50}$ per coupon, at time zero after 1 h of drying. The loss in recovery is attributed to desiccation and/or attachment to the N95 coupon. The infectivity of pH1N1 $TCID_{50}$ per coupon is represented by its respective $\log_{10}$ concentration in Fig. 2. The overall trend shows a decrease in infectivity over the 6 days for each matrix and AH. For pH1N1 in viral medium, the recovered median $\log_{10}$ per coupon concentration started at 1.80, 2.40, and 1.20 for 4.1×10^5 mPa, 6.5×10^5 mPa, and 14.6×10^5 mPa, respectively, and decreased to 0.00, 0.94, and 0.16, respectively, at the 144-h endpoint. In FBS (2%), the recovered $TCID_{50}$ $\log_{10}$ per coupon concentrations started at 1.36, 1.49, and 1.35 for 4.1×10^5 mPa, 6.5×10^5 mPa, and 14.6×10^5 mPa, respectively, and decreased to 0.00, 0.77, and 0.42, respectively, at the 144-h endpoint. In mucin (5 mg/ml), the recovered concentrations started at 1.04, 2.12, and 2.28 for 4.1×10^5 mPa, 6.5×10^5 mPa, and 14.6×10^5 mPa, respectively, and decreased to 0.16, 0.16, and 0.72, respectively, by the 144-h endpoint (except for the 4.1×10^5 mPa condition, where the last time point included was 72 h).

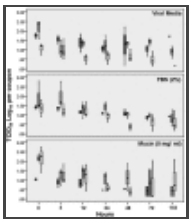


Fig 2

The pH1N1 virus $TCID_{50}$ $\log_{10}$ concentration per coupon over time (6 days) for different matrices and absolute humidity (AH) levels, where the horizontal line in the middle mark of each bar represents the median, the top and bottom of the bars represent ...

For each time point, the $\log_{10}$ change, compared to the zero time point, similarly illustrates a reduction in infectivity over time (Table 1). The range of the $\log_{10}$ change (lowest to highest) for 4.1×10^5 mPa was 0.01 to −1.33, −0.06 to −0.56, and −0.13 to −0.59 for viral medium, FBS, and mucin, respectively. The range of the $\log_{10}$ change for 6.5×10^5 mPa was −0.85 to −1.34, 0.00 to −1.40, and −0.70 to −1.72 for viral medium, FBS, and mucin, respectively. The range of the $\log_{10}$ change for 14.6×10^5 mPa was 0.07 to −0.97, −0.14 to −1.10, and −0.77 to −1.99 for viral medium, FBS, and mucin, respectively.

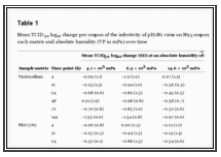


Table 1

Mean $TCID_{50}$ $\log_{10}$ change per coupon of the infectivity of pH1N1 virus on N95 coupons in relation to time zero for each matrix and absolute humidity (VP in mPa) over time

The MLE univariate analysis revealed significant differences within each of the parameter groups in regard to the $\log_{10}$ change of pH1N1 influenza A virus (Table 2). The higher VP AHs, 14.6×10^5 mPa ($P < 0.01$) and 6.5×10^5 mPa ($P < 0.0001$), resulted in significantly greater pH1N1 $\log_{10}$ reductions relative to the reference VP AH of 4.1×10^5 mPa. This translates into an overall model estimate of a −0.69- and −0.75-$\log_{10}$ reduction for 14.6×10^5 mPa and 6.5×10^5 mPa, respectively, when AH is evaluated alone regarding pH1N1 survival on N95 (Table 2). pH1N1 had a significantly larger reduction in viral medium ($P < 0.0001$), the reference matrix, than in FBS or mucin. However, the $\log_{10}$ changes of pH1N1 in FBS ($P = 0.08$) and mucin ($P = 0.07$) were not significantly different from one another. Reductions in pH1N1 infectivity at the tested time points significantly increased from the reference point of 4 h, with the exception of the 12-h time point ($P = 0.31$), where the significance levels were as follows: 24 h, $P < 0.01$; 48 h, $P < 0.01$; 72 h, $P < 0.01$; and 144 h, $P < 0.0001$. This means that there was subsequently greater loss in survival over time, where the $\log_{10}$ reductions at 4 h, 48 h, and 144 h (6 days) estimated by the model were −0.35, −0.71, and −0.97, respectively (Table 2, where the intercept was added with MLE for the individual parameter).

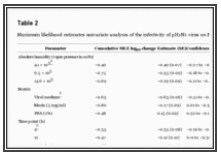


Table 2

Maximum likelihood estimates univariate analysis of the infectivity of pH1N1 virus on N95 coupons[a]

The GEE multivariate analysis data, shown in Table 3, demonstrated the overall $\log_{10}$ change of pH1N1 for each parameter when the data were simultaneously modeled, taking into account the potential correlations between the triplicate experimental runs. The parameters that had a significant impact on the $TCID_{50}$ $\log_{10}$ change of pH1N1 under the given conditions were the VP AHs of 14.6×10^5 mPa ($P < 0.01$) and 6.5×10^5 mPa ($P < 0.01$), as well as the time periods of 144 h ($P < 0.01$) and 24 to 72 h ($P < 0.01$). The GEE model illustrates that the $\log_{10}$ reduction of pH1N1 of −0.53 and −0.47 was attributed to the 6.5×10^5 mPa and 14.6×10^5 mPa VP AH values, respectively, while the $\log_{10}$ reduction of −0.74 and −0.52 might be credited to the 144-h and 24- to 72-h time periods (Table 3, where the intercept was added with GEE for the individual parameter). The matrices (viral medium, FBS, and mucin) did not have a significant impact on the survival of pH1N1.

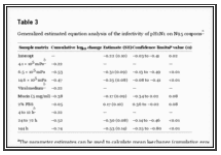


Table 3

Generalized estimated equation analysis of the infectivity of pH1N1 on N95 coupons[a]

DISCUSSION

Overall, pH1N1 (A/California/04/2009) remained infectious for 6 days with an approximately 1-$\log_{10}$ loss when deposited onto coupons of N95 respirators under the given conditions. While AH impacted survival in our experiments, the GEE multivariate analysis of factors suggested that the main component affecting survival at 6 days was elapsed time, which contributed to an overall −0.74 $TCID_{50}$ $\log_{10}$ reduction. Although the concentration of influenza virus that is potentially transferred from a respirator to hands and fingers is unknown, understanding that influenza virus might remain infectious for 6 days on the exterior side of a respirator (i.e., there might be a risk for transmission) is vital for health care personnel, patients, and visitors. Health care personnel who are in constant contact with confirmed or suspected cases of influenza should dispose of their respirators prior to leaving a patient's room.

There are three papers that have been published specifically on the persistence of the influenza virus on respirators or porous surfaces. Bean et al. researched the survival of an A/Brazil/11/78 (H1N1)-like virus on pajamas, tissues, magazines, and handkerchiefs (16). Greatorex et al. examined the survival of A/Cambridge/AHO4/2009 (H1N1) virus on various household materials, including the porous surfaces of a J Cloth, silver-containing cloth, and a soft toy (21). Sakaguchi et al. studied the survival of A/PR/8/34 (H1N1) virus on N95 respirators (Hi-Luck 350), surgical masks, and hospital gowns (20). Bean et al. and Sakaguchi et al. used cell cultures to measure infectivity with an approximate AH of 14.6×10^5 mPa (16, 20), while Greatorex et al. set AH conditions around 5×10^5 mPa (21). All three studies came to a similar conclusion that influenza A viruses show reductions in infectivity within 24 to 48 h. An additional study examined the persistence of various influenza A viruses on bank notes and found that influenza virus A/Moscow/10/99 (H3N2) remained infectious for up to 3 days and had increased survival in respiratory secretions (26).

The results from this study conflict with those of Bean et al. (16) and Sakaguchi et al. (20), which used A/Brazil/11/78 (H1N1)-like and A/PR/8/34 (H1N1) viruses, respectively. The two studies can be compared with our study in terms of influenza virus survival within viral medium at ~14.6×10^5 mPa (27.8°C to 28.3°C and 35% to 40% RH [16] and 25.2°C and 55% RH [20]). A 3-$\log_{10}$ reduction in A/PR/8/34 infectivity was observed after 24 h on N95 respirators (20), while A/Brazil/11/78 (H1N1)-like was undetectable (~3-$\log_{10}$ loss) within 8 to 12 h on porous surfaces (16). We studied the pH1N1 A/California/04/2009 virus and found only an overall 0.43-$\log_{10}$ reduction at 24 h and a 0.97-$\log_{10}$ reduction after 6 days at the 14.6×10^5 mPa VP AH (Table 1). A main component of persistence and infectivity is the interaction between the viral envelope and AH, as viruses with greater lipid content persist better under lower humidity conditions (13). Viral mutations and reassortments in the year-to-year strains provide the virus with new survival capabilities. While all three studies used cell culture infectivity methods and the three strains are all descendants of the 1918 pandemic virus, the pH1N1 virus is a reassortment of the North American swine (H3N2 and H1N2) and Eurasian H1N1 viruses (27, 28). This reassortment might be responsible for the greater persistence of pH1N1 virus than the A/Brazil/11/78 (H1N1)-like and A/PR/8/34 (H1N1) viruses under the given conditions. The unusual constellation of genes from multiple lineages (28) was a factor in its greater persistence due to the new unevaluated structural components.

As discussed previously, AH significantly impacts the survival of aerosolized influenza virus (24, 29). Besides the present study, only one other study evaluated the survival of H1N1 on respirators (20). As noted previously, Sakaguchi et al. found a marked loss of infectivity at only 24 to 48 h, and although this might largely reflect differences in the HIN1 strains, the only conditions they studied consisted of a relatively high AH of ~14.6×10^5

mPa (25.2°C and 55% RH) (20). We evaluated three different AHs and found that $\log_{10}$ reductions were significantly higher under both the 6.5×10^5 mPa ($P < 0.01$) and 14.6×10^5 mPa ($P < 0.0001$) conditions than under the lowest condition of 4.1×10^5 mPa (Table 2). The multivariate GEE analysis, where all parameters are simultaneously evaluated, further confirms the important role of increased AH in overall decreased pH1N1 survival (Table 3). Humidity is controlled in health care facilities, and the *Ventilation Standard for Health Care Facilities* lists the recommended humidity levels for a variety of health care spaces (i.e., trauma room and wound intensive care), ranging from 30 to 60% for relative humidity and 20 to 24°C for temperature (30). This equates to an absolute humidity as measured by vapor pressure at approximately 7×10^5 mPa to 18.3×10^5 mPa, of which this study examined two conditions (4.1×10^5 mPa and 6.5×10^5 mPa) at the lower end and one condition (14.6×10^5 mPa) at the higher end of the scale. Hence, our study approximated AH levels typically present in many health care settings. Extra caution, however, should be taken in U.S. temperate regions during the wintertime to properly adjust the humidity settings to stay within this approved range.

Because influenza is dispersed via small or large respiratory droplets, mucus and saliva are the most likely matrices by which the virus is deposited on surfaces. Mucus and saliva mainly consist of water, with mucous glycoproteins, free proteins, and other electrolytes as the remaining constituents (37, 38). The substrates we used to suspend the virus were meant to simulate key constituents of phlegm or saliva and did not appear to have an important impact on the overall persistence of pH1N1, as determined by the GEE analysis. This suggests that similar precautions for preventing fomite transmission of influenza virus should be taken regardless of the presence or absence of visible respiratory secretions. The Strategic National Stockpile contained a variety of National Institute of Occupational Safety and Health (NIOSH)-approved particulate N95 respirators (3M, Moldex, Moldex-Metrix, Kimberly Clark, and Gerson) that were authorized for release for emergency use during the 2009 H1N1 pandemic (31). The CDC's *Interim Guidance on Infection Control Measures for 2009 H1N1 Influenza in Healthcare Settings, Including Protection of Healthcare Personnel* recommended the use of a fit-tested N95 respirator (NIOSH-approved) for health care personnel who are within 6 feet of a patient or within a small enclosed airspace with a suspected or confirmed H1N1 patient (2). The 3M N95 respirator meets the 95% efficiency level and N series tests with NaCl, hence the name N95, and is comprised of three layers. The details of the layers are proprietary; however, the electrostatically charged polypropylene fibers play an important role in protecting the user from viruses. There are publications citing potential disinfection methods for contaminated respirators (32–35) that maintain their integrity (36), although decontamination of disposable FFRs for the purpose of reuse has not been recommended by the CDC.

The pH1N1 pandemic stressed the health care and public health infrastructures with challenges, such as how to effectively disseminate vaccines and respirators in a rapid manner to health care personnel and facilities. Although heroic efforts were put forth in addressing these challenges, it is fortunate that the 2009 H1N1 strain was a less-virulent strain than was initially anticipated. Particulate respirators (i.e., N95s) were incorporated into the CDC's *Interim Guidance on Infection Control Measures for 2009 H1N1 Influenza in Healthcare Settings, Including Protection of Healthcare Personnel* as a cautionary approach during the pandemic (2). However, as the pandemic progressed and supplies were exhausted, questions about the persistence of influenza on porous media and respirators were posed due to a desire to increase respirator supply through extended wear and reuse. This research supports discarding respirators after close contact with a person having a suspected or confirmed influenza infection due to the virus's demonstrated ability to persist for 6 days on the outer side of the FFR with only an approximate 1-$\log_{10}$ loss in infectivity. While this study examined the impact of AH on pH1N1 on the exterior of FFRs, it is worth noting that a person's respiration and water vapor on the inner side of the respirator might also play a role in its persistence and infectivity. The starting concentration of influenza on respirators, the transmission rate from fomites to hands, and the human infectious dose remain unclear. Further research is needed to determine the risk of transmission from influenza-contaminated respirators.

ACKNOWLEDGMENTS

This study was made possible by the CDC Pandemic Influenza Scientific Agenda funding.

We thank our CDC colleagues Ryan A. Fitzgerald from the Division of Healthcare Quality and Promotion, Clinical and Environmental Microbiology Laboratory, for laboratory assistance and Jorn Winter from the Influenza Division for providing protocols and consultation. We are also appreciative of the guidance from Jeffery Shaman from the Department of Environmental Health Sciences and the Mailman School of Public Health, Columbia University, on the topic of absolute humidity.

The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

FOOTNOTES

Published ahead of print 18 January 2013

ARTICLE INFORMATION

Appl Environ Microbiol. 2013 Apr; 79(7): 2148–2155.

doi: 10.1128/AEM.03850-12
PMCID: PMC3623216
PMID: 23335770

A. D. Coulliette,* K. A. Perry, J. R. Edwards, and J. A. Noble-Wang

National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
*Corresponding author.
Address correspondence to A. D. Coulliette, acoulliette@cdc.gov.

Received 2012 Dec 13; Accepted 2013 Jan 15.

REFERENCES

1. Shrestha SS, Swerdlow DL, Borse RH, Prabhu VS, Finelli L, Atkins CY, Owusu-Edusei K, Bell B, Mead PS, Biggerstaff M, Brammer L, Davidson H, Jernigan D, Jhung MA, Kamimoto LA, Merlin TL, Nowell M, Redd SC, Reed C, Schuchat A, Meltzer MI. 2011. Estimating the burden of 2009 pandemic influenza A (H1N1) in the United States (April 2009–April 2010). Clin. Infect. Dis. 52:S75–S82 [PubMed] [Google Scholar]

2. CDC 2010. Interim guidance on infection control measures for 2009 H1N1 influenza in healthcare settings, including protection of healthcare personnel. Centers for Disease Control and Prevention. http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm [PubMed]

3. CDC 2010. Prevention strategies for seasonal influenza in healthcare settings: guidelines and recommendations. Centers for Disease Control and Prevention. http://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm

4. OSHA 2009. Guidance on preparing workplaces for an influenza pandemic (OSHA 3327-05R 2009). Occupational Safety, Health Administration, U.S. Department of Labor, US Department of Human and Health Services, Washington, DC [Google Scholar]

5. The Institute of Medicine of the National Academies 2006. Reusability of facemasks during an influenza pandemic: facing the flu. Committee on the Development of Reusable Facemasks for Use During an Influenza Pandemic, The Institute of Medicine of the National Academies, The National Academies Press, Washington, DC [Google Scholar]

6. Murray M, Grant J, Bryce E, Chilton P, Forrester L. 2010. Facial protective equipment, personnel, and pandemics: impact of the pandemic (H1N1) 2009 virus on personnel and use of facial protective equipment. Infect. Control Hosp. Epidemiol. 31:1011–1016 [PubMed] [Google Scholar]

7. Qian Y, Willeke K, Grinshpun SA, Donnelly J, Coffey CC. 1998. Performance of N95 respirators: filtration efficiency for airborne microbial and inert particles. Am. Ind. Hyg. Assoc. J. 59:128–132 [PubMed] [Google Scholar]

8. Richman DD, Whitley RJ, Hayden FG, editors. (ed). 2009. Clinical virology, 3rd ed ASM Press, Washington, DC [Google Scholar]

9. Hall CB. 2007. The spread of influenza and other respiratory viruses: complexities and conjectures. Clin. Infect. Dis. 45:353–359 [PubMed] [Google Scholar]

10. Weber TP, Stilianakis NI. 2008. Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. J. Infect. 57:361–373 [PubMed] [Google Scholar]

11. Tellier R. 2006. Review of aerosol transmission of influenza A virus. Emerg. Infect. Dis. 12:1657–1662 [PMC free article] [PubMed] [Google Scholar]

12. Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. 2007. Transmission of influenza A in human beings. Lancet Infect. Dis. 7:257–265 [PubMed] [Google Scholar]

13. Assar SK, Block SS. 2001. Survival of microorganisms in the environment, p 1221–1242 *In* Block SS, editor. (ed), Disinfection, sterilization, and preservation, 5th ed Lippincott Williams & Wilkins, Philadelphia, PA [Google Scholar]

14. McDevitt JJ, Rudnick S, First M, Spengler J. 2010. Role of absolute humidity in the inactivation of influenza viruses on stainless steel surfaces at elevated temperatures. Appl. Environ. Microbiol. 76:3943–3947 [PMC free article] [PubMed] [Google Scholar]

15. Noyce JO, Michels H, Keevil CW. 2007. Inactivation of influenza A virus on copper versus stainless steel surfaces. Appl. Environ. Microbiol. 73:2748–2750 [PMC free article] [PubMed] [Google Scholar]

16. Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH. 1982. Survival of influenza viruses on environmental surfaces. J. Infect. Dis. 146:47–51 [PubMed] [Google Scholar]

17. Fisher E, Shaffer R. 2010. Survival of bacteriophage MS2 on filtering facepiece respirator coupons. Appl. Biosaf. 15:71–76 [Google Scholar]

18. Rengasamy S, Fisher E, Shaffer R. 2009. Evaluation of the survivability of MS2 viral aerosols deposited on filtering face piece respirator samples incorporating antimicrobial technologies. Am. J. Infect. Control. 38:9–17 [PubMed] [Google Scholar]

19. Fisher EM, Richardson AW, Harpest SD, Hofacre KC, Shaffer RE. 2012. Reaerosolization of MS2 bacteriophage from an N95 filtering facepiece respirator by simulated coughing. Ann. Occup. Hyg. 56:315–325 [PubMed] [Google Scholar]

20. Sakaguchi H, Wada K, Kajioka J, Watanabe M, Nakano R, Hirose T, Ohta H, Aizawa Y. 2010. Maintenance of influenza virus infectivity on the surfaces of personal protective equipment and clothing used in healthcare settings. Environ. Health Prev. Med. 15:344–349 [PMC free article] [PubMed] [Google Scholar]

21. Greatorex JS, Digard P, Curran MD, Moynihan R, Wensley H, Wreghitt T, Varsani H, Garcia F, Enstone J, Nguyen-Van-Tam JS. 2011. Survival of influenza A(H1N1) on materials found in households: implications for infection control. PLoS One 6:e27932 doi:10.1371/journal.pone.0027932 [PMC free article] [PubMed] [Google Scholar]

22. Szretter KJ, Balish AL, Katz JM. 2006. Influenza: propagation, quantification, and storage, p 15G.11.11–15G.11.22 *In* Coico R, editor. (ed), Current protocols in microbiology. John Wiley & Sons, Inc., Hoboken, NJ [Google Scholar]

23. Fisher E, Rengasamy S, Viscusi D, Vo E, Shaffer R. 2009. Development of a test system to apply virus-containing particles to filtering facepiece respirators for the evaluation of decontamination procedures. Appl. Environ. Microbiol. 75:1500–1507 [PMC free article] [PubMed] [Google Scholar]

24. Shaman J, Kohn M. 2009. Absolute humidity modulates influenza survival, transmission, and seasonality. Proc. Natl. Acad. Sci. U. S. A. 106:3243–3248 [PMC free article] [PubMed] [Google Scholar]

25. Reed LJ, Muench H. 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 27:493–497 [Google Scholar]

26. Thomas Y, Vogel G, Wunderli W, Suter P, Witschi M, Koch D, Tapparel C, Kaiser L. 2008. Survival of influenza on banknotes. Appl. Environ. Microbiol. 74:3002–3007 [PMC free article] [PubMed] [Google Scholar]

27. Taubenberger JK, Morens DM. 2010. Influenza: the once and future pandemic. Public Health Rep. 125:15–26 [PMC free article] [PubMed] [Google Scholar]

28. Trifonov V, Khiabanian H, Rabadan R. 2009. Geographic dependence, surveillance, and origins of the 2009 influenza A (H1N1) virus. N. Engl. J. Med. 361:115–119 [PubMed] [Google Scholar]

29. Myatt TA, K MH, Allen JG, Macintosh DL, Fabian MP, McDevitt JJ. 2010. Modeling the airborne survival of influenza virus in a residential setting: the impacts of home humidification. Environ. Health 9:55. [PMC free article] [PubMed] [Google Scholar]

30. ANSI/ASHRAE/ASHE 2008. Standard 170-2008: ventilation of health care facilities. American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., Atlanta, GA [Google Scholar]

31. Sharfstein JM. 2009. Letter of authorization: emergency use of disposable N95 respirators from strategic national stockpile. U.S. Food and Drug Administration, Washington, DC [Google Scholar]

32. Bergman MS, Viscusi DJ, Heimbuch BK, Wander JD, Sambol AR, Shaffer RE. 2010. Evaluation of multiple (3-cycle) decontamination processing for filtering facepiece respirators. J. Eng. Fiber. Fabr. 5:33–41 [Google Scholar]

33. Viscusi DJ, Bergman MS, Eimer BC, Shaffer R. 2009. Evaluation of five decontamination methods for filtering facepiece respirators. Ann. Occup. Hyg. 53:815–827 [PMC free article] [PubMed] [Google Scholar]

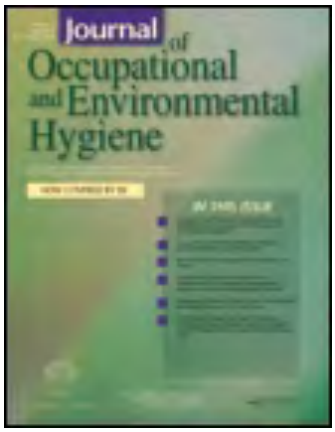
34. Heimbuch BK, Wallace WH, Kinney K, Lumley AE, Chang-Yu W, Woo MH, Wander JD. 2011. A pandemic influenza preparedness study: use of energetic methods to decontaminate filtering facepiece respirators contaminated with H1N1 aerosols and droplets. Am. J. Infect. Control 39:e1–e9 [PubMed] [Google Scholar]

35. Lore MB, Heimbuch BK, Brown TL, Wander JD, Hinrichs SH. 2012. Effectiveness of three decontamination treatments against influenza virus applied to filtering facepiece respirators. Ann. Occup. Hyg. 56:92–101 [PubMed] [Google Scholar]

36. Saltar WB, Kinney K, Wallace WH, Lumley AE, Heimbuch BK, Wander JD. 2010. Analysis of residual chemicals on filtering facepiece respirators after decontamination. J. Occup. Environ. Hyg. 7:437–445 [PubMed] [Google Scholar]

37. Fabian TK, Fejerdy P, Csermely P. 2008. Saliva in health and disease (chemical biology of), p 1–9 *In* Wiley encyclopedia of chemical biology. John Wiley & Sons, Hoboken, NJ [Google Scholar]

6



Journal of Occupational and Environmental Hygiene
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uoeh20

N95 and P100 Respirator Filter Efficiency Under High Constant and Cyclic Flow

Jonathan P. Eshbaugh [a] , Paul D. Gardner [b] , Aaron W. Richardson [a] & Kent C. Hofacre [a]
[a] Battelle Memorial Institute , Columbus, Ohio
[b] U.S. Army Edgewood Chemical Biological Center , Aberdeen Proving Ground, Maryland
Published online: 14 Nov 2008.

To cite this article: Jonathan P. Eshbaugh , Paul D. Gardner , Aaron W. Richardson & Kent C. Hofacre (2008) N95 and P100 Respirator Filter Efficiency Under High Constant and Cyclic Flow, Journal of Occupational and Environmental Hygiene, 6:1, 52-61, DOI: 10.1080/15459620802558196

To link to this article: http://dx.doi.org/10.1080/15459620802558196

Journal of Occupational and Environmental Hygiene, 6: 52–61
ISSN: 1545-9624 print / 1545-9632 online
DOI: 10.1080/15459620802558196

N95 and P100 Respirator Filter Efficiency Under High Constant and Cyclic Flow

Jonathan P. Eshbaugh,[1] Paul D. Gardner,[2] Aaron W. Richardson,[1] and Kent C. Hofacre[1]

[1]Battelle Memorial Institute, Columbus, Ohio
[2]U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, Maryland

This study investigated the effect of high flow conditions on aerosol penetration and the relationship between penetration at constant and cyclic flow conditions. National Institute for Occupational Safety and Health (NIOSH)-approved N95 and P100 filtering facepiece respirators and cartridges were challenged with inert solid and oil aerosols. A combination of monodisperse aerosol and size-specific aerosol measurement equipment allowed count-based penetration measurement of particles with nominal diameters ranging from 0.02 to 2.9 μm. Three constant flow conditions (85, 270, and 360 L/min) were selected to match the minute, inhalation mean, and inhalation peak flows of the four cyclic flow conditions (40, 85, 115, and 135 L/min) tested. As expected, penetration was found to increase under increased constant and cyclic flow conditions. The most penetrating particle size (MPPS) generally ranged from 0.05 to 0.2 μm for P100 filters and was approximately 0.05 μm for N95 filters. Although penetration increased at the high flow conditions, the MPPS was relatively unaffected by flow. Of the constant flows tested, the flows equivalent to cyclic inhalation mean and peak flows best approximated the penetration measurements of the corresponding cyclic flows.

Keywords constant flow, cyclic flow, filter, high volumetric flow, particulate penetration, respirator

Address correspondence to Jonathan P. Eshbaugh, Battelle Memorial Institute, AMSRD-ECB-RT-PR-E5604 5183 Blackhawk Rd., Aberdeen Proving Ground, MD 21010-5424; e-mail: eshbaughj@battelle.org.

INTRODUCTION

Respirators are widely used to provide protection against particulate respiratory hazards encountered by emergency responders, health care workers, and many others. The National Institute for Occupational Safety and Health (NIOSH) certifies respirator filters to ensure they provide a minimum level of protection against harmful aerosols in the workplace. The NIOSH performance requirements used to certify particulate respirator filters are provided by Title 42 *Code of Federal Regulations* Part 84.[1] Particulate hazards exist in many different sizes and shapes with different physical and chemical properties. The certification requirements are designed to provide a measure of filter performance during a worst-case or severe condition. In a less severe environment, such as the workplace, filters are expected to perform at or better than their certification level.

Particle penetration is dependent on several parameters, including face velocity (based on flow rate and available surface area); aerosol particle size; and several filter parameters including thickness, fiber diameter, and fiber packing density. Because individual filter parameters are not evaluated in certification tests, flow and particle size are important variables to consider. Previous studies have shown that an increase in flow or face velocity leads to an increase in penetration.[2–6] The most important particle size in a worst-case test condition is the most penetrating particle size (MPPS). The MPPS generally occurs between 0.05 and 0.5 μm depending on the filter properties and face velocity.[7]

NIOSH particulate filter certification tests use photometers to measure light scattering of the challenge aerosol. The light scatter provides an indication of the aerosol mass penetration in order to verify the filtration efficiency. N-series filters are challenged with a sodium chloride (NaCl) aerosol, whereas R- and P-series filters are challenged with a dioctyl phthalate (DOP) aerosol. Both NaCl and DOP aerosols are charge neutralized and have a count median diameter (CMD) of 0.075 ± 0.02 μm and 0.185 ± 0.02 μm, respectively. The filters are challenged at a constant flow rate of 85 L/min, or half that (42.5 L/min) if the respirator uses a pair of filters. Also, filters are loaded with a minimum 200 mg of aerosol.[1]

The capability of the constant 85 L/min airflow NIOSH test condition to replicate extreme real world conditions has been the center of much debate. In a study by Blackie et al.,[8] ventilation rates at work intensities ranging from moderate to exhaustive were measured. Average exhaustive (maximum) ventilation rates as high as 114 L/min were recorded. Other studies measured similar high ventilation rates.[9–11] Measurements of physiological parameters of workers have shown

instantaneous peak flow rates in the 300 to 400 L/min range for certain activities that demand high work loads.[12] Fortunately, high ventilation rates and, thus, high peak flows are likely only for a short duration at exhaustive workloads and are unlikely during normal workplace activities.[12] Nonetheless, the studies exemplify upper ventilation limits and peak flows that are above the current constant 85 L/min test flow.

The relationship between filtration performance at cyclic and constant flows has been assessed only in a limited number of studies. The most relevant, Stafford et al.,[13] measured the penetration of monodisperse polystyrene latex spheres (PSL) (0.176 to 2.02 μm) and DOP (0.3 μm) aerosols through Dust Mist (DM) and Dust Mist Fume (DMF) respirator filter cartridges at three cyclic flows with mean flow rates of 30, 35, and 53 L/min. These flows were selected to correspond to work rates of 415, 622, and 830 kg-m/min, respectively. Tests were also conducted at a constant flow rate of 16 L/min (equivalent to 32 L/min through a pair of cartridges). Results showed that the maximum penetration was considerably higher than corresponding steady-flow values based on minute volume, suggesting that tests conducted under steady-flow conditions may not accurately indicate performance under comparable cyclic-flow conditions.

Brosseau et al.[14] compared the collection of silica and asbestos aerosols by DM respirators under breathing and constant flows. The cyclic flow was sinusoidal with a minute flow of 37 L/min, a mean flow of 76 L/min, a peak flow of 100 L/min, and represented a work rate of 622 kg-m/min. The constant flow rate was 32 L/min. In general, the silica penetration under cyclic flow conditions was about one and a half times as great as that measured under steady-flow conditions. The asbestos results were inconclusive as exhalation valve leakage confounded the results. However, a straightforward relationship between constant and cyclic flow was not demonstrated in either study.

The inhalation breathing rate at rest resembles a sinusoidal flow curve.[15] As the breathing rate is increased during moderate to heavy work, the inhalation flow resembles a more trapezoidal flow curve.[15] For the sake of simplicity, in this study a sinusoidal flow curve was used to simulate all cyclic breathing rates. A sinusoidal flow curve can be simply described by the minute, mean, or peak flow. The minute volume (Minute) flow is the total air inhaled over a minute. During that minute, breaths are inhaled and exhaled.

Since in a sinusoidal flow curve inhalation and exhalation take equal time, inhalation only occurs half the time. The average flow rate during the inhalation portion or mean inhalation flow (MIF) is approximately twice the Minute flow rate. At an 85 L/min Minute flow rate the MIF is about 175 L/min. The inhalation cycle also has a peak flow rate or peak inhalation flow (PIF). In a breathing rate approximated with a sinusoidal curve, the PIF is π times the minute volume flow rate or approximately 270 L/min for an 85 L/min minute volume flow rate. The Minute, MIF, and PIF offer a simple description of a cyclic sinusoidal flow in terms of constant flow.

Many studies[2–6] have assessed the effect of flow rate and particle size on the measured aerosol penetration through particulate respirator filters. However, no studies have assessed the effect of high volumetric flow rates that may be encountered during heavy work. Only a few studies assessed filtration performance above 85 L/min and none above 100 L/min constant flow. Furthermore, few studies assessed aerosol filtration performance under cyclic flow conditions and none under high cyclic flow conditions.

The goal of this study was to evaluate the effect of high volumetric flow conditions on the filtration efficiency of select NIOSH-approved N95 and P100 particulate respirators. The effect of flow on the MPPS was evaluated at the high flow conditions. Furthermore, the relationship between the constant and cyclic flow performance was investigated. The constant flow (equivalent to the cyclic Minute, MIF, or PIF flow) penetration that best approximated the cyclic flow penetration was determined. This study was completed as part of a larger investigation into filtration efficiency against biological aerosols.[16]

METHODS AND MATERIALS

Test Materials and Conditions

The filters, summarized in Table I, included two N95 filtering facepieces, two P100 filtering facepieces, two N95 cartridges, and two P100 cartridges. The two P100 filtering facepieces used in the study had exhalation valves. The valves were not sealed during testing. The filters were not preconditioned prior to testing (i.e., tested as-received). The environmental conditions during testing were maintained at ambient temperature (25 $\pm$ 3°C) and relative humidity (40 $\pm$ 10%).

Eight particle sizes were tested with nominal diameters of 0.02, 0.05, 0.1, 0.2, 0.3, 0.7, 1.3, and 2.9 μm. Solid aerosols (i.e, NaCl and PSL) were used with the N95 filters and oil aerosols (i.e., Emery 3004 and DOP) were used with the P100 filters. Challenge concentration varied greatly

TABLE I. Cartridges and Filtering Facepieces

Filter Type	Rating	Model	Manufacturer
Cartridge	N95	Flexi-Filter	MSA (Pittsburgh, PA)
	N95	7506	North Safety Products (Cranston, RI)
	P100	HE-T	SEA (Branford, CT)
	P100	1050	Survivair (Santa Ana, CA)
Facepiece	N95	1730	Louis M. Gerson, Co., Inc. (Middleboro, MA)
	N95	Affinity Plus	MSA (Pittsburgh, PA)
	P100	8293	3M (St. Paul, MN)
	P100	2360	Moldex-Metric, Inc. (Culver City, CA)

TABLE II. Cyclic Flows Used for Testing with the Aerosol Challenges

Breathing Rate (breaths/min)	Tidal Volume (L)	Minute Volume (L/min)	PIF[A] (L/min)	MIF[B] (L/min)
25	1.6	40	130	85[C]
37	2.3	85	270[C]	175
42	2.7	115	360[C]	230
44	3.1	135	430	270[C]

[A]Peak inspiratory flow rate assuming ideal sinusoidal waveform.
[B]Mean inspiratory flow rate assuming ideal sinusoidal waveform.
[C]Selected for constant flow testing.

between particle sizes and generally ranged from 10^2 to 10^4 particles/cm^3. Although the larger particles commonly had lower concentrations, the sample times were adjusted to ensure the measurement sensitivity was adequate to measure efficiencies of at least 99.97%.

Tests were performed at four constant and three cyclic flow conditions. The cyclic flow conditions with corresponding Minute, MIF, and PIF are summarized in Table II. The flows were chosen to represent a moderate ventilation rate (40 L/min), minute volume equivalent to the NIOSH standard (85 L/min), an exhaustive ventilation rate (115 L/min), and one standard deviation above the exhaustive ventilation rate (135 L/min). These cyclic flow ventilation choices are discussed in more detail by Caretti et al.[12] The selected constant flows (85, 270, and 360 L/min) match aspects of the cyclic flows. For example, the 85 L/min constant flow matches the minute volume and MIF of the 37 breaths/min—2.3 L tidal volume and 25 breaths/min—1.6 L tidal volume cyclic flow rates, respectively.

All cartridges, except the SEA P100, were obtained from dual-cartridge respirators and thus tested at half of the tidal volume or constant flow rate stated. The SEA P100 cartridge was tested at half the flow rate stated to maintain the same test flow condition for all cartridges. Tests were not performed with the filtering facepieces at the highest cyclic flow condition for submicrometer particles because the test apparatus was not able to generate a stable challenge concentration high enough to accurately measure penetrations at the 5 or 0.03% NIOSH criterion level for N95 and P100 particulate respirators, respectively.

Test Apparatus

The large range of particles tested required the use of two test systems. A modified model 3160 Automated Filter Tester (TSI, Shoreview, Minn.) was used for the submicrometer aerosols (<0.5 μm). A separate custom test system was used for the micrometer aerosols (>0.5 μm). A complete description of both test apparatus can be found in Richardson et al.[16]

Submicrometer Test Apparatus

The model 3160 is a commercially available filter tester capable of challenging filters with a monodisperse (geometric standard deviation (GSD) <1.3) DOP or NaCl challenge aerosol to measure filtration efficiency. The main components of the 3160 are an electrostatic classifier and two condensation particle counters. Several modifications were made to the 3160 to permit efficiency measurements over the entire range of cyclic and high flow conditions. Additional high-efficiency particulate air (HEPA) filters were added to the dilution air inlet. A 1.5 hp vacuum pump (Gast Manufacturing Inc., Benton, Mich.) was added for testing at constant flows in excess of 150 L/min.

For cyclic flow testing, a breathing machine (FENZY, Villers-Cotterets, France) was connected with a check valve such that the exhaled air was exhausted into the room after being filtered. Test filters were housed in a Lucite chamber measuring 22 × 18 × 10 cm sealed between the model 3160 filter chucks. The challenge aerosol entered the chamber through a 7.5-cm hole on top. The filtering facepieces were placed over a similar sized hole in the bottom of the chamber and sealed using an adhesive. Threaded fittings were machined to mate with the cartridges.

Micrometer Test Apparatus

The micrometer test system (Figure 1) was custom built in-house and consisted of an exposure chamber, breathing machine (or vacuum pump), aerosol generator, and aerosol sampling/classification system. The chamber was a sealed Lucite enclosure approximately 75 × 75 × 60 cm. A filter holder was mounted on the bottom of the chamber. The design of the filter holder was specific to the filter being tested. For the filtering facepieces, the holder consisted of a cone with a flat plate at the top. The filtering facepiece was sealed to the flat plate using rope caulk adhesive. For the cartridges, threaded fittings were machined. The filter holder was mounted to a 2.5-cm OD stainless steel bulkhead fitting (Swagelok, Solon, Ohio) that passed through the bottom of the chamber and connected to the breathing machine (or vacuum pump) with large diameter (~2.5 cm OD) flexible tubing.

The breathing apparatus, either a breathing machine (same as used in the submicrometer apparatus) or vacuum pump (vacuum blower manufactured in-house), allowed for the adjustment of the tidal volume, breathing rate, and flow rate. The test system was also set up such that the test filter (i.e., cartridge or filtering facepiece) was bypassed during system startup to minimize aerosol loading.

The micrometer aerosol generator was challenge specific and consisted of either a 6- or 24-jet Collison nebulizer (BGI Inc., Waltham, Mass.). The challenges were PSL particles (Duke Scientific Corp., Palo Alto, Calif.) suspended in distilled water and a poly-alpha olefin oil aerosol (Emery 3004). PSL was used for N95 filters in lieu of NaCl because of ease of use, and Emery 3004 was used for P100 filters instead of DOP for safety reasons. A 24-jet Collison was used to aerosolize the 2.9 μm PSL, and a 6-jet Collison was used to aerosolize the 0.7

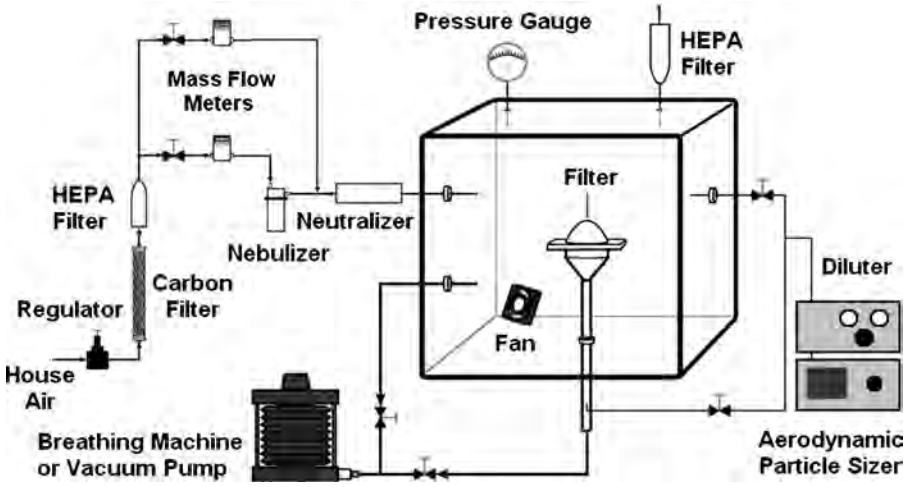


FIGURE 1. Micrometer test system schematic

and 1.3 μm PSL as well as 0.7–2.9 μm Emery 3004 oil. Aerosol exiting the nebulizer was mixed with filtered house air before passing through a neutralizer (model 3012; TSI). Additional make-up air, needed for higher filter test flow rates, was pulled through two HEPA filters located on top of the chamber.

The chamber contained mixing fans to ensure a well-mixed challenge atmosphere. Chamber pressure was measured using a magnehelic pressure gauge (Dwyer Instruments Inc., Michigan City, Ind.). Excess challenge was vented through HEPA filters and exhausted into the room. An aerodynamic particle sizer (APS, model 3321; TSI) and diluter (model 3302A, TSI) were used to measure the size-specific challenge and downstream aerosol concentrations.

Test Procedure

Submicrometer Procedure

Prior to testing, the dilution ratio on the upstream condensation particle counter (CPC) in the model 3160 was verified at a specific particle size and flow condition. This was done without a test filter to ensure no sampling bias between the up- and downstream samples (i.e., measured penetration was 100% indicating agreement between the up- and downstream CPCs). With the dilution ratio set, the cartridge or filtering facepiece was mounted in the Lucite chamber and the chamber sealed to the model 3160. The vacuum pump or breathing machine was connected to the test system and set to the proper conditions. The model 3160 was programmed to measure efficiencies sequentially at the submicrometer particle sizes from small to large. The model 3160 was started and automatically stepped through the particle sizes and measured the efficiencies. When complete, the vacuum pump or breathing machine was stopped and the filter removed from the test system and discarded.

Micrometer Procedure

The filter was mounted to the test fixture. The test chamber was sealed and the breathing or vacuum pump, as appropriate, was started. The challenge generation system was started and permitted to operate for 10 to 20 min depending on the flow condition to allow the aerosol to reach a steady-state concentration. During this time, the cartridge or filtering facepiece was bypassed to minimize filter loading. When steady-state was reached, the valves were adjusted such that flow was pulled through the test filter. A sample of the challenge aerosol was collected with the APS for 2 min.

Next, the sampling line was switched to sample downstream of the filter, and adequate time was given for the instrument to stabilize. The downstream sample duration was varied to provide adequate time to permit efficiency measurements of at least 99.97% based on the challenge concentration. For the N95 filters, the sample duration was 5 min when testing 0.7 and 1.3 μm particles and 15 min when testing with the 2.9 μm particles. The longer sample duration was needed with the 2.9 μm PSL particles because the challenge concentration was lower. The P100 filters required 15 min of downstream sampling when testing with the 0.7, 1.3, and 2.9 μm oil challenge particles.

After sampling downstream was complete, the sample line was switched to the upstream and a second 2-min sample of the challenge concentration was collected. If the two challenge samples (i.e., before and after downstream sampling) were not within 20% of each other, the test was repeated, as the challenge aerosol was not stable. After the challenge sample was collected, the aerosol generation system was stopped and the chamber flushed with clean air. The filter was removed from the test system and discarded.

Calculation of Aerosol Penetration

The percent aerosol penetration (P) was defined as the ratio of the downstream aerosol concentration (C_{Down}) to the challenge aerosol concentration (C_{Chal}):

$$P(\%) = \frac{C_{Down}}{C_{Chal}} \times 100 \quad \textbf{(1)}$$

Alternatively, the filtration efficiency (η) was defined as:

$$\eta(\%) = 100 - \mathrm{P} = \left(1 - \frac{C_{\mathrm{Down}}}{C_{\mathrm{Chal}}}\right) \times 100 \qquad \textbf{(2)}$$

The concentrations were the average number concentrations measured by the aerosol sensing instrument (i.e., APS or CPC). On the custom system, the challenge concentration was the average of the challenge measurements made before and after the downstream sample period.

Statistical Analyses

Statistical analyses were performed to assess the correlation of penetration, particle size, and flow. Because the filters were extremely efficient against the largest particle size tested (2.9 μm), resulting in many penetration measurements below the detection limit, the 2.9 μm data were not included in the analysis of variance (ANOVA). Also, because some filters were not tested at all cyclic flows, only constant flow was used in the ANOVA. All percent penetration data were converted to fractional penetration, inverted, and log transformed (base 10) for the ANOVA. A full, three-factor ANOVA model was used to determine the effect of high constant flow, particle size, and filter on penetration.

The three ANOVA factors were (1) constant flow, (2) particle size, (3) filter, and their interactions. An F-test was performed to test the hypothesis that the penetrations measured at all conditions were equal. Tukey's multiple comparison procedure was used to determine which penetration conditions differed using a 5% overall error rate. For all analyses, statistical significance was accepted at the $p < 0.05$ level.

Simple regression plots were prepared to compare the cyclic flow penetration with the constant flow penetration equivalent to the cyclic Minute, MIF, and PIF. The regression plots contain all appropriate untransformed data regardless of particle size and filter type. No regression line was calculated, since no variable (constant or cyclic) was considered dependent or independent and free of error. Perfect agreement between the constant and cyclic flows is indicated by a one-to-one line.

In addition to regression plots, Bland Altman (B-A) plots[17,18] were created to compare constant and cyclic penetration measurements. Two particle sizes were selected for the comparison (0.05 and 1.3 μm). All filter data (N95 and P100) for the two particle sizes were used in the comparison. The 0.05 μm data were selected as a representative of the MPPS, and the 1.3 μm data were selected as the largest particle size tested with most penetration data within the detection limit.

All percent penetration data were converted to fractional penetration, inverted, and log transformed (base 10) for the B-A analysis. The analysis was set up to compare the penetration measurements at a cyclic flow to penetration measurements at a constant flow equivalent to the cyclic Minute, MIF, or PIF flow. At least one data pair of a cyclic penetration and the penetration of a Minute, MIF, and PIF equivalent constant flow existed for each filter. The difference between the constant and cyclic penetration means along with the average of the means was calculated for each constant-cyclic pair.

The difference of the means and the averages were plotted on the y-axis and x-axis, respectively. The overall mean of the differences was indicated by a blue line in the plots. As the differences between constant and cyclic penetration decreased, the blue line approached zero. A blue line at zero would signify perfect agreement between constant and cyclic penetration. The B-A analysis method included the calculation of the upper limit of agreement (ULOA) and a lower limit of agreement (LLOA) (consistent with a 95% confidence interval of the measurement differences), which were included as red lines in the plots.

RESULTS

The particle size with the highest mean penetration for each filter/flow condition was selected as the MPPS. The N95 and P100 MPPS and the MPPS mean penetration are summarized in Tables III and IV, respectively. Because only a discrete number of particle sizes were tested, the actual MPPS may be slightly above or below the particle size selected. As seen in the tables, the MPPS penetration increased with flow.

The full, three-factor ANOVA revealed all factors (constant flow, particle size, and filter), and all factor interactions significantly affected penetration. It should be noted that the penetration data were not normal, even though it was log transformed. A normality plot illustrated that the deviation occurred at the extreme upper and lower penetrations. This was expected since the penetration measurements have an upper and lower (100 and 0.0001%) measurement limit. Tukey's multiple comparison procedure confirmed all three constant flows were significantly different, most filters were significantly different, and most particle sizes were significantly different. Only two sets of filters and two sets of particle penetrations were not found to be significantly different (MSA N95 Facepiece/Gerson N95 Facepiece and Gerson N95 Facepiece/North N95 Cartridge, 0.02 μm / 0.3 μm and 0.1 μm/0.05 μm).

Two filters were selected as examples to show penetration as a function of size at 85 L/min constant flow and 85 L/min minute volume cyclic flow. The Gerson N95 filtering facepiece and the Survivair P100 cartridge are displayed in Figures 2A and 2B, respectively. The percent penetration, standard deviation, and a line connecting the data points (for illustrative purposes) are displayed in each figure. Both figures provide a visual example of the filtration performance. Each penetration curve contains a maximum point where penetration decreases when particle size is increased or decreased. Also, note that the penetration of the 85 L/min minute volume cyclic flow is greater than the penetration of the 85 L/min constant flow.

Regression plots for the Minute, MIF, and PIF comparisons are shown in Figures 3A, 3B, and 3C, respectively. As an example, the penetrations measured at the 37 breaths/min – 2.3 L tidal volume cyclic flow and the 85 L/min constant flow are plotted in the Minute comparison plot. Keeping with the example, the penetration of a 0.05 μm particle (MPPS as shown

TABLE III. N95 MPPS Percent Penetration

	Test Condition						
	Constant Flow (L/min)			Cyclic Flow (L/min)			
Filter	85	270	360	40	85	115	135
North[A]							
MPPS (μm)	0.05	0.05	0.05	0.05	0.05	0.05	0.05
x	8.8	17.1	20.8	7.0	12.2	17.3	18.8
σ	0.3	2.0	1.2	1.3	2.6	0.9	2.9
MSA Flexi-Filter[A]							
MPPS (μm)	0.05	0.05	0.05	0.05	0.05	0.05	0.05
x	0.7	4.6	6.0	1.0	3.3	7.5	8.1
σ	0.2	1.2	2.8	0.2	1.2	2.6	2.4
MSA Affinity[B]							
MPPS (μm)	0.05	0.05	0.05	0.05	0.05	0.05	—[C]
x	2.8	7.6	8.6	3.1	8.7	13.4	—
σ	0.5	2.2	1.9	0.3	1.7	0.7	—
Gerson[B]							
MPPS (μm)	0.05	0.05	0.1	0.05	0.05	0.05	—
x	4.2	9.7	8.6	7.9	14.4	16.4	—
σ	0.2	1.3	0.8	0.8	1.3	2.0	—

[A] Cartridge filter. Filter tested at half the flow rate stated.
[B] Filtering facepiece. No outlet valve.
[C] Test not performed.

TABLE IV. P100 MPPS Percent Penetration

	Test Condition						
	Constant Flow (L/min)			Cyclic Flow (L/min)			
Filter	85	270	360	40	85	115	135
SEA HE-T[A]							
MPPS (μm)	0.2	0.2	0.1	0.2	0.2	0.2	0.2
x	0.0004	0.0120	0.0290	0.0003	0.0060	0.0080	0.0120
σ	0.0001	0.0009	0.0010	0.0000	0.0030	0.0040	0.0050
Survivair[A]							
MPPS (μm)	0.2	0.1	0.1	0.1	0.1	0.1	0.1
x	0.0080	0.0980	0.1600	0.0110	0.0480	0.0800	0.1200
σ	0.0010	0.0050	0.0970	0.0040	0.0010	0.0160	0.0180
3M[B]							
MPPS (μm)	0.05	0.02	0.02	0.05	0.05	0.05	—[C]
x	0.010	0.220	0.540	0.028	0.300	0.470	—
σ	0.003	0.047	0.098	0.003	—	0.045	—
Moldex[B]							
MPPS (μm)	0.05	0.05	0.05	0.05	0.05	—	—
x	0.048	0.940	1.200	0.096	0.730	—	—
σ	0.019	0.350	1.000	0.013	0.057	—	—

[A] Cartridge filter. Filter tested at half the stated flow rate.
[B] Filtering facepiece. Respirator tested with exhalation valve as-received.
[C] Test not performed.

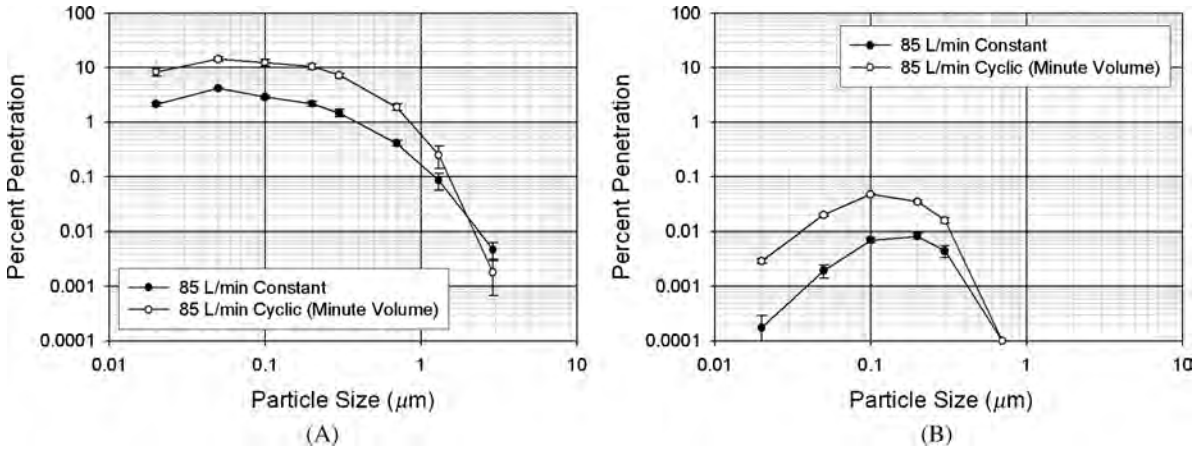


FIGURE 2. Penetration curves of (A) Gerson N95, and (B) Survivair P100 filters

in Table III) through a North filter cartridge (North Safety, Cranston, R.I.) is represented by a point located at 12.2 on the x-axis (cyclic flow) and 8.8 (constant flow) on the y-axis in this plot. The regression plots illustrate a correlation between the two flow types (as cyclic penetration increases equivalent constant penetration increases). Also, for equivalent Minute and MIF comparisons, most points are below the one-to-one line, and for the equivalent PIF comparison, most points are

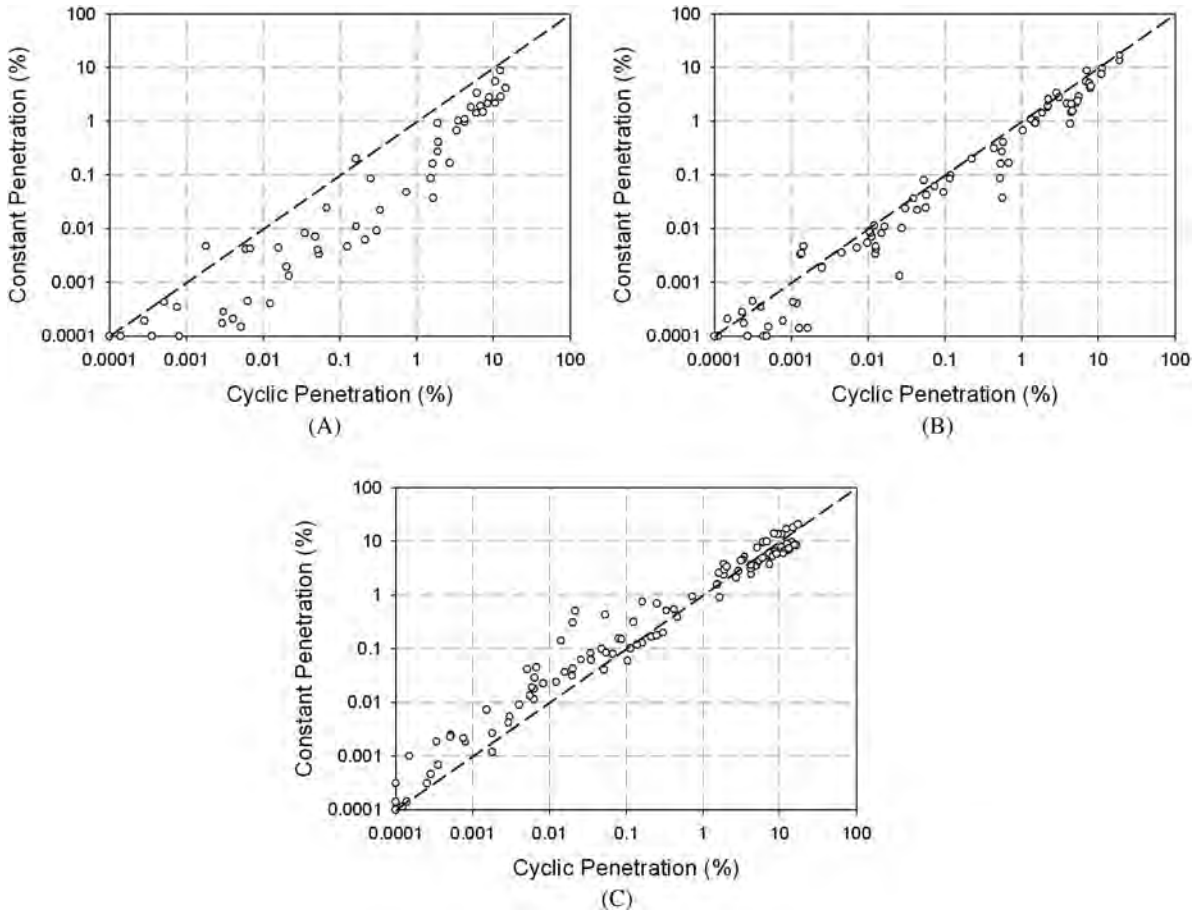


FIGURE 3. Regression plots comparing cyclic and equivalent constant (A) minute, (B) MIF, and (C) PIF flows

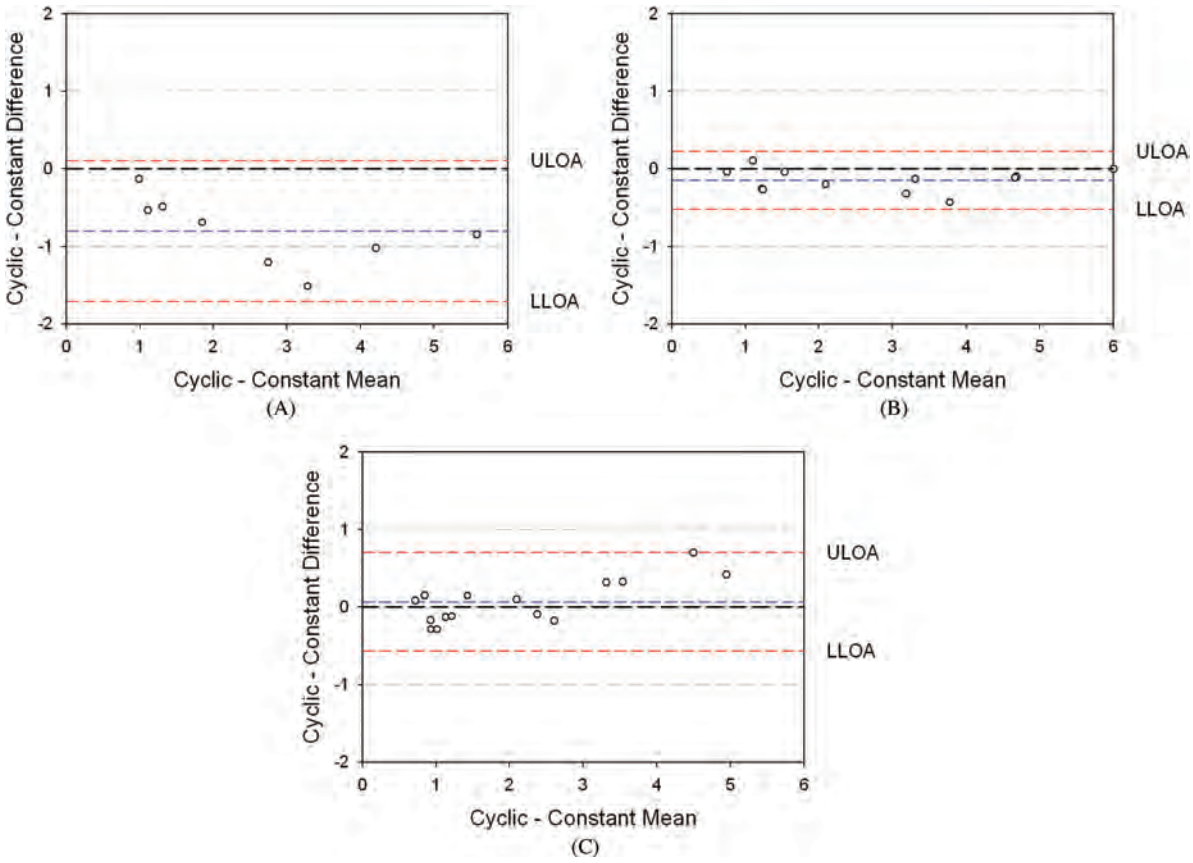


FIGURE 4. 0.05 μm Bland Altman plots for (A) minute, (B) MIF, and (C) PIF measurement comparisons

on or above the one-to-one line. Thus, penetration tends to be somewhat less at the MIF and slightly higher at the PIF compared with the corresponding cyclic flow condition.

The 0.05 and 1.3 μm B-A plots are shown in Figures 4 and 5, respectively. Each figure contains a plot for the Minute, MIF, and PIF comparison. As mentioned previously, only 0.05 and 1.3 μm data were used in the plots. The 0.05 μm B-A analysis mean differences for the Minute, MIF, and PIF comparisons were −0.81, −0.15, and 0.06, respectively. Similarly, the 1.3 μm B-A analysis mean differences for the Minute, MIF, and PIF comparisons were −0.45, −0.23, and 0.03, respectively. The mean differences can be uninverted and untransformed to provide a factor that demonstrates the difference between penetration measurement at constant and cyclic flows. The 0.05 μm data results in factors of 6.4, 1.4, and 0.9, and the 1.3 μm data results in factors of 2.8, 1.7, and 0.9. For example, the 0.05 μm cyclic penetration measured is approximately 6.4 times the constant penetration measured at the equivalent minute volume.

DISCUSSION

This study focused on assessing the performance of NIOSH-approved N95 and P100 filtering facepiece respirators and cartridges against particles within the MPPS range at high flow rates with an emphasis on comparing constant and cyclic measured penetrations. Previous studies had shown that an increase in face velocity at moderate flow rates (<100 L/min) caused an increase in penetration.[2–6] Diffusion, interception, and electrostatic attraction that become less effective at an increased flow are the main filtration mechanisms within the MPPS range.[7] As seen in the results, penetration increased with increased flow rate. Even at the high flow rates tested, diffusion, interception, electrostatic attraction and, thus, penetration are affected by flow.

The MPPS ranged from 0.02 to 0.2, as seen in Tables III and IV. Although an increase in filtration face velocity decreases the MPPS,[7] an actual decrease in MPPS was difficult to observe at the discrete particle sizes tested. A decrease in MPPS was not seen in N95 filters but was observed in three of the four P100 filters tested. The N95 filters all had an MPPS of approximately 0.05 μm, consistent with literature,[6,19] at all flows tested. Alternatively, the P100 cartridges had a larger MPPS ranging from 0.1 to 0.2 μm. Several observations were made to help explain the larger MPPS of the P100 cartridges. The P100 cartridges contained pleated filter media made from uncharged mechanical filtration media. Pleated media contains a greater amount of filter surface area than the other filters. Thus, when all filters are tested at the same flow, the pleated filters have a much lower face velocity. As discussed in other

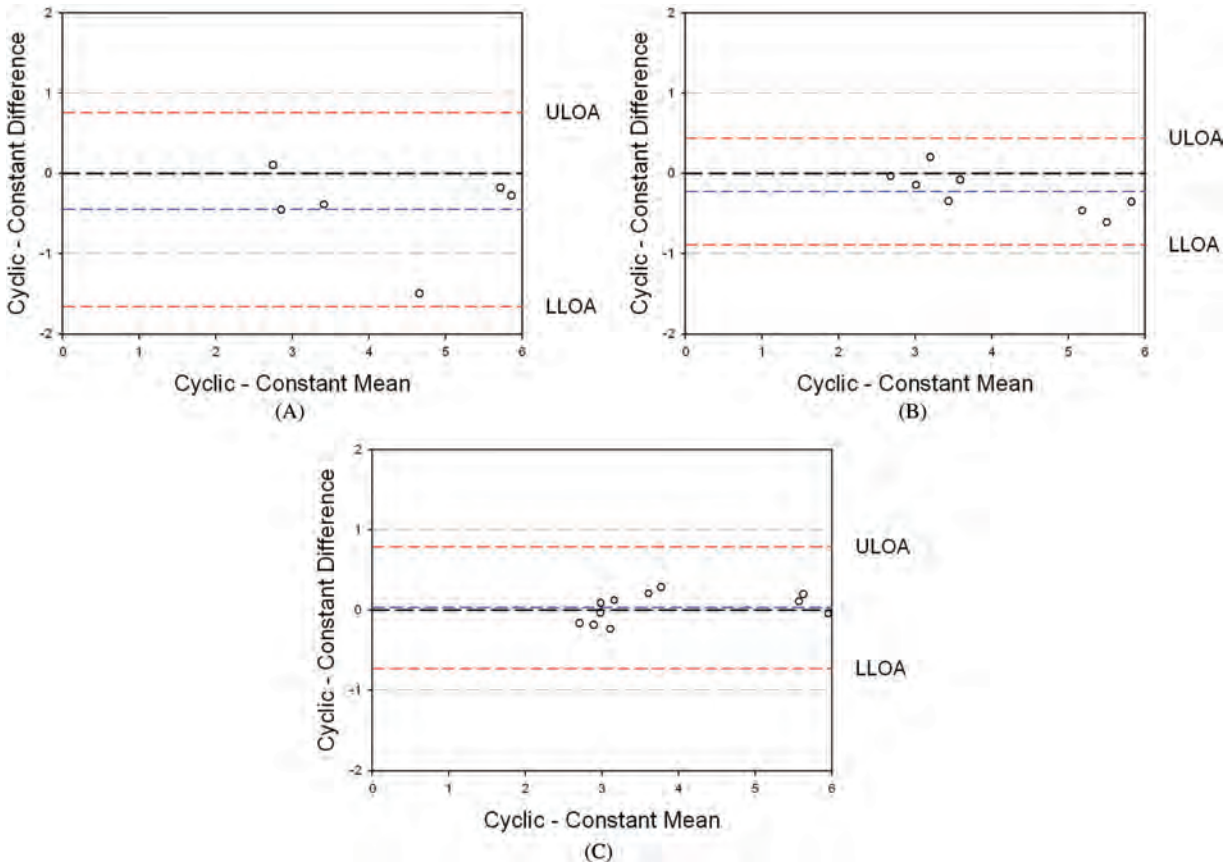


FIGURE 5. 1.3 μm Bland Altman Plots for (A) minute, (B) MIF, and (C) PIF measurement comparisons

texts,[2,7] a decrease in face velocity will increase the diffusion filtration efficiency, thus increasing filtration efficiency and the MPPS. Also, the filtration material itself can make a difference when considering MPPS. All the filter elements tested except the P100 cartridges contained some type of electret media. Previous studies have shown that the MPPS of electret filters is smaller than that of mechanical filters.[2,6]

The B-A analysis treated the two flow conditions (constant and cyclic) as measurement methods of the same parameter (penetration) and compared the measurement differences. The difference between the two methods should have been close to zero if the two methods were similar. Although there were only a few data points for each penetration measurement comparison, the limited data were sufficient to reveal differences between the measurement methods.[17,18] For the 0.05 μm data, the PIF penetration measurement (0.06 mean difference) best approximates the cyclic flow penetration measurement, followed by the MIF (–0.15 mean difference) and Minute (−0.81 mean difference) penetration measurements.

Furthermore, the mean difference positive or negative value represents a measured penetration greater than or less than that of a cyclic penetration measurement, respectively. The MIF and PIF mean difference magnitudes and signs suggest that a true representation of the cyclic penetration measurement, assuming a sinusoidal flow, is somewhere between the MIF and PIF flow. Hence, a penetration measurement at 85 L/min constant test flow actually simulates penetration measurement at a cyclic flow with a minute volume between 30 and 40 L/min. This is consistent with previous research that has shown constant flow equivalent to the MIF or Minute flow results in a lower penetration than the corresponding cyclic flow.[13,14]

Although the mean differences were not exactly the same, the 0.05 and 1.3 μm B-A comparisons provided similar results. The PIF best approximated cyclic penetration, followed by the MIF and minute flow. Furthermore, the factors by which the constant and cyclic measured penetration varied were fairly consistent between the 0.05 and 1.3 μm sizes. This is of interest considering the 0.05 μm particle penetration can be several orders of magnitude above the 1.3 μm penetration.

It is also interesting because, assuming the flow causes the penetrations to differ by some similar factor, as the penetration approaches zero (usually with larger or smaller particle sizes), the influence of flow has less of an effect on penetration. For example, a small penetration of 0.001% would be less affected than a larger penetration of 1% if both were multiplied by the same factor (the difference in penetration before and after the multiplication would be greater for the larger penetration). Thus, when considering the difference in

penetration between constant and cyclic flow or any flow, it is best to look at the MPPS. The MPPS allows an easily observable difference in penetration, while another particle size with a smaller penetration will require more sensitive measurement equipment.

The observations of the 0.05 and 1.3 μm results should not be applied to all particle sizes. The constant-cyclic flow relationship most likely holds true only with particles sized within the MPPS range. Larger particles are filtered with different mechanisms such as impaction and may not respond the same to a change in flow type.

All N95 and P100 filters, except for the SEA P100 (Safety Equipment America, Branford, Conn.), exceeded 5 or 0.03% penetration on a count basis on at least one of the higher flow conditions. However, these results should not be used as an indication of performance during NIOSH certification tests because different test apparatus and conditions were used. Notably, NIOSH particulate filter efficiency measurements are based on mass penetration and, thus, cannot be directly compared to the count penetration performed in this study.

CONCLUSION

The particle capture mechanisms in the MPPS range are diffusion, interception, and electrostatic attraction. As demonstrated in the results, the particle capture efficiency decreased with an increase in flow rate. A large difference in the MPPS at increased flows was not observed. However, only a discrete range of particle sizes were tested. The MPPS generally ranged from 0.05 to 0.2 μm for P100 filters and was approximately 0.05 μm for N95 filters.

The MPPS penetration measurements under constant and cyclic flow conditions were compared. Constant flows equivalent to the cyclic MIF or PIF approximated the cyclic flow penetration measurement more closely than the Minute flow. The MIF penetration was slightly lower, whereas the PIF penetration was slightly higher than the equivalent cyclic penetration measurement. Although an aerosol penetration test with a cyclic flow more closely simulates a real-world breathing condition, a reasonable approximation of the cyclic flow can be achieved by selecting an equivalent constant flow rate within the range of the MIF and PIF.

ACKNOWLEDGMENTS

The authors would like to thank the Office of Law Enforcement Standards, National Institute of Standards and Technology, Gaithersburg, Md., and the National Personal Protective Technology Laboratory (NPPTL), National Institute for Occupational Safety and Health, Pittsburgh, Pa., for supporting this project. Special appreciation goes to Dr. Samy Rengasamy, NPPTL, for his expertise and assistance with planning this study.

REFERENCES

1. "Respirator Protection," *Code of Federal Regulations Title 42*, Part 84. 1995. pp. 30382–30383.
2. **Fardi, B., and B.Y.H. Liu:** Performance of disposable respirators. *Part. Part. Syst. Charact. 8*:308–314 (1991).
3. **Chen, C.C., M. Lehtimaki, and K. Willeke:** Aerosol penetration through filtering facepieces and respirator cartridges. *Am. Ind. Hyg. Assoc. J. 53*:566–574 (1992).
4. **Stevens, G.A., and E.S. Moyer:** Worst case aerosol testing parameters: I. Sodium chloride and dioctyl phthalate aerosol filter efficiency as a function of particle size and flow rate. *Am. Ind. Hyg. Assoc. J. 50*:257–264 (1989).
5. **Qian, Y., K. Willeke, S.A. Grinshpun, J. Donnelly, and C.C. Coffey:** Performance of N95 respirators: Filtration efficiency for airborne microbial and inert particles. *Am. Ind. Hyg. Assoc. J. 59*:128–132 (1998).
6. **Balazy, A., M. Toivola, T. Reponen, A. Podgorski, A. Zimmer, and S.A. Grinshpun:** Manikin-based performance evaluation of N95 filtering-facepiece respirators challenged with nanoparticles. *Ann. Occup. Hyg. 50*(3):259–269 (2006).
7. **Hinds, W.C.:** *Aerosol Technology; Properties, Behavior, and Measurement of Airborne Particles*. New York: John Wiley & Sons, 1999. pp. 182–205.
8. **Blackie, S.P., M.S. Fairbarn, N.G. McElvaney, P.G. Wilcox, N.J. Morrison, and R.L. Pardy:** Normal values and ranges for ventilation and breathing pattern at maximal exercise. *Chest 100*:136–142 (1991).
9. **Neder, J.A., L.E. Nery, C. Peres, and B.J. Whipp:** Reference values for dynamic responses to incremental cycle ergometry in males and females aged 20 to 80. *Am. J. Respir. Crit. Care Med. 164*:1481–1486 (2001).
10. **Sue, D.Y., and J.E. Hansen:** Normal values in adults during exercise testing. *Clin. Chest Med. 5*:89–98 (1984).
11. **Wasserman, K., J.E. Hansen, D.Y. Sue, and B.J. Whipp:** *Principles of Exercise Testing and Interpretation*. Philadelphia: Lea & Febiger, 1987. pp. 100–123.
12. **U.S. Army Edgewood Chemical Biological Center:** *Workplace Breathing Rates: Defining Anticipated Values and Ranges for Respirator Certification Testing* by D.M. Caretti, P.D. Gardner, and K.M. Coyne (ECBC-TR-316). Aberdeen Proving Ground, Md.: U.S. Army Edgewood Chemical Biological Center, 2004.
13. **Stafford, R.G., H.J. Ettinger, and T.J. Rowland:** Respirator cartridge filter efficiency under cyclic- and steady-flow conditions. *Am. Ind. Hyg. Assoc. J. 5*:182–192 (1973).
14. **Brosseau, L.M., M.J. Ellenbecker, and J.S. Evans:** Collection of silica and asbestos aerosols by respirators at steady and cyclic flow. *Am. Ind. Hyg. Assoc. J. 51*:420–426 (1990).
15. **Johnson, A.T.:** How much work is expended for respiration? *Front. Med. Biol. Eng. 5(4)*:265–287 (1993).
16. **U.S. Army Edgewood Chemical Biological Center:** *Respirator Filter Efficiency Testing Against Particulate and Biological Aerosols Under Moderate to High Flow Rates* by A.W. Richardson, J.P. Eshbaugh, K.C. Hofacre, and P.D. Gardner (ECBC-CR-085). Aberdeen Proving Ground, Md.: U.S. Army Edgewood Chemical Biological Center, 2006.
17. **Bland, M.J., and D.G. Altman:** Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet 1(8476)*:307–310 (1986).
18. **Altman, D.G., and J.M. Bland:** Measurement in medicine: The analysis of method comparison studies. *The Statistician 32*:307–317 (1983).
19. **Rengasamy, S., R. Verbofsky, W.P. King, and R.E. Shaffer:** Nanoparticle penetration through NIOSH-approved N95 filtering-facepiece respirators. *J. Intern. Soc. Respir. Prot. 24*:49–59 (2007).

7

HHS Public Access

Author manuscript

J Occup Environ Hyg. Author manuscript; available in PMC 2015 October 19.

Published in final edited form as:

J Occup Environ Hyg. 2014 ; 11(8): D115–D128. doi:10.1080/15459624.2014.902954.

Commentary Considerations for Recommending Extended Use and Limited Reuse of Filtering Facepiece Respirators in Health Care Settings

Edward M. Fisher and **Ronald E. Shaffer**

National Personal Protective Technology Laboratory, National Institute for Occupational Safety and Health, Pittsburgh, Pennsylvania

Abstract

Public health organizations, such as the Centers for Disease Control and Prevention (CDC), are increasingly recommending the use of N95 filtering facepiece respirators (FFRs) in health care settings. For infection control purposes, the usual practice is to discard FFRs after close contact with a patient ("single use"). However, in some situations, such as during contact with tuberculosis patients, limited FFR reuse (i.e., repeated donning and doffing of the same FFR by the same person) is practiced. A related practice, extended use, involves wearing the same FFR for multiple patient encounters without doffing. Extended use and limited FFR reuse have been recommended during infectious disease outbreaks and pandemics to conserve FFR supplies. This commentary examines CDC recommendations related to FFR extended use and limited reuse and analyzes available data from the literature to provide a relative estimate of the risks of these practices compared to single use.

Analysis of the available data and the use of disease transmission models indicate that decisions regarding whether FFR extended use or reuse should be recommended should continue to be pathogen- and event-specific. Factors to be included in developing the recommendations are the potential for the pathogen to spread via contact transmission, the potential that the event could result in or is currently causing a FFR shortage, the protection provided by FFR use, human factors, potential for self-inoculation, the potential for secondary exposures, and government policies and regulations. While recent findings largely support the previous recommendations for extended use and limited reuse in certain situations, some new cautions and limitations should be considered before issuing recommendations in the future. In general, extended use of FFRs is preferred over limited FFR reuse. Limited FFR reuse would allow the user a brief respite from extended wear times, but increases the risk of self-inoculation and preliminary data from one study suggest that some FFR models may begin to lose effectiveness after multiple donnings.

Background

The Centers for Disease Control and Prevention (CDC)—including the National Institute for Occupational Safety and Health (NIOSH), as well as the Occupational Safety and Health

Address correspondence to: Ronald E. Shaffer, Technology Research Branch, National Personal Protective Technology Lab, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 626 Cochrans Mill Road, Building 20, P.O. Box 18070, Pittsburgh, PA15236; RShaffer@cdc.gov.

Administration (OSHA) and the Food and Drug Administration (FDA)—develop regulations and/or recommendations for the use of respiratory protection in health care settings, and each agency plays a different role which impacts the use of them in health care. CDC develops recommendations for the use of respirators to reduce the spread of disease in health care settings. NIOSH certifies respirators and develops recommendations on the use of respiratory protection in health care workplaces to protect workers. OSHA develops and enforces workplace regulations on respiratory protection. FDA clears the sale of certain types of respirators as medical devices.

The most commonly used type of respirator in health care settings are NIOSH certified N95 filtering facepiece respirators (FFRs). These devices are disposable, tight-fitting air-purifying respirators that have a filter efficiency of 95% or greater for a standard test aerosol.[1] FFRs are also used by workers in many industries to reduce the amount of harmful dusts and aerosols they inhale. Workers are expected to wear their FFR during all periods of exposure. However, there are times of non-exposure when workers need to remove their FFR (e.g., take a drink of water, use the restroom, or go on a rest break) or situations during use when their FFR must be replaced.

Employers have several options for FFR usage to handle these situations. During "single use," users put on ("don") a new FFR each time they need one and discard their used FFR each time they take it off ("doff"). Another option is commonly referred to as "FFR reuse." Reuse involves donning and doffing the same FFR more than once until the FFR is discarded. Employers benefit from FFR reuse compared to single use by extending the lifetime of the FFR so that fewer need to be purchased. There is no specific restriction on the number of uses or donnings. Rather, historical guidance is focused on the length of time the FFR can be used and identifying situations when the FFR should be discarded. In general, NIOSH[2] specifies that the service life of all filters on NIOSH-approved respirators is limited by considerations of hygiene, damage, and breathing resistance and that any filter should be replaced if it becomes soiled, damaged, or causes noticeably increased breathing resistance. In workplaces that could produce high cumulative particulate filter loading (i.e., >200 mg), the service time for N95 FFRs should only be extended beyond 8 hr of use (continuous or intermittent) by performing an evaluation that demonstrates that continued use will not reduce the filter efficiency.

FFR Use in Health care

FFRs have been used in industrial settings such as construction, manufacturing, and mining since the 1970s. Starting in the 1990s, these devices found new applications in health care settings.[3] Initially, FFRs were recommended as the minimum level of protection to reduce exposure to infectious aerosols from patients with tuberculosis[4–7] Later, similar recommendations[8] were made for outbreaks and pandemics involving pathogens with potential for aerosol transmission.

FFR use in health care settings has unique challenges and risks. Unlike industrial settings, some models of NIOSH-certified FFRs (commonly called "surgical N95 respirators") are also cleared for sale by the FDA as medical devices.[9] According to the FDA's 510(k) Premarket Notification Database, [10] the first clearance for a surgical N95 respirator

(product code = MSH) occurred in 1996, after FFRs were first recommended by CDC as the minimum level of protection for health care workers (HCWs) treating patients with tuberculosis[4] and NIOSH updated its certification requirements to create the N95 class of filters.[1] Most (22/31 = 71%) of the surgical N95 respirator models in the FDA database were cleared after 2005, which coincides with a period of increased interest in these types of products due to concerns about an infectious disease pandemic.

Because of the concerns that previously used FFRs may be contaminated with infectious material (i.e., act as a fomite), the factors that a health care employer considers in formulating FFR use policies (e.g., single vs. reuse) for its employees are also different from employers in industrial settings. Despite this concern, FFRs are reused under certain conditions in health care.[11] In the health care context, reuse is defined as a HCW donning the same FFR for a series of close patient contacts and doffing it at the end of each of the close patient contacts before it is discarded. Even when FFR reuse is practiced or recommended (discussed in the next section), restrictions are in place (e.g., discard when FFR is contaminated or damaged, becomes difficult to breathe through, and so on) which limits the number of times the same FFR is reused. Thus, FFR reuse is sometimes referred to as "limited FFR reuse." Options for limited FFR reuse were provided when FFRs were first introduced as the minimum level of respiratory protection for HCWs in close contact with patients with tuberculosis.[4–6]

Another related FFR use practice, termed "extended use," involves donning a FFR and wearing it for multiple patient encounters without doffing and redonning between patient visits. Thus, the same FFR is worn continuously (for up to several hours) across multiple patient encounters before it is doffed. This practice is only practical when bundled with the practice of cohorting, which involves locating patients with a common diagnosis in the same unit, ward, or zone. Extended use can be implemented separately from reuse (i.e., like single use, discard the FFR once it is doffed) or combined with reuse. Compared to single use and reuse, recommendations for extended use in health care are fairly recent. The first time extended use of FFRs was identified as an option was during the 2009 H1N1 pandemic.[12]

Both extended use and limited reuse of FFRs allow the employer to reduce its consumption of FFRs, prolonging existing supplies during a pandemic or respiratory pathogen outbreak or to save money and reduce waste during day-to-day operations (e.g., close contact with tuberculosis patients) by using fewer FFRs,[13] similar to the benefits found for industrial settings. This commentary examines recommendations related to extended use and limited reuse of FFRs in health care. Key scientific and policy issues are highlighted along with considerations for policy makers to weigh when making decisions on whether to recommend extended use and/or limited reuse of FFRs during routine health care situations and for public health emergencies involving respiratory pathogens that have the potential for aerosol transmission. Finally, key knowledge gaps are discussed to identify additional data needs that could enhance understanding of the risks for transmission of diseases associated with FFR extended use and limited reuse.

Current and Past FFR Extended Use and Limited Reuse Recommendations

Table I summarizes past and current recommendations for extended use and limited reuse of FFRs. CDC recommendations were selected for this analysis because of their widespread recognition in health care. In 2007, CDC published general infection control guidance for isolation precautions, which included a list of all pathogens and medical procedures in which respiratory protection was recommended.[14] For certain pathogens affecting defined populations (e.g., TB) or infectious agents of special interest to health care (e.g., epidemiologically important organisms such as severe acute respiratory syndrome (SARS)] and influenza), CDC publishes detailed specialized infection control guidance. For this analysis, we selected all of the respiratory pathogens in which specialized infection control guidance was published as either interim or final recommendations and included the use of respiratory protection (N95 FFR or higher). This strategy provided a diversity of respiratory pathogens for analysis. These situations include two recent outbreaks/pandemics (2004 SARS and 2009 H1N1 flu), two routine situations (TB and seasonal influenza), and two pathogens of concern (Avian Influenza A (H5N1) and Avian Influenza A (H7N9)).

Cost can be a consideration for adopting extended use and limited reuse practices as it was in adopting the recommendation to allow limited reuse of FFRs when working in close contact with TB patients. However, the CDC recommendations on limited reuse and extended use have primarily considered the specific pathogens involved and the specific characteristics of the event. The first key factor is whether contact transmission is possible for the pathogen. Contact transmission of pathogens occurs through direct or indirect contact with the patient or the patient's environment via blood or body fluids (e.g., respiratory secretions). For pathogens in which contact transmission (e.g., fomites) is not a concern, limited reuse of FFRs has been determined to be a viable option. For TB, the CDC maintains that “a respirator classified as disposable can be reused by the same HCW as long as it remains functional and is used in accordance with local infection control procedures.” [15] Infection control guidelines for TB [14] recommend only airborne precautions; contact isolation precautions are only needed if extrapulmonary lesions are draining, which occurs rarely. Contact transmission of TB is thought to be highly unlikely.[16]

This contrasts with the recommendations for seasonal influenza where contact with contaminated surfaces and objects is considered a possible mode of transmission.[17] In situations where airborne precautions are recommended, and contact precautions are recommended or contact transmission is possible, the second key factor in the CDC recommendations is the likelihood of a localized shortage of the FFRs needed to protect HCWs during high-risk procedures. The use of FFRs for protection of HCWs during routine infectious disease procedures generally does not result in a FFR shortage, as evidenced by CDC's guidance to wear a FFR during aerosol generating procedures (AGPs) on patients diagnosed with seasonal influenza; this does not include an option for FFR extended use or reuse.[17]

CDC recommendations for Avian Influenza A (H7N9)[18] indicate that FFRs should be discarded after leaving the patient room or patient care area (i.e., “single use”). CDC recommendations for Avian Influenza A (H5N1)[19] do not specifically mention single use,

extended use, or limited reuse, but instead refer back to the general CDC infection control guidance[14] which specifies single use. These recommendations are consistent with the other four recommendations in Table I based on the potential for contact transmission of these pathogens and that FFR shortages are unlikely in the near-term.

However, during periods of high usage (e.g., public health emergencies such as an influenza pandemic[20] or widespread respiratory pathogen outbreak), supplies of FFRs can quickly become depleted because most hospitals maintain only a small inventory of FFRs. Not surprisingly, shortages were reported at the hospital level during both the 2004 SARS outbreak and the 2009 H1N1 influenza pandemic.[21–23] In a recent evaluation of respiratory protection programs in California hospitals, it was reported that half of the hospital managers interviewed (n = 48) reported shortages of FFRs during the 2009 H1N1 outbreak due to increased demand and supplier lag time in filling orders.[11] During the 2004 SARS and 2009 H1N1 events, recommendations were made allowing the option for extended use and limited reuse, although both recommendations acknowledged situations in which these strategies would not be appropriate.

For SARS, CDC stated in its interim guidance that "health care facilities may consider reuse as long as the device has not been obviously soiled or damaged (e.g., creased or torn)" and "if a sufficient supply of respirators is not available."[24] The recommendation recognized the importance of preventing contamination through contact with infectious material on the outside of the respirator. CDC also addressed concerns about a shortage of FFRs during the 2009 H1N1 flu pandemic with supply-conserving strategies for hospitals that included the possibility of extended use and limited reuse of FFRs, with extended use preferred over limited reuse.[12,25] Reuse of FFRs was reported to occur quite often in California hospitals during 2009 H1N1 as either a response to shortages or as standard practice; 81% of survey respondents indicated that their hospital had a plan to implement reuse, while only 12.5% indicated plans to apply extended use.[11]

Scientific Evidence On FFR Extended Use and Limited Reuse

As shown in Table I and discussed above, prior and current CDC recommendations made for FFR extended use and reuse were largely based on the type of infection control precautions or transmission mode(s) associated with that pathogen and whether shortages of FFRs were observed or anticipated. Those recommendations were based upon the data available at that time, which often lacked evidence to answer key questions regarding the effectiveness of extended use or limited reuse and the risk of disease transmission from handling potentially contaminated FFRs. In 2006, the Institute of Medicine (IOM) addressed [26] the reusability of facemasks, and summarized the data available to support previous recommendations. The committee agreed with the previous CDC guidance and recommended that "avoiding contamination [*of FFRs*] will allow for limited reuse." The IOM also identified key knowledge gaps that served as a catalyst for increasing awareness of the research needs.

Since publication of the IOM report, numerous research groups have attempted to address some of these knowledge gaps. In the following sections, we discuss studies published since

2006 that address key areas of FFR extended use and reuse, including FFR protection, human factors (e.g., physiological/psychological effects), self-inoculation, and secondary exposures (e.g., from particle reaerosolization and co-contaminants). Some earlier studies (pre-2006) are also discussed to provide context where needed. The purpose of this analysis is to improve the scientific basis for future recommendations for employers in health care settings to consider when implementing FFR extended use and/or limited reuse. For each of the issues below, a qualitative assessment of the risks of extended use and limited reuse versus single use is presented (see Table II).

FFR Protection

One possible concern with FFR extended use and reuse is that extending the useful life of a FFR could reduce its protective effectiveness (i.e., when worn properly and used in a complete respiratory protection program it provides exposure reduction consistent with the assigned protection factor for this class of respirator). The protection provided by a properly used FFR results primarily from a combination of its ability to filter out (remove) biological aerosols from the inhalation air stream of the wearer and seal tightly to the face (i.e., "fit"). Each of these concerns has been studied (to some extent) or can be assessed using existing data.

Filter Media—Most N95 FFRs contain a polypropylene electret filtering medium within the layers of a FFR (Figure 1). The electret filtering medium has been shown to capture and retain a majority of airborne biological particles compared to the layers next to the face and farthest from the face, although particle size could affect particle deposition location.[27,28] Electrets and other similar types of nonwoven air filter media are not unique to FFRs.[29] They are commonly found in various dust collection systems (e.g., vacuum cleaners, clean rooms, and home heat ventilation and air conditioning (HVAC) systems). Recommended replacement life for electret filters in air cleaning systems is typically 3 months of normal use, as the fundamental mechanisms (diffusion, interception, impaction, electrostatic, and so on) of these types of filters do not readily degrade over time with normal use.

Only a few studies have been done to verify FFR performance in extended use or reuse type scenarios. Moyer and Bergman[30] conducted a laboratory evaluation of the intermittent use (short-term use once per week) of N95 filters over several months. Filtration efficiency was reduced to below 95% for filters from 2 of the 3 manufacturers after 9 and 13 weeks of simulated reuse. Researchers at the Institut de recherché Robert-Sauvé en santé et en sécurité du travail (IRSST) validated the long-term filter performance of a single N95 FFR model. [31] For inert particles below 200 nm, filter efficiency levels remained above 97.3%, even after 5 hr of particle loading (i.e., continuous use). Not surprisingly, another study found that samples from 19 of 21 N95 FFR models stored for up to 10 years had expected levels of filtration performance.[32]

Fit—Fit is a measurement of the efficacy of the seal between the FFR and the face of the wearer. Components of the FFR, such as straps, face seal material, shape, and adjustable nose bands influence FFR fit. Several studies have analyzed strap performance and fit for multiple donnings of FFRs. Roberge et al. measured the restorative forces of straps for five

simulated donnings and reported reduction in the strap load for each successive donning with the majority of the reduction occurring after the first donning.[33] However, the FFR model with the lowest restorative strap performance load was still able to pass fit-testing. Bergman et al. examined the effect of FFR reuse on fit by measuring the fit factors of 6 FFR models donned by 10 subjects up to 20 times with wear times of approximately 2 min between each donning.[34] FFR fit gradually decreased over multiple consecutive donnings; however, good fit was observed for some subjects on some models even after 20 donnings. The best levels of fit were observed for the first five donnings, likely because of the relatively little wear on FFR components (e.g., head straps and nosepieces) compared with later donnings.

It was concluded from that study that five donnings could be performed before fit factors started to drop below 100. Catastrophic failure of the FFR (e.g., complete head strap breakage, nosepiece becomes damaged, and so on) should have no effect on risk, if users diligently perform device inspection procedures required during the FFR donning process. Fit of FFRs is also a concern for extended use where the FFR may become wet and deformed due to moist exhaled breath and facial perspiration. Hauge et al. measured real-time fit while HCWs performed three 10-min simulated patient care scenarios. It was determined that initial fit was predictive of fit during the tasks as the five subjects with initial fit factors greater than 200 registered simulated workplace protection factors greater than 400, and the three subjects with initial fit factors less than 200 had simulated workplace protection factors ranging from 132 to 326.[35] Although the tasks were only a combined 30 min, the study design could be considered an extended use scenario covering three patient encounters.

Workplace Protection Factors—Few studies in health care settings measure workplace protection factors (WPF). WPF is a measure of the protection provided by a properly functioning respirator when correctly worn and used in the workplace and is determined as the ratio of the particle concentration outside the respirator over the particle concentration inside the respirator. Infectious bioaerosols are hard to detect and differentiate from non-infectious bioaerosols.[36] Furthermore, assuring compliance during all periods of exposure in the health care setting is challenging.[37] Several studies in other workplaces have assessed protection over extended periods of continuous use by measuring the WPFs: up to 224 min in a steel foundry[38]; 172 min in a concrete factory [39]; and 60 min on farms.[40] All three studies concluded that the N95 FFRs provided levels of protection consistent with expectations (i.e., protection factors were ≥ the assigned protection factor of 10), with reported geometric mean WPF values ranging from 18 to 223. No evidence of reduced protection as a function of time was noted in these studies. The aerosol challenge encountered at the farm locations consisted of biological aerosols such as endotoxins and fungal spores which are more closely related to the bioaerosols in a hospital than to the dust encountered at the foundry and concrete factory.

Summary—Overall, the scientific studies provide evidence that extended use is unlikely to reduce the protection afforded by a FFR (see Table II) and support the CDC TB infection control guidance which states in the Frequently Asked Questions section, “Disposable

respirators can be functional for weeks to months." However, as noted in Table II, some additional cautions may apply for reuse. Reuse involves multiple repeated uses (donnings) of the same device, and it is possible that some components (straps, nose clips, and so on) could begin to degrade over time and reduce protection. These effects are likely specific to each model of FFR, but the only study published[34] to date on this topic suggests that limiting FFR reuse to no more than five donnings or reuses would provide an adequate safety margin.

Human Factors

One of the consequences of extended use is the need to wear the FFR continuously for up to several hours, compared with single use or reuse in which the FFR would only be worn during the period of close contact with the patient (typically less than 15–20 min). Thus, questions have been raised regarding the safety of long-term FFR use and, if safe, how long HCWs can physiologically and psychologically tolerate extended use.

NIOSH researchers found that FFR use caused no or minimal increases in heart rate, respiratory rate, and transcutaneous carbon dioxide as well as no differences in oxygen saturation on test subjects during 1 hr of low-moderate treadmill exercise when compared with wearing no respirator (control).[41–43] They also reported that 2 hr of continuous FFR use at low-moderate work rate did not cause a change in core body temperature,[42] and there was no significant increase in FFR deadspace heat or humidity after the first hour.[44] Taken together, these studies suggest that FFR use for 1–2 hr should cause minimal physiological stress to individuals medically cleared to wear FFRs.

A study by researchers affiliated with Department of Veterans Affairs reported how long 27 HCWs could tolerate multiple bouts of 2-hr-long extended use periods, interspersed with 15–30 min breaks.[45] Median tolerance times of 6.6 hr and 5.8 hr were reported for the two FFR models without exhalation valves. Only 16 and 18 of the 27 subjects using those two models were able to complete all four 2-hr use periods of continuous use; the most reported reason for stopping use was head and facial discomfort (e.g., heat). In a follow-up analysis of the same data, it was concluded that FFR discomfort negatively affects respirator tolerance over time, but respirator intolerance is not associated with perceived self-reported exertion.[46]

Although the number of participants was small, a recent study reports greater tolerance of extended use of FFRs among HCWs.[47] They reported that 9 of 10 study participants (nurses) were willing to wear FFRs for the entirety of two full 12-hr shifts, stopping only to eat and drink, because it was the end of their shift, or because the FFR was too uncomfortable. The nurses tolerated FFR continuous wear for an average of 223 min on day 1 and 145 min on day 2 and experienced little physiological burden; however, discomfort increased with time, and the nurses reported feeling more short of breath the longer they wore respiratory protection. Transcutaneous carbon dioxide levels increased over time, but were not clinically relevant in that carbon dioxide levels did not reach the requirement for clinically defined hypercapnia.

A study conducted in a teaching hospital in Brazil considered changes in appearance and possible physical damage resulting from FFR reuse.[48] A new N95 FFR was distributed to each nurse once per month and reused as needed until the next new N95 FFR was provided. The researchers found that within 5 days, the majority of the distributed cone-shaped FFRs exhibited visible "wear and tear," indicating possible physical damage (caused by folding them for storage in a pocket) and visible stains/dirt on the FFR interior and exterior surfaces. Although the performance of the respirators was not assessed, the data suggest that some models may be more suitable for reuse (e.g., those that fold easily) or that hospitals should enforce some restrictions on reuse (e.g., replace every 5 days, rather than every 30 days).

Overall, the available scientific studies provide evidence that HCWs will experience greater discomfort during periods of extended continuous wear of FFRs, but this discomfort will likely be tolerable for most HCWs. Continuous FFR use over extended periods of time up to 12 hr is unlikely to harm workers (see Table II) who have been medically cleared for respirator use. Furthermore, because HCWs need to take occasional breaks during their work shift (e.g., to use the rest room, eat or drink, and so on) FFR extended use of greater than 4 consecutive hours is unlikely in most settings.

Self-Inoculation Hazard

One knowledge gap often cited against allowing FFR extended use and limited reuse is whether a FFR worn during close contact with an infected patient is likely to serve as a fomite. Historically, little data were available to assess the transfer potential of respiratory pathogens from the FFR to the hands of the HCW, resulting in the potential for self-inoculation. Similar to other potential fomites (e.g., surfaces, medical devices, and stethoscopes[49]) assessing the level of risk of self-inoculation associated with touching a used FFR is complex. It is very difficult to trace a specific hospital-acquired infection to a particular object. Thus, while no studies have identified the use of a contaminated FFR as a source of infection, the possibility cannot be ruled out.

Nicas and Sun and Nicas and Jones have provided models for transmission of pathogens, including influenza, in health care settings.[50,51] Nicas and Sun considered fomite hazards of textile and nontextile surfaces and in room air to estimate the expected pathogen dose to a HCW's mucous membranes and respiratory tract.[51] Nicas and Jones modeled four influenza virus exposure pathways including fomite transmission. A similar approach is used below to estimate the potential fomite hazard of used FFRs. Factors that influence the risk of self-inoculation directly associated with handling a contaminated FFR include the quantity of respiratory pathogens deposited on the FFR surface (i.e., contamination levels), viability of the pathogen, transfer efficiency of the pathogen from FFRs to the hands of the wearer, and area of hand contact with the contaminated surface.

FFR Contamination Levels—There are no published studies that quantify the amount of pathogens on FFRs used in clinical settings. However, simple mathematical models can be used to provide some estimates. In one study, influenza aerosol concentration, breathing rate of the wearer, time of patient interaction/FFR use, and particle retention efficiency of the FFR were used as inputs to a linear model to estimate influenza contamination levels inside

and on the surface of the FFR (C_{FFR}).[52] Using this model, an increase in any parameter results in higher levels of C_{FFR} (i.e., total number of pathogens on the FFR). For a typical HCW scenario, using model input values estimated from the peer-reviewed literature, the model calculated that C_{FFR} would be approximately 4500 influenza viruses given an influenza aerosol concentration of 12,000 viruses m^{-3}, a breathing rate of 1.140 m^3hr^{-1}, a particle retention efficiency of the FFR of 0.991, and a 20-min patient interaction/FFR use time. The values for influenza aerosol concentration and wear time found in the literature varied more than other parameters used in the model. Thus, for extended use which involves longer wear times, the number of pathogens available for transfer to the hands is increased.

This model illustrates the need to take into account HCW procedures (e.g., AGPs) which can *increase* C_{FFR} by up to 2200% and the potential for administrative controls such as source control of patients (e.g. asking patients to wear face-masks) which can *reduce* C_{FFR} by up to 71%.[53,54] In addition to administrative controls, the use of engineering controls such as local exhaust ventilation might reduce C_{FFR}. Similarly, previous recommendations issued during the SARS outbreak suggested the use of a surgical mask or faceshield on top of a FFR to reduce C_{FFR}[26]; although subsequent work has identified a number of potential concerns, including regulatory compliance with this approach.[55] While developed for influenza, this model could be used to approximate C_{FFR} resulting from any respiratory pathogen if estimates of the concentrations of the pathogen near the breathing zone of the HCW could be obtained.

Pathogen Survival—Given that FFRs can become contaminated with pathogens when used in close contact with infectious patients, the next factor under consideration is how long these pathogens can survive (remain infectious) and, for some types of microorganisms, grow (propagate) on the FFR surface. Some studies in the early 1990s found that under ideal conditions (e.g., humidity >78%), fungi and certain bacteria could grow on filters made of cellulose because they are capable of digesting cellulose.[56,57] However, modern (post-1995) FFRs are made of polypropylene, which cannot serve as a nutrient for bacteria.[58]

Studies confirmed that surrogates for TB were not able to grow on polypropylene-based filter media, even under incubation conditions.[58–61] Although bacteria were found to survive for several days, this was not considered a concern because contact transmission for TB is considered unlikely (see Table I). These studies lent support for the FFR reuse guidance being drafted at that time.

Respiratory viruses have received more recent attention. While growth is not an issue because these pathogens require a host organism to propagate, their persistence or survival on surfaces is a concern. In general, the evidence indicates that viruses are more persistent on nonporous substrates compared with porous materials such as FFRs. Bean et al. reported laboratory-grown influenza A and influenza B survived for 24–48 hr on hard, nonporous surfaces but survived for <8-12 hr on porous substrates.[62] Similarly, another study[63] found that influenza remained viable for 8 hr on FFR samples, but infectivity dropped below detection limits at <24hr.

However, Tiwari et al. examined the persistence of two avian respiratory viruses including influenza H13N7 on various substrates and although they found that both viruses survived longer on nonporous surfaces than on porous ones, the viruses remained active for up to 6 days.[64] In one laboratory study, pH1N1 was detected on FFRs for up to 6 days with an average of 90% reduction (1 log) in viability during this time period.[65] Similar findings were found using MS2 phage as a surrogate for respiratory viruses.[66] A surrogate for SARS coronavirus, transmissible gastroenteritis virus, was shown to remain viable for 24 hr on FFR samples with an estimated 99% (>2 log) reduction in titer.[67])

Another study found that inactivation of Φ6 bacteriophage spiked on a N95 FFR surface was highly sensitive to environmental conditions, with a ~1 log reduction over 24 hr at 40% relative humidity versus ~4 log reduction over 24 hr at 60% relative humidity.[68] Although it is difficult to generalize from these conflicting findings, it is clear that for reuse during a work shift with short storage times (< 1 hr) most of the trapped pathogen will remain viable. Some reduction in viability might occur for overnight (>12 hr) or weekend (>24 hr) storage depending upon storage conditions (temperature, humidity, light, and so on) and pathogen type and strain.

In many cases where pathogens remain persistent and pose a contact threat, cleaning and disinfection regimens are routinely used. For example, countertops, exam tables, and other surfaces of patient rooms are often cleaned when a patient is discharged. Research has been conducted on cleaning and disinfecting procedures for FFRs for possible reuse by the same HCW. Although the results appear to be promising, the practice is not currently recommended[69-72] and thus is not a viable solution at this time to reduce the fomite potential of a reused FFR.

Recent improvements in antimicrobial chemistries have allowed some manufacturers to begin incorporating these technologies into FFRs. There are now a few surgical N95 FFRs incorporating antimicrobial technologies (product code = ONT) that have been cleared by the FDA as medical devices.[9] Interestingly, one device has been cleared by the FDA with claims of 8 hr of continuous use. Unfortunately, none of these devices has been evaluated in the peer-reviewed literature for claims regarding their ability to reduce fomite potential. However, several studies[73–77] have looked at the ability of prototypes or devices, not cleared by FDA, that incorporate some type of antimicrobial chemistry in them to render trapped pathogens inactive over time (i.e., storage time between uses). These studies suggest that efficacy of antimicrobial FFRs for this application is dependent on the pathogen, antimicrobial agent, storage conditions, and specific test method used which makes generalization of findings difficult.[73,78–81] Although promising, the lack of conclusive evidence suggests that additional work is needed before FFRs incorporating antimicrobial technologies can be factored into FFR reuse recommendations.

Transfer Efficiency—Because FFRs can become contaminated with pathogens likely to remain infectious during typical extended use and reuse scenarios, the next factor to assess is the likelihood of pathogens transferring from the FFR to the hands of HCWs. Unfortunately, no studies exist that quantify the percentage of pathogen transferred from the FFR to the hands of HCWs. However, similar to estimating contamination levels, models can be used

where estimates of the key input parameters are available. A simple model for estimating the amount of pathogen transferred to the hands (C_{hand}) of HCWs from contaminated FFRs uses C_{FFR} (the number of pathogens on the FFR as discussed above), transfer efficiency of the pathogen (E_t), and contact area of the hands (A_h) and the FFR (A_{FFR}).

$$C_{hand}=C_{FFR}\frac{A_h}{A_{FFR}}\times E_t$$

Unfortunately, no peer-reviewed sources are available on the transfer efficiency of relevant pathogens from a FFR to skin and others surfaces. However, an unpublished conference presentation reports the transfer efficiency of a bacterium, *Bacillus atrophaeus*, from FFRs to synthetic skin as 0.005% and 0.05% for touching and rubbing, respectively.[82] Other microbial transfer studies for porous surfaces have shown similar results. For example, Rusin et al. reported transfer efficiencies for a bacterium, *Micrococcus luteus*, of 0.13% from a 100% cotton substrate and 0.06% from a 50:50 cotton/polyester substrate. Even lower transfer efficiencies (<0.01%) from those surfaces were reported for bacteriophage PRD-1.[83] Another recent study compared the transfer efficiency of bacteria and viruses from several porous and nonporous surfaces to the fingers. [84] In general, the lowest transfer efficiencies were found for porous surfaces under low relative humidity. Isoelectric point and hydrophobicity of the surface were also important factors.

As discussed previously, C_{FFR} can be estimated. For simplicity, we use the influenza values reported above from Fisher et al. as a surrogate for all respiratory pathogens.[52] The contact area of the hands depends upon the action of the HCW (Table III). For extended use, it is likely that only the finger tips are used to touch the FFR surface (e.g., to reposition the FFR). The total surface area of the volar portion of the fingertips has been estimated to be 7.34 cm^2.[85] However, when implementing FFR reuse, the proper donning process requires a user seal check step, which requires the user to cover the entire FFR surface by cupping both hands around the filter surface. In this situation, A_h would be very similar to A_{FFR}, which has been estimated to be approximately 175 cm^2, but varies among the various FFR models. Assuming uniform deposition of the pathogen over the surface of the FFR, applying input values of 4,500 FFR^{-1} for C_{FFR} and 0.1% as an approximation for E_t to the equation results in an estimated 4.5 pathogens being transferred to the hands of the HCW during the user seal check step and <1 pathogen for each touch involving a fingertip.

Summary—While the model above indicates that some pathogens from a contaminated FFR could transfer to the hands, other factors also affect the risk of infection. Steps in the fomite pathway such as the transfer of viable pathogens from hands to respiratory tract ports of entry, transport of viable pathogens to the site of infection, and the infectious dose of the pathogen are not unique to extended use and reuse of FFRs, but are common to any potential fomite. A full assessment that takes into account these steps is beyond the scope of this commentary. However, the model developed by Nicas and Sun indicates that each successive step in the fomite pathway further reduces the number of infectious pathogens

reaching the site where infection can occur, reducing the risk of self-inoculation from practicing FFR extended use and/or limited reuse.[51]

In theory, extended use should not present a significant self-inoculation hazard, as ideally, the HCW's hands should never come in contact with the contaminated filtering surface when proper doffing protocols are followed.[86] However, the Rebmann study[47] reported that HCWs touched or adjusted their FFR on average 10–20 times per 12 hr. shift. Even with this amount of contact, our analysis, based on the data and the models discussed above, suggests that very few pathogens are likely to make it to the site of infection each time the hand or fingertip comes in contact with the FFR. Thus, extended use is considered minimal risk for typical patient interactions (Table II) when coupled with training and education to reinforce proper use (e.g., don't touch the FFR surface) and adherence to hand hygiene recommendations.

Reusing FFRs provides multiple opportunities for the hands of HCWs to come in contact with any infectious microbes on the respirator surface and thus involves a higher level of risk compared to extended use (Table II). HCWs' hands would presumably contact the contaminated FFR surface when placing the FFR onto the face, adjusting the position of the FFR and flexible strap across the nasal bridge (if applicable), and when performing the user seal check, a requirement after donning a respirator and after each adjustment to the respirator. Similar to extended use, fomite risks from FFR reuse can be mitigated through training and education to reduce unnecessary touching of the FFR and rigorous adherence to hand hygiene. Steps to limit FFR contamination (e.g., masking patients, use of engineering controls, face shields, and so on) can also limit fomite risks, as C_{hand} is proportional to C_{FFR}.

Risk to Others (secondary exposures)

Concerns have been raised that extended use of FFRs could result in additional opportunities for pathogen transmission to co-workers and patients due to reaerosolization of trapped pathogens to the environment from a sneeze, cough, or through rough handling. Several studies have addressed this issue. Most recently, Fisher et al. examined virus reaerosolization from FFRs and concluded that the risk of virus transfer to the environment from the FFR was negligible, a finding key to extended use and reuse.[87] FFRs were challenged with virus-containing droplet nuclei with a size range of 0.65 to 7.0 μm (with the majority <1.1 μm) and challenged with reversed airflow to simulate a sneeze or cough. The highest reaerosolization of 0.21% occurred with a droplet nuclei challenge while a droplet challenge led to reaerosolization of less than 0.0001%. These findings are consistent with earlier studies that examined reaerosolization of bacteria and inert particles. Qian et al. and Willeke and Qian reported the reaerosolization of less than 0.2% for bacteria deposited on N95 FFRs as aerosols and challenged with a reverse airflow consistent with a violent sneeze or cough.[88,89] Kennedy and Hines found that less than 0.3% of polystyrene latex microspheres reaerosolized from FFRs when dropped from a height of 3 feet[90] while Birkner et al reported the average release of 0.006% polystyrene latex microspheres were released from FFRs dropped from heights up to 1.37 m.[91]

Overall, these data provide evidence that the risks of secondary exposure due to reaerosolization or rough handling associated with FFR extended use or limited reuse can be considered negligible (Table II). Similar to the fomite concerns discussed above, secondary exposure risks could increase as C_{FFR}, the number of pathogens on the FFR, increases (i.e., higher C_{FFR} = higher levels of reaerosolized pathogen), so steps taken to limit FFR contamination (e.g., masking patients, faceshields, local exhaust ventilation systems) should be implemented where possible.

In situations where patients are under contact precautions, such as those co-infected with common health care pathogens with the ability for prolonged environmental survival (e.g., Vancomycin-resistant enterococci, *Clostridium difficile*, and norovirus), it may be prudent to have HCWs discard FFRs after close contact because these pathogens could be transferred to other patients via the unclean hands of the HCW.

Sharing FFRs among HCWs could also result in a secondary risk if at least one of the users is infectious (symptomatic or asymptomatic). For example, a specialized face mask containing electret filter media (similar to those found in N95 FFRs) was worn in one study [92] to successfully collect infectious virus from the exhaled breath of symptomatic test subjects. Because of respirators' ability to trap respiratory pathogens, sharing a contaminated FFR could result in disease transmission. However, proper labeling, training, and education can be effective at limiting any inadvertent sharing of FFRs during reuse.

Other Regulatory and Policy Considerations

We also conducted an Internet search and reviewed FFR extended use and reuse recommendations issued by other United States agencies (e.g., FDA and OSHA) and professional organizations (e.g., Association for Professionals Infection Control and Epidemiology).[93,94] In terms of FFR extended use and limited reuse, we identified no major discrepancies among the recommendations from the Association for Professionals in Infection Control and Epidemiology (APIC), OSHA, and the CDC recommendations in (Table I). For example, OSHA TB guidance[7,95] indicates that disposable respirators (i.e., FFRs) can be reused by the same HCW, as long as the functional and structural integrity of the respirator is maintained and the outside of the filter is inspected before each use for signs of physical damage or soiling, and discarded if signs are present.

While OSHA is responsible for regulating employers to provide a safe workplace for their employees and CDC makes public health recommendations that are often adopted by hospitals, FDA has a different role in health care settings. The FDA regulates the manufacture and labeling of medical devices.[96] Medical devices are cleared by the FDA under the Food, Drug, and Cosmetic Act based upon data submitted by the manufacturer to support the claimed intended use of the product. Under 21 CFR 878.4040, FDA classifies surgical N95 respirators as a type of surgical apparel, intended to be worn by operating room personnel during surgical procedures to protect both the surgical patient and the operating room personnel from transfer of microorganisms, body fluids, and particulate material. As part of the labeling requirement, FDA recommends that manufacturers state whether a device is intended to be a reusable device or a single-use disposable device.[9]

Some surgical N95 respirator models are cleared by the FDA with claims of being a single-use device, while other manufacturers do not make such claims.[10] For surgical N95 respirators labeled as "single use only," extended use or limited reuse could be considered as an "off label" use of these products. FDA has specific requirements for reuse ("reprocessing") of single-use medical devices.[97] Unfortunately, as discussed earlier in this manuscript, some hospital use practices for these types of medical devices such as limited FFR reuse were first recommended[4–7] and put into practice prior to FDA's involvement. There is also a general lack of awareness among infection control professionals and safety/employee health administrators in understanding FDA's role in regulating surgical N95 respirators.[98] These factors contribute to the prevalence of "industrial N95 FFRs" used in health care settings. These industrial N95 FFRs are NIOSH-certified FFRs, but have not been cleared by the FDA as medical devices. Several of these industrial N95 FFRs were stockpiled by the CDC in the Strategic National Stockpile.[99]

In the future the different regulatory and policy perspectives will need to be factored into FFR extended use and limited reuse recommendations. For example, recommendations for operating rooms, where soiling and potential contamination from blood borne pathogens will likely occur, might be different. In those situations, limited reuse should only be considered after consultation with the surgical N95 respirator manufacturer and local hospital infection professionals.

Knowledge Gaps

While significant progress has been made since 2006, some knowledge gaps remain to be filled further enhancing an understanding of the risks involved with FFR extended use and limited reuse. Various models related to fomite transfer were presented where little experimental data are available for use as inputs. In particular, data on actual FFR contamination levels from various health care situations and transfer efficiency of pathogens from FFRs to the hands are limited. While several papers have been published on survivability of various respiratory pathogens on FFRs and the effectiveness of antimicrobial technologies, it is not known how generalizable the results are, which makes it difficult to fully assess risk. Well-designed and carefully controlled studies carried out using consistent test methods appropriate to FFR reuse might reduce some of these uncertainties.

Moreover, the infectious dose of various pathogens for the various transmission routes is not well understood, an issue further complicated by newly emerging pathogens and strains. Research and development efforts such as Project BREATHE (Better Respiratory Equipment using Advanced Technology for Health care Employees)[3] that promote the development of better respirators for health care workers are needed to identify novel technologies and designs (e.g., launderabilty, a "no touch" user seal check, and so on) to address some of the additional concerns posed by extended use and reuse. The paucity of data on many of the practical aspects of FFR extended use and reuse also suggests that additional studies are needed to validate preliminary findings regarding the acceptable number of donnings and to develop best practices for storage, labeling, and education/training. Surveillance data on FFR usage, including extended use and reuse, during routine operations and public health emergencies are needed to better understand the possible

benefits (e.g., cost savings, ability to extend existing supplies, reducing the "burn rate," and so on) of FFR extended use and limited reuse.

Limitations

The primary purpose of this article is to assess recent scientific findings to assist policy makers when making decisions on whether to recommend that employers in health care settings permit FFR extended use and/or limited reuse during routine operations and for future public health emergencies. The authors acknowledge that the evidence discussed above is not always as sufficient as desired to develop evidence-based policy decisions. However, decisions on how to protect exposed workers must be made in the present and cannot wait until additional evidence is available. In the interim the available evidence can be useful for policy-based and pragmatic public health decision ideologies.[100] As discussed by Rosella and coauthors, [100] emerging public health situations require a balance between various factors. Both evidential and policy considerations are important. Policy makers need to use the best evidence available to them, even when it has substantial limitations, acknowledge the uncertainties, and account for them in as practical a way as possible.

Conclusion

For recommending FFR extended use and/or limited reuse for routine events, policy makers should weigh the increased risks for disease transmission from FFR extended use and limited reuse against the inconvenience, cost, and waste of single use. In public health emergencies, policies on FFR extended use and limited reuse should weigh the risks for disease transmission against the risk of disease transmission associated with sacrificing because of FFR shortages (e.g., foregoing respiratory protection or using surgical masks for pathogens or activities where N95 FFRs are recommended). Decisions regarding whether FFR extended use or limited reuse should be recommended need to continue to be pathogen- and event-specific. The two most important factors driving this decision should be whether the pathogen is likely to spread (in part) via contact transmission and whether the event could result in or is currently causing a FFR shortage.

This analysis of recent research (post-2006) generally supports CDC guidance issued since 2004 for FFR extended use and limited reuse for routine events such as TB and seasonal influenza (during AGP) as well as the public health emergencies such as the 2004 SARS and 2009 H1N1 flu pandemics. While recent findings largely support these CDC recommendations, some new cautions and limitations should be considered in recommendations issued in the future as discussed subsequently.

Extended use offers a lower risk of self-inoculation compared to limited reuse given that the HCWs hands should ideally rarely contact the contaminated FFR surface. Training and education should be stressed to reinforce the need for strict adherence to guidance to minimize unnecessary contact with the FFR surface and strict adherence to hand hygiene practices. Extended use poses no additional health risk to a medically cleared respirator user and despite the additional discomfort should be tolerable for most HCWs. For these reasons,

extended use should be preferred over limited reuse, even though FFR reuse requires the least change to current practices.

Limited FFR reuse would allow the HCW to doff the FFR to provide a brief respite from the psychological and physiological factors that decrease FFR comfort, but increases the potential for contact transfer when donning the used FFR and performing the user seal check. However, fomite transfer models indicate that the potential for transfer of pathogens from FFRs to the hands of the wearer is small suggesting that limited FFR reuse can be employed with minimal additional risk in most cases. An exception is reuse of FFRs after AGPs, where higher FFR contamination levels are likely to occur. Education and training should be emphasized to reinforce the need for proper hand hygiene when redonning the FFR, including inspection of the device for physical damage and performing a user seal check. Strict adherence to these steps should further reduce the potential to transfer virus from the hands to the points of entry of infection.

While limited FFR reuse remains a viable option for reducing usage rates and for situations involving a pathogen that does not spread via contact transmission, data suggest that FFR protection can begin to be reduced for some models after multiple donnings or uses. Guidance should emphasize the need for the employer to consult with the respirator manufacturer regarding the maximum number of donnings or uses suggested for the FFR models used in that location or to presumptively limit the number of reuses to no more than five to ensure an adequate safety margin, in the absence of new information to the contrary.

Acknowledgments

The authors wish to express our sincere gratitude to members of the NPPTL H7N9 working group as well as Jeff Hageman (CDC's Division of Health Care Quality Promotion), Lisa Koonin (CDC's Influenza Coordination Unit), John Noti (NIOSH's Health Effects Laboratory Division), Teresa Seitz (NIOSH's Division of Surveillance, Hazard Evaluations, and Field Studies), and Caroline Ylitalo (3M Company) for their helpful suggestions and contributions. The findings and conclusions in this manuscript have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

References

1. Respiratory Protective Devices. Code of Federal Regulations Title. 1995; 42(Part 84):30336–30404.
2. National Institute for Occupational Safety and Health (NIOSH). [accessed January 28, 2013] NIOSH Guide to the Selection and Use of Particulate Respirators. Available at http://www.cdc.gov/niosh/docs/96–101/
3. Gosch ME, Shaffer RE, Eagan AE, Roberge RJ, Davey VJ, Radonovich LJ Jr. B95: A new respirator for health care personnel. Am J Infect Control. 2013; 41(12):1224–1230. [PubMed: 23726655]
4. Centers for Disease Control and Prevention (CDC). Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Facilities, 1994. MMWR Recomm Rep. 1994; 43(RR-13):1–132.
5. Harber P, Barnhart S, Boehlecke BA, et al. Respiratory Protection Guidelines, American Thoracic Society March 1996. Am J Respir Crit Care Med. 1996; 154(4 Pt 1):1153–1165. [PubMed: 8887621]
6. National Institute for Occupational Safety and Health (NIOSH). [accessed December 4, 2013] TB Respiratory Protection Program In Health Care Facilities - Administrator's Guide. Available at http://www.cdc.gov/niosh/docs/99–143/

7. Occupational Safety and Health Administration (OSHA). [accessed December 5, 2013] Enforcement Procedures and Scheduling for Occupational Exposure to Tuberculosis CPL2–00–106. Available at https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=DIRECTIVES&p_id=1586
8. Larson, EL.; Liverman, CT. Preventing Transmission of Pandemic Influenza and Other Viral Respiratory Diseases: Personal Protective Equipment for Healthcare Workers: Update 2010. Washington, D.C.: National Academies Press; 2011.
9. Food and Drug Administration (FDA). [accessed December 4, 2013] Guidance for Industry and FDA Staff: Surgical Masks—Premarket Notification [510(k)] Submissions. Available at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm072549.htm
10. Food and Drug Administration (FDA). [accessed December 5, 2013] 510(k) Premarket Notification. Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm
11. Beckman S, Materna B, Goldmacher S, et al. Evaluation of respiratory protection programs and practices in California hospitals during the 2009–2010 H1N1 influenza pandemic. Am J Infect Control. 2013; 41(11):1024–1031. [PubMed: 23932825]
12. Centers for Disease Control and Prevention (CDC). [accessed July 17, 2013] Questions and Answers Regarding Respiratory Protection for Preventing 2009 H1N1 Influenza Among Healthcare Personnel. Available at http://www.cdc.gov/h1n1flu/guidelines_infection_control_qa.htm
13. Rivera P, Louther J, Mohr J, Campbell A, DeHovitz J, Sepkowitz KA. Does a cheaper mask save money? The cost of implementing a respiratory personal protective equipment program. Infect Control Hosp Epidemiol. 1997; 18(1):24–27. [PubMed: 9013242]
14. Siegel JD, Rhinehart E, Jackson M, Chiarello L. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. Am J Infect Control. 2007; 35(10 Suppl 2):S65–164. [PubMed: 18068815]
15. Jensen, PA.; Lambert, LA.; Iademarco, MF.; Ridzon, R. [accessed July 19, 2013] Guidelines for Preventing the Transmission of Mycobac-terium tuberculosis in Health-Care Settings, 2005. Available at http://www.cdc.gov/MMWR/PREVIEW/mmwrhtml/rr5417a1.htm
16. Centers for Disease Control and Prevention (CDC). [accessed July 19,2013] Core Curriculum on Tuberculosis: What the Clinician Should Know. Available at http://www.cdc.gov/tb/education/corecurr/
17. Centers for Disease Control and Prevention (CDC). [accessed July 2, 2013] Prevention Strategies for Seasonal Influenza in Healthcare Settings Guidelines and Recommendations. Available at http://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm
18. Centers for Disease Control and Prevention (CDC). [accessed July 2, 2013] Interim Guidance for Infection Control Within Healthcare Settings When Caring for Patients with Confirmed, Probable, or Cases Under Investigation of Avian Influenza A(H7N9) Virus Infection. Available at http://www.cdc.gov/flu/avianflu/h7n9-infection-control.htm
19. Centers for Disease Control and Prevention (CDC). [accessed January 28, 2014] Interim Recommendations for Infection Control in Health-Care Facilities Caring for Patients with Known or Suspected Avian Influenza. Available at http://www.cdc.gov/flu/avian/professional/infect-control.htm
20. Murray M, Grant J, Bryce E, Chilton P, Forrester L. Facial protective equipment, personnel, and pandemics: Impact of the pandemic (H1N1) 2009 virus on personnel and use of facial protective equipment. Infect Control Hosp Epidemiol. 2010; 31(10):1011–1016. [PubMed: 20731598]
21. Commins, J. [accessed July 19, 2013] Nurses Protest H1N1 Respirator Mask Shortage. Available at http://www.healthleadersmedia.com/content/NRS-241429/Nurses-Protest-H1N1-Respirator-Mask-Shortage.html
22. Mascarenhas, R. [accessed July 2, 2013] Swine-flu Worries Spur Shortage of Hand Sanitizer, Respirator Supplies in N J. Available at http://www.nj.com/news/index.ssf/2009/11/swine_flu_concerns_cause_short.html

23. Srinivasan A, Jernign DB, Liedtke L, Strausbaugh L. Hospital preparedness for severe acute respiratory syndrome in the United States: Views from a national survey of infectious diseases consultants. Clin Infect Dis. 2004; 39(2):272–274. [PubMed: 15307038]
24. Centers for Disease Control and Prevention (CDC). [accessed July 19, 2013] Interim Domestic Guidance on the Use of Respirator to Prevent the Transmission of SARS. Available at http://www.cdc.gov/sars/clinical/respirators.html
25. Centers for Disease Control and Prevention (CDC). [accessed July 19, 2013] Interim Guidance on Infection Control Measures for 2009 H1N1 Influenza in Healthcare Settings, Including Protection of Healthcare Personnel. Available at http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm
26. National Research Council (NRC). Reusability of Facemasks During an Influenza Pandemic: Facing the Flu. Washington, D.C.: National Academies Press; 2006.
27. Fisher E, Rengasamy S, Viscusi D, Vo E, Shaffer R. Development of a test system to apply virus containing particles to filterins facepiece respirators for the evaluation of decontamination procedures. Appl Environ Microbiol. 2009; 75(6):1500–1507. [PubMed: 19139225]
28. Noti JD, Lindsley WG, Blachere FM, et al. Detection of infectious influenza virus in cough aerosols generated in a simulated patient examination room. Clin Infect Dis. 2012; 54(11):1569–1577. [PubMed: 22460981]
29. Hutten, IM. Handbook of Nonwoven Filter Media. Oxford: Elsevier; 2007. p. 325-367.
30. Moyer ES, Bergman MS. Electrostatic N-95 respirator filter media efficiency degradation resulting from intermittent sodium chloride aerosol exposure. Appl Occup Environ Hyg. 2000; 15(8):600–608. [PubMed: 10957815]
31. Haghighat, F.; Bahloul, A.; Lara, J.; Mostofi, R.; Mahdavi, A. [accessed July 2, 2013] Development of a Procedure to Measure the Effectiveness of N95 Respirator Filters Against Nanoparticles Report R-754. Available at http://www.irsst.qc.ca/media/documents/PubIRSST/R-754.pdf
32. Viscusi DJ, Bergman M, Sinkule E, Shaffer RE. Evaluation of the filtration performance of 21 N95 filtering face piece respirators after prolonged storage. Am J Infect Control. 2009; 37(5):381–386. [PubMed: 19188003]
33. Roberge R, Niezgoda G, Benson S. Analysis of forces generated by N95 filtering facepiece respirator tethering devices: A pilot study. J Occup Environ Hyg. 2012; 9(8):517–523. [PubMed: 22746194]
34. Bergman MS, Viscusi DJ, Zhuang Z, Palmiero AJ, Powell JB, Shaffer RE. Impact of multiple consecutive donnings on filtering facepiece respirator fit. Am J Infect Control. 2012; 40(4):375–380. [PubMed: 21864945]
35. Hauge J, Roe M, Brosseau LM, Colton C. Real-time fit of a respirator during simulated health care tasks. J Occup Environ Hyg. 2012; 9(10):563–571. [PubMed: 22924959]
36. Janssen L, Zhuang Z, Shaffer R. Principles for the collection of useful respirator performance data in the workplace. J Occup Environ Hyg. 2013; 11(4):218–226. [PubMed: 24579751]
37. Janssen L, Ettinger H, Graham S, Shaffer R, Zhuang Z. The use of respirators to reduce inhalation of airborne biological agents. J Occup Environ Hyg. 2013; 10(8):D97–D103. [PubMed: 23767796]
38. Janssen LL, Nelson TJ, Cuta KT. Workplace protection factors for an N95 filtering facepiece respirator. J Occup Environ Hyg. 2007; 4(9):698–707. [PubMed: 17654225]
39. Bidwell J, Janssen L. Workplace performance of an N95 respirator in a concrete block manufacturing plant. J Int Soc Resp Prot. 2004; 21(Fall/Winter):94–102.
40. Cho KJ, Jones S, Jones G, et al. Effect of particle size on respiratory protection provided by two types of N95 respirators used in agricultural settings. J Occup Environ Hyg. 2010; 7(11):622–627. [PubMed: 20835946]
41. Kim JH, Benson SM, Roberge RJ. Pulmonary and heart rate responses to wearing N95 filtering facepiece respirators. Am J Infect Control. 2013; 41(1):24–27. [PubMed: 22944510]
42. Roberge R, Benson S, Kim JH. Thermal burden of N95 filtering facepiece respirators. Ann Occup Hyg. 2012; 56(7):808–814. [PubMed: 22294505]

43. Roberge RJ, Coca A, Williams WJ, Powell JB, Palmiero AJ. Physiological impact of the N95 filtering facepiece respirator on healthcare workers. Respir Care. 2010; 55(5):569–577. [PubMed: 20420727]
44. Roberge RJ, Kim JH, Benson S. N95 filtering facepiece respirator deadspace temperature and humidity. J Occup Environ Hyg. 2012; 9(3):166–171. [PubMed: 22413894]
45. Radonovich LJ Jr, Cheng J, Shenal BV, Hodgson M, Bender BS. Respirator tolerance in health care workers. JAMA. 2009; 301(1):36–38. [PubMed: 19126810]
46. Shenal BV, Radonovich LJ Jr, Cheng J, Hodgson M, Bender BS. Discomfort and exertion associated with prolonged wear of respiratory protection in a health care setting. J Occup Environ Hyg. 2012; 9(1):59–64. [PubMed: 22168256]
47. Rebmann T, Carrico R, Wang J. Physiologic and other effects and compliance with long-term respirator use among medical intensive care unit nurses. Am J Infect Control. 2013; 41(12):1218–1223. [PubMed: 23768438]
48. Duarte LRP, Miola CE, Cavalcante NJF, Bammann RH. Maintenance status of N95 respirator masks after use in a health care setting. Rev Esc Enferm USP. 2010; 44(4):1007–1012.
49. Campos-Murguía A, León-Lara X, Munñoz JM, Macías AE, Álvarez JA. Stethoscopes as potential intrahospital carriers of pathogenic microorganisms. Am J Infect Control. 2013; 42(1):82–83. [PubMed: 24176606]
50. Nicas M, Jones RM. Relative contributions of four exposure pathways to influenza infection risk. Risk Anal. 2009; 29(9):1292–1303. [PubMed: 19558389]
51. Nicas M, Sun G. An integrated model of infection risk in a healthcare environment. Risk Anal. 2006; 26(4):1085–1096. [PubMed: 16948699]
52. Fisher EM, Noti JD, Lindsley WG, Blachere FM, Shaffer RE. Validation and application of models to predict facemask influenza contamination in healthcare settings. Risk Anal. Published online March 4, 2014.
53. Milton DK, Fabian MP, Cowling BJ, Grantham ML, McDevitt JJ. Influenza virus aerosols in human exhaled breath: Particle size, culturability, and effect of surgical masks. PLoS Pathog. 2013; 9(3):e1003205. [PubMed: 23505369]
54. Thompson KA, Pappachan JV, Bennett AM, et al. Influenza aerosols in UK hospitals during the H1N1 (2009) pandemic—The risk of aerosol generation during medical procedures. PLoS ONE. 2013; 8(2):e56278. [PubMed: 23418548]
55. Roberge RJ. Effect of surgical masks worn concurrently over N95 filtering facepiece respirators: Extended service life versus increased user burden. J Pub Health Manag Pract. 2008; 14(2):E19–26. [PubMed: 18287908]
56. Pasanen AL, Nikulin M, Berg S, Hintikka EL. Stachybotrys-Atra Corda may produce mycotoxins in respirator filters in humid environments. Am Indus Hyg Assoc J. 1994; 55(1):62–65.
57. Pasanen AL, Keinanen J, Kalliokoski P, Martikainen PI, Ruuskanen J. Microbial growth on respirator filters from improper storage. Scand J Work Environ Health. 1993; 19(6):421–425. [PubMed: 8153595]
58. Wang Z, Reponen T, Willeke K, Grinshpun SA. Survival of bacteria on respirator filters. Aerosol Sci Tech. 1999; 30(3):300–308.
59. Reponen TA, Wang Z, Willeke K, Grinshpun SA. Survival of mycobacteria on N95 personal respirators. Infect Control Hosp Epidemiol. 1999; 20(4):237–241. [PubMed: 10219873]
60. Johnson B, Winters DR, Shreeve TR, Coffey CC. Respirator filter re-use test using the laboratory simulant mycobacterium tuberculosis (H37RA strain). J Am Biol Saf Assoc. 1998; 3:105–116.
61. Brosseau LM, NV MC, Vesley D. Bacterial survival on respirator filters and surgical masks. J Am Biol Saf Assoc. 1997; 2(3):32–43.
62. Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH Jr. Survival of influenza viruses on environmental surfaces. J Infect Dis. 1982; 146(1):47–51. [PubMed: 6282993]
63. Sakaguchi H, Wada K, Kajioka J, et al. Maintenance of influenza virus infectivity on the surfaces of personal protective equipment and clothing used in healthcare settings. Environ Health Prev Med. 2010; 15(6):344–349. [PubMed: 21432565]
64. Tiwari A, Patnayak DP, Chander Y, Parsad M, Goyal SM. Survival of two avian respiratory viruses on porous and nonporous surfaces. Avian Dis. 2006; 50(2):284–287. [PubMed: 16863083]

65. 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(7):2148–2155. [PubMed: 23335770]

66. Fisher EM, Shaffer RE. Survival of bacteriophage MS2 on filtering facepiece respirator coupons. Appl Biosaf. 2010; 15(2):71.

67. Casanova L, Rutala WA, Weber DJ, Sobsey MD. Coronavirus survival on healthcare personal protective equipment. Infect Control Hosp Epidemiol. 2010; 31(5):560–561. [PubMed: 20350196]

68. Casanova LM, Waka B. Survival of a surrogate virus on N95 respirator material. Infect Control Hosp Epidemiol. 2013; 34(12):1334–1335. [PubMed: 24225628]

69. Fisher EM, Williams JL, Shaffer RE. Evaluation of microwave steam bags for the decontamination of filtering facepiece respirators. PLoS ONE. 2011; 6(4):e18585. [PubMed: 21525995]

70. Heimbuch BK, Wallace WH, Kinney K, et al. Apandemic influenza preparedness study: Use of energetic methods to decontaminate filtering facepiece respirators contaminated with H1N1 aerosols and droplets. Am J Infect Control. 2011; 39(1):E1–E9. [PubMed: 21145624]

71. Lore MB, Heimbuch BK, Brown TL, Wander JD, Hinrichs SH. Effectiveness of three decontamination treatments against influenza virus applied to filtering facepiece respirators. Ann Occup Hyg. 2012; 56(1):92–101. [PubMed: 21859950]

72. 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(7):426–436. [PubMed: 21732856]

73. Rengasamy S, Fisher E, Shaffer RE. Evaluation of the survivability of MS2 viral aerosols deposited on filtering face piece respirator samples incorporating antimicrobial technologies. Am J Infect Control. 2010; 38(1):9–17. [PubMed: 19896238]

74. Borkow G, Zhou SS, Page T, Gabbay J. A novel anti-influenza copper oxide containing respiratory face mask. PLoS ONE. 2010; 5(6):e11295. [PubMed: 20592763]

75. Lee JH, Wu CY, Lee CN, et al. Assessment of iodine-treated filter media for removal and inactivation of MS2 bacteriophage aerosols. J Appl Microbiol. 2009; 107(6):1912–1923. [PubMed: 19508297]

76. Lee JH, Wu CY, Wysocki KM, Farrah S, Wander J. Efficacy of iodine-treated biocidal filter media against bacterial spore aerosols. J Appl Microbiol. 2008; 105(5):1318–1326. [PubMed: 18564344]

77. Li Y, Leung P, Yao L, Song QW, Newton E. Antimicrobial effect of surgical masks coated with nanoparticles. J Hosp Infect. 2006; 62(1):58–63. [PubMed: 16099072]

78. Zuo Z, de Abin M, Chander Y, Kuehn TH, Goyal SM, Pui DY. Comparison of spike and aerosol challenge tests for the recovery of viable influenza virus from non-woven fabrics. Influenza Other Respir Viruses. 2013; 7(5):637–644. [PubMed: 23398976]

79. McCabe KM, Turner J, Hernandez MT. A method for assessing the disinfection response of microbial bioaerosols retained in antimicrobial filter materials and textiles. J Microbiol Meth. 2012; 92(2013):11–13.

80. Majchrzycka K, Gutarowska B, Brochocka A. Aspects of tests and assessment of filtering materials used for respiratory protection against bioaerosols. Part II: Sweat in the environment, microorganisms in the form of a bioaerosol. Intern J Occup Safe Ergon. 2010; 16(2):275–280.

81. Majchrzycka K, Gutarowska B, Brochocka A. Aspects of tests and assessment of filtering materials used for respiratory protection against bioaerosols. Part I: Type of active substance, contact time, microorganism species. Intern J Occup Safe Ergon. 2010; 16(2):263–273.

82. Fisher, EM.; Ylitalo, CM.; Stepanova, N.; Shaffer, RE. Assessing Filtering Facepiece Respirator Contamination During Patient Care in Flu Season: Experimental and Modeling Approaches; ISRP —Sixteenth International Conference: A Global View on Respiratory Protection; Boston. September 23–27, 2012;

83. Rusin P, Maxwell S, Gerba C. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. J Appl Microbiol. 2002; 93(4):585–592. [PubMed: 12234341]

84. Lopez GU, Gerba CP, Tamimi AH, Kitajima M, Maxwell SL, Rose JB. Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. Appl Environ Microbiol. 2013; 79(18):5728–5734. [PubMed: 23851098]

85. Murai M, Lau HK, Pereira BP, Pho RW. A cadaver study on volume and surface area of the fingertip. J Hand Surg Am. 1997; 22(5):935–941. [PubMed: 9330158]

86. Centers for Disease Control and Prevention (CDC). [accessed July 19, 2013] Sequence for Donning Personal Protective Equipment PPE/Sequence for Removing Personal Protective Equipment. Available at http://www.cdc.gov/HAI/pdfs/ppe/ppeposter148.pdf

87. Fisher EM, Richardson AW, Harpest SD, Hofacre KC, Shaffer RE. Reaerosolization of MS2 bacteriophage from an N95 filtering facepiece respirator by simulated coughing. Ann Occup Hyg. 2012; 56(3):315–325. [PubMed: 22127875]

88. Qian Y, Willeke K, Grinshpun SA, Donnelly J. Performance of N95 respirators: Reaerosolization of bacteria and solid particles. Am Ind Hyg Assoc J. 1997; 58(12):876–880. [PubMed: 9425648]

89. Willeke K, Qian Y. Tuberculosis control through respirator wear: Performance of National Institute for Occupational Safety and Health-regulated respirators. Am J Infect Control. 1998; 26(2):139–142. [PubMed: 9584808]

90. Kennedy NJ, Hinds WC. Release of simulated anthrax particles from disposable respirators. J Occup Environ Hyg. 2004; 1(1):7–10. [PubMed: 15202151]

91. Birkner JS, Fung D, Hinds WC, Kennedy NJ. Particle release from respirators, part I: Determination of the effect of particle size, drop height, and load. J Occup Environ Hyg. 2011; 8(1):1–9. [PubMed: 21132594]

92. Huynh KN, Oliver BG, Stelzer S, Rawlinson WD, Tovey ER. A new method for sampling and detection of exhaled respiratory virus aerosols. Clin Infect Dis. 2008; 46(1):93–95. [PubMed: 18171219]

93. Occupational Safety and Health Administration (OSHA). [accessed July 19, 2013] Pandemic Influenza Preparedness and Response Guidance for Healthcare Workers and Healthcare Employers. Available at https://www.osha.gov/Publications/3328-05-2007-English.html

94. Rebmann, T.; Alexander, S.; Cain, T.; Citarella, B.; Cloughessy, M.; Coll, B. [accessed July 19, 2013] APIC Position Paper: Extending the Use and/or Reusing Respiratory Protection in Healthcare Settings During Disasters. Available at http://www.apic.org/Resource_/TinyMceFileManager/Advocacy-PDFs/APIC_Position_Ext_the_Use_and_or_Reus_Resp_Prot_in_Hlthcare_Settin-gs1209l.pdf

95. Occupational Safety and Health Administration (OSHA). [accessed August 12, 2013] Healthcare Wide Hazards Tuberculosis. Available at https://www.osha.gov/SLTC/etools/hospital/hazards/tb/tb.html#RespiratoryProtection

96. Food and Drug Administration (FDA). [accessed January 28, 2014] FDA's Role in Regulating PPE. Available at http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/GeneralHospitalDevicesandSupplies/PersonalProtectiveEquipment/ucm056084.htm

97. Food and Drug Administration (FDA). [accessed December 5, 2013] Reprocessing of Single-Use Devices. Available at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/ReprocessingofSingle-UseDevices/default.htm

98. Figueroa, M. School of Public Health. New Brunswick, N.J.: University of Medicine and Dentistry of New Jersey and Graduate School-New Brunswick Rutgers; 2013. Respiratory Protection Programs and Healthcare Professionals.

99. Food and Drug Administration (FDA). [accessed December 4, 2013] Summary Fact Sheet for Disposable Respirators for Use During the Swine Flu Emergency. Available at http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161612.htm

100. Rosella LC, Wilson K, Crowcroft NS, Chu A, Upshur R, Willison D, et al. Pandemic H1N1 in Canada and the use of evidence in developing public health policies—A policy analysis. Soc Sci Med. 2013; 83:11–9.

101. Roy CJ, Milton DK. Airborne transmission of communicable infection—The elusive pathway. N Engl J Med. 2004; 350(17):1710–1712. [PubMed: 15102996]

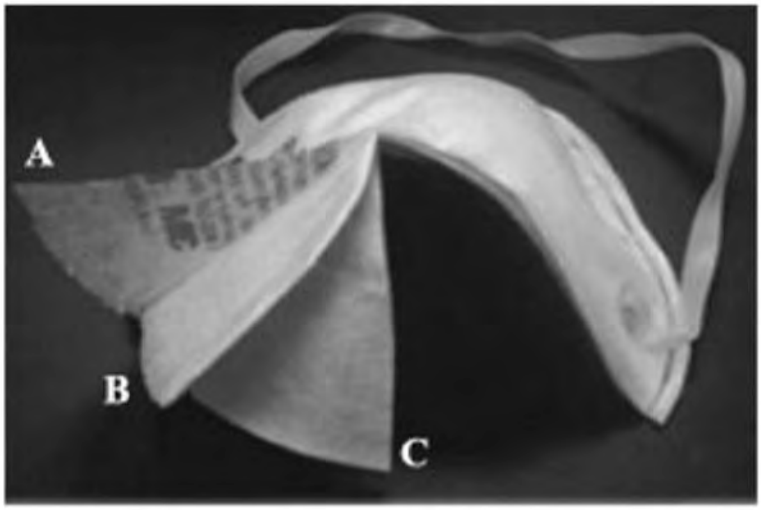


Figure 1.
Photograph of a NIOSH certified N95 FFR cut open to show the different layers. A, polypropylene material (outermost layer); B, electret filtering medium (typically made from melt-blown or electrospun polypropylene); and C, polypropylene material (innermost layer).

Table I

Current and Past CDC Recommendations for Limited Reuse and Extended Use of FFRs in Health Care for Select Respiratory Pathogens

Respiratory pathogen	Contact precautions	Possibility of contact transmission[A]	Possibility of an FFR shortage	Extended use/Limited reuse recommended
TB	No	No	No	Yes
SARS	Yes	Yes	Yes	Yes
Avian Influenza A (H5N1)	Yes	Yes	No	No
2009 H1N1 Flu	No	Yes	Yes	Yes
Seasonal Influenza (AGP Only)	No	Yes	No	No
Avian Influenza A(H7N9)	Yes	Yes	No	No[B]

[A] The scientific community continues to debate the primary mode(s) of transmission for many respiratory viruses. However, most experts acknowledge that contact transmission cannot be ruled out.(101)

[B] Interim recommendation, subject to change

Table II

Qualitative Assessment of Increased Risks of FFR Extended Use and Limited Reuse Compared with Single Use

Issue	FFR Extended Use	Limited FFR Reuse
FFR Protection	• Negligible risk of decreased protection	• Minimal risk of decreased protection, but can be mitigated through limiting the number of reuses.
Human Factors	• Increased discomfort, but no additional health risk to a medically cleared respirator user	• No additional health risk to a medically cleared respirator user
Self-inoculation	• Minimal risk for typical patient interactions, but can be mitigated through training and education • Risks can increase during/after AGP but can be reduced by limiting contamination	• Moderate risk for typical patient interactions but can be mitigated through training and education and limiting the number of reuses • Risks can increase during/after AGP but can be reduced by limiting contamination
Secondary Exposures	• Negligible for typical patient interactions • Minimal following AGP but can be reduced by limiting contamination	• Negligible for typical patient interactions • Minimal following AGP but can be reduced by limiting contamination

Table III

Steps in the Donning and Doffing Process Involving Potential Contact with FFR Surface

Strategy	Donning	User Seal Check	Doffing
FFR Reuse	Yes	Yes	No[A]
FFR Extended Use	No	No	No[A]

[A] HCWs hands should not contact the surface if proper doffing technique is used.

8

HHS Public Access

Author manuscript

Infect Control Hosp Epidemiol. Author manuscript; available in PMC 2015 November 16.

Published in final edited form as:

Infect Control Hosp Epidemiol. 2013 May ; 34(5): 494–499. doi:10.1086/670225.

Challenge of N95 Filtering Facepiece Respirators with Viable H1N1 Influenza Aerosols

Delbert A. Harnish, MS[1], **Brian K. Heimbuch, MS**[1], **Michael Husband, MS**[2], **April E. Lumley**[1], **Kimberly Kinney, BS**[1], **Ronald E. Shaffer, PhD**[3], and **Joseph D. Wander, PhD**[4]

[1]Applied Research Associates, Panama City, Florida

[2]Office of the Secretary, Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, US Department of Health and Human Services, Washington, DC

[3]National Institute for Occupational Safety and Health, Pittsburgh, Pennsylvania

[4]Air Force Research Laboratory, Tyndall Air Force Base, Florida

Abstract

OBJECTIVE—Specification of appropriate personal protective equipment for respiratory protection against influenza is somewhat controversial. In a clinical environment, N95 filtering facepiece respirators (FFRs) are often recommended for respiratory protection against infectious aerosols. This study evaluates the ability of N95 FFRs to capture viable H1N1 influenza aerosols.

METHODS—Five N95 FFR models were challenged with aerosolized viable H1N1 influenza and inert polystyrene latex particles at continuous flow rates of 85 and 170 liters per minute. Virus was assayed using Madin-Darby canine kidney cells to determine the median tissue culture infective dose ($TCID_{50}$). Aerosols were generated using a Collison nebulizer containing H1N1 influenza virus at 1×10^8 $TCID_{50}$/mL. To determine filtration efficiency, viable sampling was performed upstream and downstream of the FFR.

RESULTS—N95 FFRs filtered 0.8-μm particles of both H1N1 influenza and inert origins with more than 95% efficiency. With the exception of 1 model, no statistically significant difference in filtration performance was observed between influenza and inert particles of similar size. Although statistically significant differences were observed for 2 models when comparing the 2 flow rates, the differences have no significance to protection.

CONCLUSIONS—This study empirically demonstrates that a National Institute for Occupational Safety and Health–approved N95 FFR captures viable H1N1 influenza aerosols as well as or better than its N95 rating, suggesting that a properly fitted FFR reduces inhalation exposure to

Address correspondence to Delbert A. Harnish, MS, Applied Research Associates, 430 West 5th Street, Suite 700, Panama City, FL 32401 (dharnish@ara.com).

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article. All authors submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and the conflicts that the editors consider relevant to this article are disclosed here.

The findings and conclusions of this article are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health or the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, US Department of Health and Human Services.

airborne influenza virus. This study also provides evidence that filtration efficiency is based primarily on particle size rather than the nature of the particle's origin.

Pandemic influenza poses a significant health threat to the international community as novel strains emerge that vary widely in virulence and infectivity.[1,2] Which of the primary modes of human transmission of influenza[3–6]—direct contact, inspiration, inhalation, and direct spray—are responsible for spreading influenza is a subject of active debate. As a consequence, specification of the appropriate personal protective equipment (PPE) for respiratory protection against influenza is likewise controversial. For direct-spray transmission, a surgical mask may be appropriate for reducing the risk of infection, but it is not recommended for protection against aerosol transmission via inhalation or inspiration. In accordance with guidance provided by the Centers for Disease Control and Prevention, the Occupational Safety and Health Administration (OSHA) mandates that healthcare workers wear PPE at least as protective as a properly fitted National Institute for Occupational Safety and Health (NIOSH)–certified N95 filtering facepiece respirator (FFR) when exposed to some inhalable or inspirable infectious aerosols (eg, severe acute respiratory syndrome, tuberculosis, and 2009 H1N1 pandemic influenza).[7,8] For use in clinical settings, N95 FFRs are sometimes also cleared for sale by the Food and Drug Administration as a medical device having fluid-resistant properties and certified by NIOSH. Devices carrying a NIOSH certification have shown the ability to remove 95% or more of particles of the conventional most-penetrating particle size (MPPS), 0.3 μm (with larger or smaller particles being removed more efficiently).[9] However, the MPPS for FFRs employing electret media (media possessing an electrical charge) is smaller.[10]

The mechanisms used by FFRs to remove particles from the air are well understood.[11] It is also well accepted that the composition of particles of similar density does not affect particle capture efficiency. Thus, viable and inert particles of equivalent size and mass should be removed with the same filtration efficiency. Many studies have been performed to evaluate the filtration efficiency of viable microorganisms.[12–16] Without exception, they all show that viable microorganisms are removed at similar or slightly greater rates than inert particles of the same size, supporting the idea that FFR effectiveness against aerosol transmission does not need to be reevaluated for every new disease-causing agent. However, even with this consistent knowledge base, end users of FFRs still want confirmation that the device is capable of removing actual infective agents of interest. We found limited studies evaluating FFR performance when challenged with viable influenza aerosols. Zuo et al[17] challenged N95 FFRs with viable aerosols of human adenovirus serotype 1 and swine influenza H3N2 but were able to obtain viable data for the adenovirus only upstream of the FFR. Borkow et al[18] evaluated the antimicrobial efficacy of copper-impregnated N95 FFRs by challenging with viable H1N1 aerosols. Their results showed greater than 99% viable filtration efficiency (VFE), but they did not correlate their data to inert particles. Our study challenged 5 FFR models (Table 1) with viable H1N1 influenza aerosols representative of human respiratory secretions and compares the VFE to the inert particle filtration efficiency (PFE) at 2 flow rates.

METHODS

H1N1 Virus

Influenza A/PR/8/34 VR-1469 (ATCC VR-95) was propagated in embryonic chicken eggs by means of standard World Health Organization protocols.[19] Virus titers were determined by a median tissue culture infectious dose ($TCID_{50}$) assay using Madin-Darby canine kidney cells (ATCC CCL-34) and cell culture techniques approved by the World Health Organization.[19] For aerosolization studies, the H1N1 influenza virus was diluted to a concentration of 1×10^8 $TCID_{50}$/mL in an artificial saliva buffer.[20] The count median diameter (CMD) of the particle size distribution (PSD) of the influenza aerosol in the artificial saliva buffer was 0.83 μm, as previously determined using an Aerodynamic Particle Sizer (APS) 3321 (TSI).[21]

Filtration Studies

Five models of NIOSH-approved N95 FFRs, of which 2 models contained antimicrobial components (GlaxoSmithKline [GSK] Actiprotect and SafeLife T5000), were used for this study (Table 1). The 3 nonantimicrobial models were chosen for their common use in the healthcare workplace. The 2 antimicrobial models were selected because they were the only 2 such models that were commercially available and NIOSH approved. All models were tested in triplicate under 2 conditions: (1) an aerosol challenge at the NIOSH-specified standard flow rate of 85 liters per minute (LPM) and (2) a morestrenuous aerosol challenge of 170 LPM to evaluate FFR performance under extreme conditions. A laboratory-scale aerosol tunnel (LSAT; Figure 1) was used to challenge the FFRs with viable influenza and inert beads. A complete description of the LSAT has been reported elsewhere.[20–22] Prior to each test, the LSAT was flushed with purified air for 30 minutes at a flow rate of 50 LPM. For each independent test (1 FFR at 1 condition), a FFR was glue-sealed into a 6-inchdiameter sample holder as described elsewhere[21] and then secured into the LSAT via stainless steel sanitary fittings. Each FFR was first challenged with 0.8-μm polystyrene latex beads (Thermo Scientific). The beads were suspended in sterile water and then placed in a 6-jet Collison nebulizer (BGI), operating at 20 psi to generate the aerosol. Following a 10-minute equilibration period, 3 alternating upstream and downstream samples were collected using the APS. The air flow was then redirected to a high-efficiency particulate air (HEPA) filter, while the Collison nebulizer was replaced with another Collison nebulizer containing 30 mL of H1N1 influenza diluted to a concentration of 1×10^8 $TCID_{50}$/mL in artificial saliva.

Following a 10-minute equilibration period, alternating viable samples were collected through the upstream and downstream ports. All-glass impingers (AGI-30; Ace Glass) containing 20 mL of serum-free Eagle's minimum essential medium (Hyclone Laboratories) supplemented with 1% 100× penicillin-streptomycin and 1% 200 mM L-glutamine (Sigma-Aldrich) were used for collection. To minimize particle loss, the AGI-30s were directly attached to the isokinetic sampling ports on the LSAT. Sampling was initiated by opening the valve on the port and then applying a vacuum source to the AGI-30, which sampled at approximately 12.5 LPM. After 5 minutes, the sampling port was closed, the vacuum was turned off, and the AGI-30 was placed on ice until viable plating was performed. A total of 6

samples (3 upstream and 3 downstream, alternately sampled) were collected for each FFR. Following each run, the FFR was removed and HEPA filters were connected to the sampling ports. The LSAT was subsequently flushed with purified air at 60 ± 10 LPM for 3 hours. A manometer was used to monitor the pressure drop across the filter during each run.

Data Analysis

Upstream and downstream measurements for the 0.8-μm bead study were collected using data from the 0.723–0.925-μm size bins of the APS. The concentration of viable virus (log $TCID_{50}$ per milliliter of extract) collected in the upstream and downstream AGI-30s was determined using the Spearman-Kärber formula.[23] Equation (1) was used to determine the total amount of virus recovered from each sample (20-mL impinger volume). For samples with no detectable downstream viable data, half the detection limit (2.5 $TCID_{50}$ infectious dose units) of the viable assay was used to calculate the reduction.[24] The VFE of the FFRs was determined using equation (2), and the PFE was determined using equation (3). A 2-tailed paired *t* test was used to compare the inert (0.8-μm bead) and viable (H1N1 influenza) filtration data for each N95 FFR model. The average PFE and VFE values for the 2 flow rates were compared using a 2-tailed unpaired *t* test. A 1-way analysis of variance (ANOVA) test with a Bonferroni posttest was used to compare data obtained from the antimicrobial and nonantimicrobial FFR models.

Equation (1) is as follows:

$$\text{virus concentration per sample} = L_S = 10^{[L+\log(V)]}, \quad (1)$$

where L is viable H1N1 expressed in units of $\log_{10}$ $TCID_{50}$ per milliliter and V is sample volume. If no viable viruses are present ($L = -\infty$), then L_S will be half the detection limit. Equation (2) is

$$\text{VFE} = \frac{\sum_{1-n}^{i}[1 - (DL_S/UL_S)] \times 100\%}{n}, \quad (2)$$

where DL_S is downstream $\log_{10}$ $TCID_{50}$, UL_S is upstream $\log_{10}$ $TCID_{50}$, and n is the number of determinations, and equation (3) is

$$\text{PFE} = \frac{\sum_{1-n}^{i}[1 - (D/U)] \times 100\%}{n}, \quad (3)$$

where U is the upstream particle concentration and D is the downstream particle concentration.

RESULTS

The average upstream challenge for all FFR replicates was 1.8×10^3 $TCID_{50}$per liter of air. Under standard flow (85 LPM) parameters, the mean PFE for all FFR models ranged from 99.72% to 99.999%, and the mean VFE ranged from 98.93% to 99.996% (Table 2). A statistical comparison of the 2 data sets demonstrated that there is a significant difference ($P < .05$) between inert and viable particle filtration for only the Kimberly-Clark model ($P = .$

02). The SafeLife T5000 provided 1–2 orders of magnitude higher filtration performance, exceeding the NIOSH standard for an N100 FFR. Four of the 6 SafeLife T5000 replicates produced no detectable virus downstream.

Under high flow (170 LPM) parameters, the mean PFE for all FFR models ranged from 98.37% to 99.994%, and the mean VFE ranged from 96.29% to 99.995% (Table 3). The SafeLife T5000 again provided 1–2 orders of magnitude higher filtration performance. A statistical comparison of the 2 data sets demonstrated a significant difference between inert and viable particle filtration for only the Kimberly-Clark FFR ($P = .02$).

A comparison of performances at 85 and 170 LPM was conducted. The Kimberly-Clark model demonstrated statistically significant different filtration efficiencies for both inert and viable aerosol challenges ($P = .003$ and .002, respectively). The GSK Actiprotect model was found to demonstrate a significant difference only for the inert particles ($P = .0006$). A 1-way ANOVA test demonstrated a statistically significant difference between the nonantimicrobial FFR models and both the Safelife T5000 and GSK Actiprotect for VFE at the 170-LPM condition ($P = .0001$ and .05, respectively). A significant difference was also observed for PFE ($P = .0002$ and .0003, respectively). No significant difference was found between the nonantimicrobial and antimicrobial FFRs at the 85-LPM condition.

DISCUSSION

Previous experimental studies, supported by filtration theory, demonstrate that PFE increases with particle size above the MPPS. While it is possible in a laboratory setting to artificially generate an influenza aerosol near the MPPS of most FFRs, particles in this size range (approximately 0.1 μm) are relatively unstable and are unlikely to exist in practice. In actual workplace settings, influenza expelled from humans via respiratory sections is typically much larger (approximately 0.8 μm) than the bare virus. We acknowledge the existence of divergent reports pertaining to the assessment of particles/droplets derived from human respiratory secretions,[25–28] but we maintain that use of a 0.8-μm particle is justified on the basis of the literature.[20]

Each N95 FFR model tested as part of this study yielded equivalent VFE and PFE values that exceeded 95% (Tables 2 and 3). As NIOSH certification is based on removal of 0.3-μm particles, these higher removals are to be expected for the larger particles studied here. Although determined to be statistically significant, the differences between PFE and VFE at 85 LPM for the Kimberly-Clark model (less than 2.6%) are not considered to be meaningful because the 95% NIOSH benchmark was met and actual protection is driven more by differences in fit (leakage) than filtration performance. Thus, the statistical analysis in this case is not instructive and indicates only that the low variability among replicate measurements obtained by the particle sizer allows discrimination of the slightly higher filtration efficiencies of inert particles from the generally greater variability associated with capturing and assaying viable biological particles.

A possible bias introduced in this study is that the methods of analysis for inert and viable challenges are different, which may influence the comparison of the VFE and PFE. The PFE

is determined using the APS and accounts only for particles whose aerodynamic particle size ranges from 0.723 to 0.925 μm. In contrast, the VFE accounts for all particles in the PSD. Another bias may be present in the sampling procedure because AGI-30 impingers collect larger particles more efficiently,[29] as do FFRs. The particles most likely to penetrate the FFR fall into a smaller size range, in which capture efficiency by the impinger is lower. Another factor that must be considered is the distribution of viable particles within the overall PSD, which is not known and may introduce another bias that cannot be accounted for. Our data are consistent with values reported by Borkow et al,[18] who demonstrated more than 95% reduction of VFE in an aerosol (approximately 3.0-μm CMD) containing viable influenza, although they sampled by means of impaction rather than impingers and performed their testing at a lower flow rate, 28 LPM. Zuo et al[17] also provided data showing that viable influenza can be removed from the airstream but provided particle-count data derived only from a viable challenge of much smaller particles (CMD of less than 0.1 μm), which behave much differently.[20]

The effect of flow rate on N95 FFR performance was assessed by incorporating 2 flow conditions into the experimental design. According to 42 CFR 84 subpart K, section 84.181, the 85-LPM flow rate is the condition specified by NIOSH for evaluating the performance of FFRs. This flow rate was selected to represent a worker's inhalation at a high work rate. However, peak inhalation flow during breathing may be greater than 85 LPM for brief periods of time[30] and exacerbated further as work intensity is increased. For these reasons, we also tested at 170 LPM to provide an extreme challenge to the filter. The overall filtration numbers were slightly lower in the higher flow rate (Tables 2 and 3), as would be expected for particles smaller than 1 μm. Critical inspection of the data shows that the actual difference in filtration performance between the 85- and 170-LPM conditions for the particle size studied is negligible (1%–2%). Although statistically significant, these differences are merely an indicator of low variability in the data sets and not a physically meaningful distinction.

Although the antimicrobial FFR models (SafeLife T5000 and GSK Actiprotect) demonstrated considerably higher filtration efficiencies than the nonantimicrobial models, they did not provide a significantly greater reduction in viable penetration compared with inert particles, and we attribute the increased filtration efficiency to physical means rather than antimicrobial properties. These results are in line with what was observed by Borkow et al,[18] who found no improvement in VFE for FFRs impregnated with copper oxide. The SafeLife T5000's filtration performance actually exceeds the rating for a N100, observed for both the inert and the viable H1N1 particles. For the GSK FFR, the lack of reduction in VFE due to the antimicrobial was expected, as the manufacturer claims only that the antimicrobial is a surface decontaminant. It is of interest to note that the GSK FFR had the highest variability for VFE ($\sigma = \pm 2.5\%$) among all FFRs tested (Tables 2 and 3). The reason for this is unclear; it is possible that the citric acid present on the FFR interferes with the viable assay, but as the VFE is lower than the PFE, it might suggest that citrate is protective, acting to shield the virus downstream of the FFR. Additional research is necessary to isolate the mechanism causing the variability.

The significance of these findings to healthcare workers is that the data provide a basis to estimate the level of protection that a healthcare worker can expect from a respirator during exposure to infectious aerosols. Inhalation exposures received by a respirator wearer come from a combination of leakage around the face seal, direct penetration through the filter, and leakage through other apertures (eg, holes in filters from staples used to secure FFR straps). Numerous workplace studies have shown that a properly fitted NIOSH-certified N95 FFR will reduce toxic inhalation exposures by a factor of 10 or more.[31,32] Controlled leak studies conducted using manikin headforms have shown that leak size is the dominant factor affecting respirator inward leakage.[33] In the workplace, an OSHA-mandated fit test is required to ensure that the respirator is capable of fitting the healthcare worker (ie, seals tightly to the face to minimize leakage in the face seal area). Because the FFR was sealed (ie, a perfect fit) in our experiments, capture efficiencies for viable H1N1 influenza exceeding 98.9% at the lowest flow rate represent a best-case scenario in terms of fit. However, when some inward leakage during routine respirator wear is factored in, these data, combined with the workplace studies cited above, suggest that an N95 FFR is capable of reducing inhalational exposure to H1N1 influenza or other infectious aerosols by a factor of 10 or greater if properly fitted and used as expected, similar to the attenuation of other workplace aerosols.

In conclusion, this study empirically demonstrates that a NIOSH-approved N95 FFR captures viable H1N1 influenza aerosols with an efficiency equal to or greater than its N95 rating, suggesting that a properly fitted FFR reduces inhalation exposure to airborne influenza virus. Only 5 FFR models were tested as part of this study, but the findings have broad applicability to all properly fitted NIOSH-approved N95 FFRs. This study also demonstrates that the N95 FFR models tested remove particles from the airstream, indiscriminate of viability. Particles that contain H1N1 influenza are equally affected by filtration mechanisms as inert particles of the same size. Although the antimicrobial FFRs demonstrated significantly higher VFE, they also showed significantly higher PFE; thus, their enhanced performance must be attributed to physical means rather than antimicrobial activity.

ACKNOWLEDGMENTS

Financial support. This research was funded by the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, US Department of Health and Human Services, through an interagency agreement with the Air Force Research Laboratory (AFRL). This work was also partly funded by the National Institute for Occupational Safety and Health through an interagency agreement with the AFRL. Representatives of both funding agencies were involved in the development of the experimental design, data interpretation, and manuscript preparation.

REFERENCES

1. Tellier R. Aerosol transmission of influenza A virus: a review of new studies. J R Soc Interface. 2009; 6:783–790.
2. Masaki I, Watanabe T, Masato H, et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortment H5 HA/H1N1 virus in ferrets. Nature. 2012; 486(7403):420–428. [PubMed: 22722205]
3. Jones RM, Adida E. Influenza infection risk and predominate exposure route: uncertainty analysis. Risk Anal. 2011; 31(10):1622–1631. [PubMed: 21418085]

4. Institute of Medicine. Preventing Transmission of Pandemic Influenza and Other Viral Respiratory Diseases: Personal Protective Equipment for Healthcare Personnel, Update 2010. Washington, DC: National Academies Press; 2011.
5. Tellier R. Review of aerosol transmission of influenza A virus. Emerg Infect Dis. 2006; 12:1657–1662. [PubMed: 17283614]
6. Bridges CB, Kuehnert MJ, Hall CB. Transmission of influenza: implications for control in health care settings. Clin Infect Dis. 2003; 37(8):1094–1101. [PubMed: 14523774]
7. Siegel JD, Rhinehart E, Jackson M, Chiarello L. 2007 Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings. Am J Infect Control. 2007; 25:S65–S164. [PubMed: 18068815]
8. [Accessed December 10, 2011] Pandemic influenza preparedness and response guidance for healthcare workers and healthcare employers. Occupational Safety and Health Administration (OSHA) website. Publication no. OSHA 3328-05. http://www.osha.gov/Publications/3328-05-2007-English.html. Published 2007.
9. Birkner, JS.; Colton, CE. Respiratory protective equipment. In: Rose, V.; Cohrssen, B., editors. Patty's Industrial Hygiene. 6th ed.. New York: Wiley; 2010. p. 1169-1233.
10. Shaffer RE, Rengasamy S. Respiratory protection against airborne nanoparticles: a review. J Nanopart Res. 2009; 11:1661–1672.
11. Hinds, WC. Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles. 2nd ed.. New York: Wiley; 1999.
12. Balazy A, Toivola M, Adhikari A, et al. Do N95 respirators provide 95% protection level against airborne viruses, and how adequate are surgical masks? Am J Infect Control. 2006; 34(2):51–57. [PubMed: 16490606]
13. Heimbuch, BK.; Wander, JD.; Wu, CY.; Wallace, WH.; Kinney, K.; Lumley, AE. Defense Technical Information Center. Tyndall Air Force Base, FL: AFRL-ML-TY-TP-2006-4527, Air Force Research Laboratory; 2006. Bioaerosol challenges to antimicrobial surface treatments: enhanced efficacy against MS2 coli phage of air filter media coated with polystyrene-4-methyltrimethylammonium triiodide. Accession no. ADA444909.
14. Heimbuch, BK.; Wu, CY.; Wander, JD. Defense Technical Information Center. Tyndall Air Force Base, FL: AFRL-RX-TY-TP-2009-4567, Air Force Research Laboratory; 2009. Viral penetration of high efficiency particulate air (HEPA) filters. Accession no. ADA505315.
15. Gardner, PD.; Richardson, AW.; Eshbaugh, JP.; Hofacre, KC. Defense Technical Information Center. Aberdeen Proving Ground, MD: ECBC-CR-085, Edgewood Chemical and Biological Center, US Army Research Development and Engineering Command; 2006. Respirator filter efficiency testing against particulate and biological aerosols under moderate to high flow rates. Accession no. ADA457318.
16. Eninger RM, Adhikari A, Reponen T, Grinshpun SA. Differentiating between physical and viable penetrations when challenging respirator filters with bioaerosols. Clean—Soil AirWater. 2008; 36(7):615–621.
17. Zuo Z, Kuehn TH, Pui DYH. Performance evaluation of filtering facepiece respirators using virus aerosols. Am J Infect Control. 2013; 41(1):80–82. [PubMed: 22483237]
18. Borkow G, Zhoi SS, Page T, Gabbay J. A novel anti-influenza copper oxide containing respiratory face mask. PLoS ONE. 2010; 5(6):e11295. [PubMed: 20592763]
19. World Health Organization. [Accessed December 14, 2011] WHO Manual on Animal Influenza Diagnosis and Surveillance. http://www.who.int/csr/resources/publications/influenza/whocdscsrncs20025rev.pdf. Published 2002.
20. American Society for Testing and Materials (ASTM). Standard Test Method for Effectiveness of Decontamination of Air-Permeable Materials Challenged with Biological Aerosols Containing Human Pathogenic Viruses. West Conshohocken, PA: ASTM International; 2010. Standard E2720-10
21. Heimbuch BK, Wu CY, Wander JD, et al. A pandemic influenza preparedness study: use of energetic methods to decontaminate filtering facepiece respirators contaminated with H1N1 aerosols and droplets. Am J Infect Control. 2010; 38(1):3–8. [PubMed: 20022406]

22. Foarde, K.; Heimbuch, BK.; Maxwell, A.; VanOsdell, D. Test Operating Procedure under the Army Test and Evaluation Command. Aberdeen Proving Ground, MD: Edgewood Chemical and Biological Center; 2009. Method for evaluating air purification technologies for collective protection using viable microbial aerosols. (forthcoming).
23. Finney, DJ. Statistical Methods in Biological Assays. 2nd ed.. New York: Hafner; 1964.
24. 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.
25. Duguid JP. The size and duration of air-carriage of respiratory droplets and droplet-nuclei. J Hyg. 1946; 4:471–480. [PubMed: 20475760]
26. Yang S, Lee GWM, Chen CM, Wu CC, Yu KP. The size and concentration of droplets generated by coughing in human subjects. J Aerosol Med. 2007; 20(4):484–494. [PubMed: 18158720]
27. Chao CYH, Wan MP, Morawska L, et al. Characterization of expiration air jets and droplets size distributions immediately at the mouth opening. Aerosol Sci. 2009; 40:122–133.
28. Morawska L, Johnson GR, Ristovski Z, et al. Size distribution and sites of origin of droplets expelled from the human respiratory tract during expiratory activities. Aerosol Sci. 2009; 40:256–269.
29. Lin X, Willeke K, Ulevicius V, Grinshpun SA. Effect of sampling time on the collection efficiency of all-glass impingers. AIHA J. 1997; 58(7):480–487.
30. Caretti, DM.; Gardner, PD.; Coyne, KM. Defense Technical Information Center. Aberdeen Proving Ground, MD: ECBC-TR-316, Edgewood Chemical Biological Center, US Army Research, Development, and Engineering Command; 2004. Workplace breathing rates: defining anticipated values and ranges for respirator certification testing. Accession no. ADA465595.
31. Janssen LL, Nelson TJ, Cuta KT. Workplace protection factors for an N95 filtering facepiece respirator. J Occup Environ Hyg. 2007; 4(9):698–707. [PubMed: 17654225]
32. Cho KJ, Reponen T, McKay R, et al. Comparison of workplace protection factors for different biological contaminants. J Occup Environ Hyg. 2011; 8(7):417–425. [PubMed: 21732855]
33. Rengasamy S, Eimer BC. Total inward leakage of nanoparticles through filtering facepiece respirators. Ann Occup Hyg. 2011; 55(3):253–263. [PubMed: 21292731]

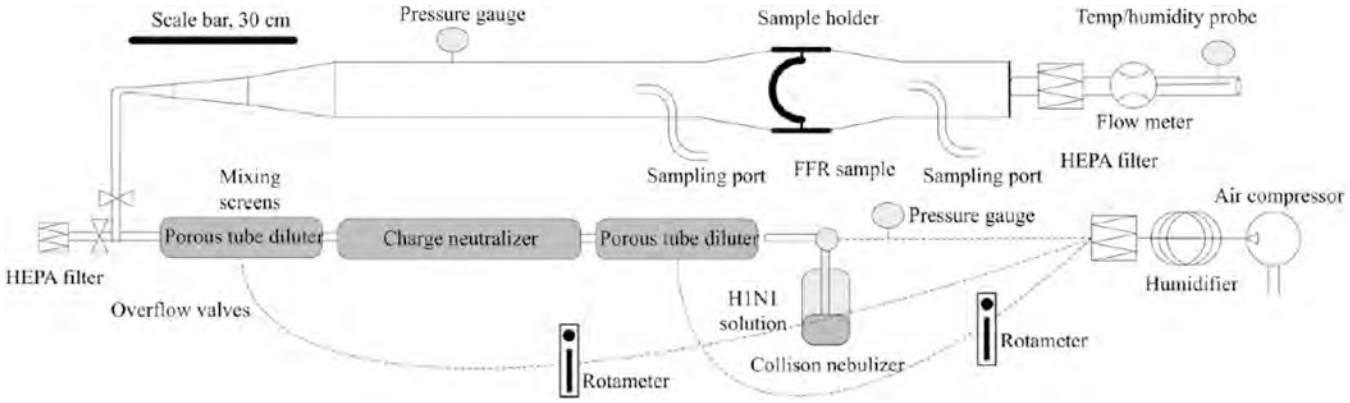


FIGURE 1.

Laboratory-scale aerosol tunnel. FFR, filtering facepiece respirator; HEPA, high-efficiency particulate air.

TABLE 1

Filtering Facepiece Respirators (FFRs) Used in This Study

Manufacturer	Model	Rating	FFR shape	Antimicrobial
3M	1860S	N95	Cup	None
3M	1870	N95	Flat fold	None
Kimberly-Clark	PFR95	N95	Duck bill	None
SafeLife	T5000	N95	Cup	Triosyn (iodine)
GlaxoSmithKline	Actiprotect	N95	Cup	Virucoat (citric acid)

TABLE 2

Average Removal Efficiencies of 0.8-μm Particles at 85 Liters per Minute

FFR model	Inert, %	H1N1 influenza, %	*P*
3M 1860S	99.85 ± 0.10	99.27 ± 0.38	.08
3M 1870	99.90 ± 0.09	99.13 ± 1.36	.45
Kimberly-Clark PFR95	99.72 ± 0.16	98.93 ± 0.36	.02
SafeLife T5000	99.999 ± 0.001	99.996 ± 0.002[a]	.09
GlaxoSmithKline Actiprotect	99.94 ± 0.06	99.23 ± 1.00	.19

[a]The data for 1 filtering facepiece respirator (FFR) replicate were below the detection limit.

TABLE 3

Average Removal Efficiencies of 0.8-μm Particles at 170 Liters per Minute

FFR model	Inert, %	H1N1 influenza, %	*P*
3M 1860S	99.37 ± 0.39	98.56 ± 0.87	.13
3M 1870	99.96 ± 0.03	99.59 ± 0.27	.14
Kimberly-Clark PFR95	98.37 ± 0.32	96.29 ± 0.56	.02
SafeLife T5000	99.994 ± 0.009	99.995 ± 0.002[a]	.90
GlaxoSmithKline Actiprotect	99.23 ± 0.15	96.29 ± 2.49	.09

[a]The data for all 3 filtering facepiece respirator (FFR) replicates were below the detection limit.

9

AFRL-RX-TY-TP-2009-4594

A PANDEMIC INFLUENZA PREPAREDNESS STUDY: USE OF ENERGETIC METHODS TO DECONTAMINATE FILTERING FACEPIECE RESPIRATORS CONTAMINATED WITH H1N1 AEROSOLS AND DROPLETS

POSTPRINT (Code 20)

Brian K. Heimbuch, William H. Wallace, Kimberly R. Kinney, April E. Lumley
Applied Research Associates
P.O. Box 40128
Tyndall Air Force Base, FL 32403

Chang Yu Wu, Myung-Heiu Woo
University of Florida
Gainesville, FL

Joseph D. Wander
Airbase Sciences Division
Air Force Research Laboratory
139 Barnes Drive, Suite 2
Tyndall Air Force Base, FL 32403-5323

June 2012

AIR FORCE RESEARCH LABORATORY
MATERIALS AND MANUFACTURING DIRECTORATE

■ **Air Force Materiel Command** ■ **United States Air Force** ■ **Tyndall Air Force Base, FL 32403-5323**

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
12-JUN-2012	Journal Article POSTPRINT	01-FEB-2009 - 30-NOV-2009

4. TITLE AND SUBTITLE

A Pandemic Influenza Preparedness Study: Use of Energetic Methods to Decontaminate Filtering Facepiece Respirators Contaminated with H1N1 Aerosols and Droplets (POSTPRINT)

5a. CONTRACT NUMBER

FA4819-07-D-0001

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

0909999F

6. AUTHOR(S)

+Heimbuch, Brian K.; +Wallace, William H.; +Kinney, Kimberly R.; +Lumley, April E.; ++Wu, Chang-Yu; ++Woo, Myung-Heui; *Wander, Joseph D.

5d. PROJECT NUMBER

DODT

5e. TASK NUMBER

L0

5f. WORK UNIT NUMBER

Q220L7AD

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

+Applied Research Associates, P.O. Box 40128, Tyndall Air Force Base, FL 32403
++University of Florida, Gainesville, FL

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

*Air Force Research Laboratory
Materials and Manufacturing Directorate
Airbase Technologies Division
139 Barnes Drive, Suite 2
Tyndall Air Force Base, FL 32403-5323

10. SPONSOR/MONITOR'S ACRONYM(S)

AFRL/RXQL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

AFRL-RX-TY-TP-2009-4594

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution Statement A: Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

Ref AFRL/RXQ Public Affairs Case #10-003. Document contains color images. Published 10.1016/j.ajic.2010.07.004.
Distribution Code 20: JOURNAL ARTICLES; DTIC USERS ONLY. Replaces PREPRINT copy (ADA514657).

14. ABSTRACT

A major concern among healthcare 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 must be evaluated to verify the effectiveness of this strategy: biocidal efficacy, filtration performance, pressure drop, fit, and residual toxicity. The focus of this research effort was to evaluate the ability of microwave/steam energy, low-temperature moist heat, and ultraviolet germicidal irradiation at 254 nm to decontaminate H1N1 influenza virus loaded onto FFRs as either aerosols or droplets. Our data indicate that all three decontamination technologies provide > 4-log reduction of viable H1N1 virus --in 93% of our experiments, the virus was removed to levels below the method detection limit. These data are encouraging and may contribute to the evolution of effective strategies for decontamination and reuse of FFRs.

15. SUBJECT TERMS

disinfection, reuse, infection control, microwave, respirator, steam, UVG1, virus

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Joseph D. Wander
U	U	U	UU	9	19b. TELEPHONE NUMBER *(Include area code)*

Reset

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

AJIC major article

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.

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 ≥95% of airborne particles ~0.3 μm in diameter and has been demonstrated to effectively remove infectious microorganisms from the air.[6,7] Particles larger and smaller than 0.3 μm 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

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 al[15] 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 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.

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

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 nonbiocidal.[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 viruses[28-31] 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

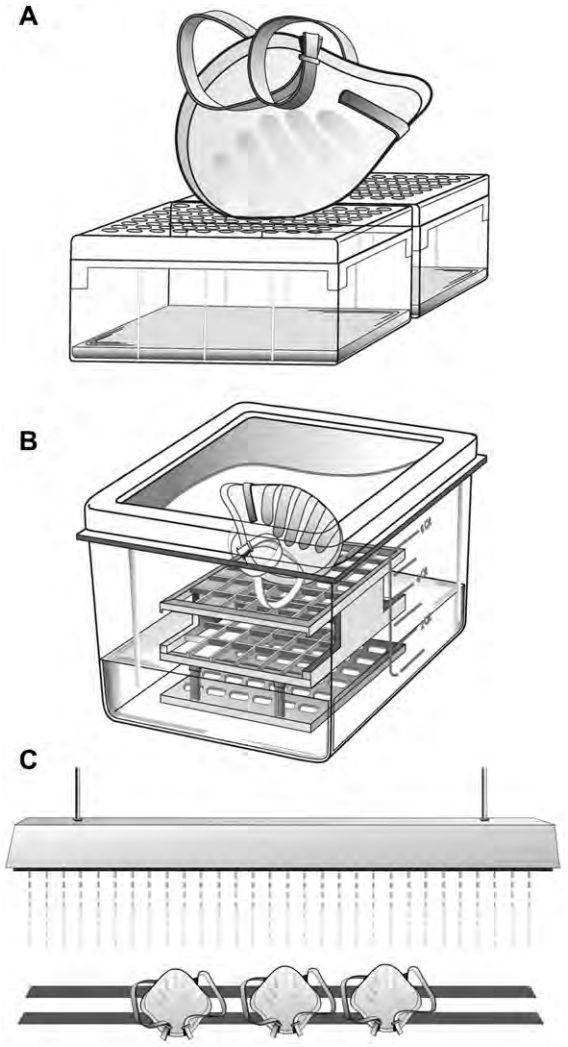


Fig 1. Devices for decontaminating FFRs. (A) MGS device for decontamination of individual FFRs. (B) Chamber for applying WMH to FFRs. (C) Decontamination of FFRs using UVGI.

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 ($TCID_{50}$) 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 $MgCl_2 \cdot 7\,H_2O$, 0.13 g of $CaCl_2 \cdot H_2O$, 0.42 g of $NaHCO_3$, 7.70 mL of 0.2 M KH_2PO_4, 12.3 mL of 0.2 M K_2HPO_4, 0.11g of NH_4Cl, 0.19 g of KSCN, 0.12 g of $(NH_2)_2CO$, 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 deionized water (pH 7)] to a concentration of ~8 $\log_{10}$ $TCID_{50}$/mL. The virus solution was added to a 6-jet Collison 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

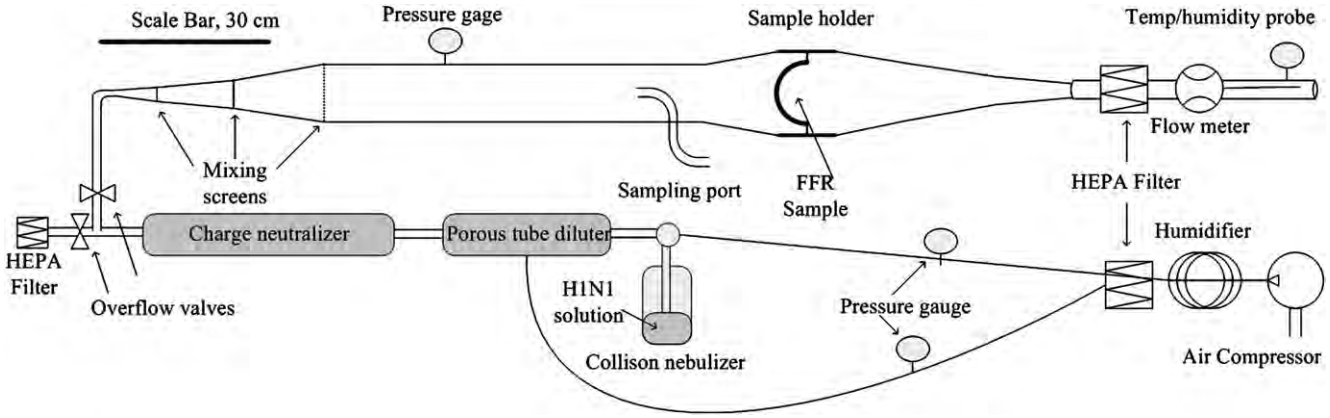


Fig 2. The LSAT device used to apply aerosols to the FFRs. The LSAT is fabricated with 10-cm-diameter stainless steel sanitary fittings and a 15-cm filter holder to accommodate the FFR. The biological aerosol is generated by a 6-jet Collison nebulizer. Dilution air, conditioned by passing the air through 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 ft^3/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/cm^2 to 2.2 mW/cm^2. The exterior surface of H1N1-contaminated FFRs was irradiated for 15 minutes, which provided an average dose of 18 kJ/m^2. 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 $TCID_{50}$ 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

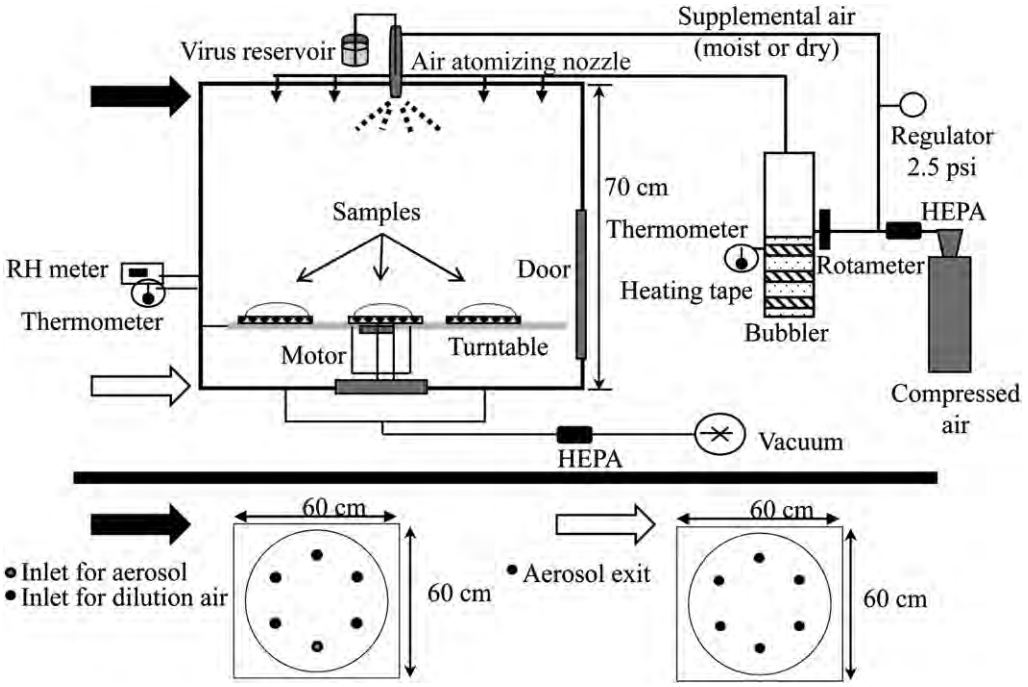


Fig 3. The droplet loader device used to apply droplets to the FFRs. The device is composed of a stainless steel shell (60 cm l × 60 cm w × 90 cm h). Droplets are created by applying compressed air to an air-atomizing nozzle that produces a droplet at the source with a CMD of ~40 μm. Uniform dispersion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

at 5% CO_2/37°C before cytopathic effects were analyzed.

Data analysis

The Spearman-Karber formula[40] was used to determine the concentration of viable virus per mL of extract (L, expressed in units of $\log_{10}$TCID$_{50}$/mL). The following equation was used to determine the total amount of virus recovered from each sample (45.6 cm^2):

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

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 TCID$_{50}$ infectious dose unit. The upper 95% CI, assuming a mean of <1 live viruses in each sample, was 3.47 ($\log_{10}$ = 0.55) TCID$_{50}$ 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 $\log_{10}$ TCID$_{50}$ 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 $\log_{10}$TCID$_{50}$, 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 (log_{10} $TCID_{50}$ 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 $TCID_{50}$ 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)

	UVGI		MGS		WMH	
Respirator*	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 al[44] 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 al[31] 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 al[15] 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

1. Kilbourne ED. Influenza pandemics of the 20th century. Emerg Infect Dis 2006;12:9-14.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
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.
8. Bridges CB, Kuehnert MJ, Hall CB. Transmission of influenza: implications for control in healthcare settings. Clin Infect Dis 2003;37:1094-101.
9. Tellier R. Review of aerosol transmission of influenza A virus. Emerg Infect Dis 2006;12:1657-62.
10. 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.
11. 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.
12. Bailar JC, Burke DS, Brosseau LM, Cohen HJ, Gallagher EJ, Gensheimber KF, et al. Reusability of facemasks during an influenza pandemic. Washington, DC: Institute of Medicine, National Academies Press; 2006.
13. 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.
14. Viscusi DJ, Bergman MS, Eimer BC, Shaffer RE. Evaluation of five decontamination methods for filtering facepiece respirators. Ann Occup Hyg 2009;53:815-27.
15. 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.
16. 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.
17. 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.
18. 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.
19. 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.
20. 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.
21. 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.
22. 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.
23. 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.
24. Velva GR, Wu JF. Mechanism of lethal action of 2450-MHz radiation on microorganisms. Appl Environ Microbiol 1979;37:550-3.
25. Jeng DK, Kaczmarek KA, Woodworth AG, Balasky G. Mechanism of microwave sterilization in the dry state. Appl Environ Microbiol 1987;53:2133-7.
26. 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.
27. Swayne DE, Beck JR. Heat inactivation of avian influenza and Newcastle disease viruses in egg products. Avian Pathol 2004;33:512-8.
28. Deshmukh DR, Pomeroy BS. Ultraviolet inactivation and photoreactivation of avian viruses. Avian Dis 1969;13:596-602.
29. Jakab GJ, Knight ME. Decreased influenza virus pathogenesis by infection with germicidal UV-irradiated airborne virus. Environ Int 1982;8:415-8.
30. Jensen M. Inactivation of airborne viruses by ultraviolet irradiation. Appl Microbiol 1964;12:418-20.
31. 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.
32. 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.
33. 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.
34. Boone SA, Gerba CP. Significance of fomites in the spread of respiratory and enteric viral disease. Appl Environ Microbiol 2007;73:1687-96.
35. Aps JKM, Martens LC. Review: the physiology of saliva and transfer of drugs into saliva. Forensic Sci Int 2005;150:119-31.
36. Bean B, Moore BM, Sterner B. Survival of influenza viruses on environmental surfaces. J Infect Dis 1982;146:47-51.
37. 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.
38. 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 antivirus 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.

10

The Use of Respirators to Reduce Inhalation of Airborne Biological Agents

Larry Janssen, Harry Ettinger, [...], and Ziqing Zhuang

OVERVIEW

The use of respiratory devices to protect against potentially hazardous biological aerosols that are transmittable via inhalation has increased in recent years. When in an environment containing this potential hazard, both surgical masks (SM) and N95 filtering facepiece respirators (FFR) have been used by the general public as well as health care workers. While the superior filtration and fit characteristics of N95 FFR over surgical masks have been demonstrated in laboratory and workplace studies with inert (non-biological) particles, their superiority in reducing disease transmission in clinical/field settings is still questioned by some members of the health care/infection control industry. Attempts to study the relative efficacy of the two devices in the field using clinical outcomes have yielded inconclusive results because of limitations in experimental design and implementation.

This commentary examines the differences between the two devices and identifies considerations necessary to study their performance properly. No study to date has been conducted in a manner that would allow the performance of the two types of devices to be differentiated. In particular, study subjects failing to wear the assigned device during all times of potential exposure, along with a lack of continuous observation of subjects' use, compromise the superior protection the N95 FFR can provide. Additionally, the lack of formalized, complete respiratory protection programs negates the superior filtration and fit characteristics of the N95 FFR. As has been shown in industrial workplaces, one may reasonably expect that N95 FFR will effectively reduce health care workers' inhalation exposures to airborne biological agents when complete, effective respiratory programs are in place. Because voluntary users and the general public will not likely use respirators under the guidance of a formal program, the benefit of respirator use alone is likely to be minimal.

INTRODUCTION

Respiratory protection devices are an important element of an overall contagion control strategy when infectious biological aerosols are potentially present in an occupational environment. In health care facilities, N95 class FFR certified by the National Institute for Occupational Safety and Health (NIOSH) are typically the minimum class of respiratory protection recommended.[1,2] In contrast, studies exist that suggest that surgical masks normally used in health care settings may be equally effective in reducing disease transmission.[3,4] The "respirator versus surgical mask" debate continues in both health care and non-health care settings.

This article describes and evaluates the findings of recent studies examining the role of respirators and surgical masks in reducing disease transmission. Established principles of evaluating respiratory protective device performance are used to explain inconclusive results. This article also makes recommendations to maximize respiratory protection from biological aerosols.

RESPIRATORS AND SURGICAL MASKS

While similar in appearance, N95 FFR and SM are designed to serve different purposes. Stated briefly, surgical masks (SM) are intended to prevent bacteria and other particles exhaled by the wearer from contaminating a sterile field (e.g., patient's wound). This device also serves as a barrier to prevent the wearer from touching his/her oronasal region with contaminated hands or gloves as well as to protect that region from direct sprays and splashes. SM are regulated by the Food and Drug Administration (FDA). Particle filtration performance evaluation is recommended, but no minimum level of filtration efficiency is required.[5] SM are not mandated to form a seal against the user's face; any leakage provides a route for biological particles to enter the wearer's breathing zone.

FFRs also serve as a barrier to touching of the oronasal region, and some of them also act as a barrier to direct sprays and splashes. However, FFRs' primary function is to reduce the wearer's exposure to particles with aerodynamic diameters in the inhalable ($\leq$100 μm) size fraction, including those in the respirable size range ($\leq$10 μm).[6] Numerous studies have demonstrated that biological and non-biological particles are filtered in the same manner, with equivalent efficiency.[7–13] Filtration efficiency criteria for N95 FFR are set by NIOSH and are measured under rigorous test conditions.[14] Any certified particulate respirator must be at least 95% efficient when tested according to NIOSH criteria. In addition, FFR must be capable of forming a seal to the user's face in order to be worn in an occupational setting. The Occupational Safety and Health Administration (OSHA) has specific test criteria for demonstrating acceptable respirator fit on each individual user.[15] OSHA also regulates FFR selection, use, and care in workplaces, including health care facilities.[15,16]

The filtration and fit characteristics of SM were evaluated by Oberg and Brosseau.[17] Nine surgical masks, six of which met all FDA performance criteria, were subjected to the NIOSH filtration efficiency test and OSHA-mandated fit tests. The filters ranged from approximately 10% to 96% efficiency under the NIOSH test conditions; only one SM met the NIOSH minimum requirement for filter efficiency. This finding was consistent with

research done by NIOSH, which also found a wide range of filtration performance for SM tested at the NIOSH filtration test conditions.[18] Furthermore, quantitative fit tests conducted by Oberg and Brosseau resulted in only two acceptable fits out of 40 trials. Consequently, small particles are likely to enter the wearer's breathing zone via both the SM itself (poor filtration) and the gaps between the SM and the skin of the face (poor fit). As such, SM cannot be expected to significantly reduce the inhalation of infectious aerosols.

ASSESSMENT OF FFR AND SM EFFECTS ON DISEASE TRANSMISSION

Recent studies have attempted to measure the ability of FFR, SM, or both in a variety of occupational and community settings.[3,4,19–25] To understand the results of these studies, it is important to identify several factors that confound the assessment of how well either type of device performs.

Multiple Routes of Exposure

Aerosol transmission of biological particles is only one of several routes of exposure for some diseases for which respiratory protection may be used. Recent field studies[26,27] suggested that long-range transmission of influenza is possible via aerosols in the respirable size range. Additionally, investigations of disease outbreaks[28,29] suggest proximity to the index (first) case as a major factor in respiratory disease transmission. This may indicate increased inhalation exposure to small particles, and/or transmission of a virus (e.g., influenza) by particles >100 μm (droplets, sprays) produced when an infected person coughs or sneezes. It is traditionally believed that droplet spray transmission occurs only within a radius of approximately ~3 feet from the infected person, although recent recommendations have suggested that 6 to 10 feet may be prudent for emerging or highly virulent pathogens.[2] Transmission of some viruses may occur by touching contaminated surfaces or objects with the hands and subsequently touching the eyes, nose, or mouth. Exposure of unprotected eyes to airborne viruses may also contribute to infection.[19] Importantly, the relative contribution of each mode of transmission is not clear for many diseases.[30,31]

By limiting droplet spray and hand contact with the nose and mouth, both FFR and SM may limit disease transmission by these routes. Because only FFR are designed and tested to filter small aerosols and effectively seal to the user's face (demonstrated by individual fit testing), they are expected to be more effective than SM in controlling transmission of disease via particle inhalation. It is also critical that gloves, gowns, and eye protection be used in conjunction with hand washing to control the non-inhalation exposure routes if the efficacy of either FFR or SM is to be assessed. This "bundling" of interventions can, in itself, confound the evaluation of FFR or SM performance.

Lack of Airborne Exposure Limits

Human dose-response curves for some respiratory pathogens, including influenza, have been developed [32] and used to estimate the infectious dose of influenza A in humans.[33] In these studies, both the likelihood of infection and the severity of symptoms increased with an increasing inhalation dose of influenza virus. These findings are consistent with the pattern seen with other hazardous aerosols, and the same industrial hygiene principles of control apply to both inert (i.e., non-biological) and biological aerosols.

Nonetheless, while quantitative airborne exposure limits do exist for the inert particulate hazards (dusts, fumes, and so on) for which FFR are commonly worn, these limits have not been established for biological hazards. Accordingly, no field study of FFR or SM performance against pathogens such as influenza has attempted to measure airborne biological particles either outside (C_o) or inside (C_i) the device during periods of exposure. This means there is no assurance that the device under evaluation was tested with a sufficient concentration of airborne infectious agents, or how much the device was able to reduce the inhaled exposure. In contrast, workplace studies of FFR performance against inert hazards use C_o and C_i measurements to define the device's efficacy: the calculated C_o:C_i ratio represents performance, i.e., how much the FFR reduces exposure and is called the workplace protection factor (WPF).[34–39] For contaminants with exposure limits, FFR performance is adequate when C_i measurements are below that limit. While C_o:C_i ratios for biological contaminants may not be convenient (or even feasible) to measure at this time, they would provide reasonable estimates of the actual exposure reduction provided by the devices in use.

Multiple Exposure Venues

Infectious agents can be present in health care facilities and other workplaces, in the homes of infected individuals, and in general community environments such as schools, theaters, and mass transit vehicles. Because the end point of FFR or SM performance studies is typically infection (or a marker of infection), it is critical that participants are not potentially exposed to the infectious agent in *any* venue outside that in which the device is being tested. Clearly, infections that are acquired outside the environment in which the FFR or SM is used cannot be attributed to poor performance of the device.

Non-Compliance and Lack of Subject Observation

Respiratory protection for airborne biological or chemical hazards can be effective only when properly worn during all times of exposure. Overall

protection is rapidly reduced when the FFR is not worn during even short periods of exposure. The term Effective Protection Factor (EPF) describes the amount by which the challenge atmosphere is reduced by FFR, taking into account periods of non-wear time in the contaminated atmosphere.[39] It is calculated as follows:

$$EPF = \frac{T_s}{T_w + \frac{T_{nw}}{WPF}}$$

T_s = Shift or exposure duration

T_w = Time the respirator is worn

T_{nw} = Time the respirator is not worn

WPF = Workplace protection factor

Figure 1 illustrates the dramatic decrease in protection with increasing periods of non-wear time. The EPF of 10 is equivalent to the minimum level of protection normally expected when a properly fitted and used FFR is worn, i.e., a 10-fold reduction in exposure. As shown, even FFR with the potential to reduce exposures 100- to 500-fold are unable to provide the expected level of protection when non-wear time exceeds 10%. As non-wear time increases to approximately 50%, the EPF for the three respirators shown is 2, or little better than no protection at all.

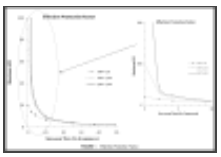


FIGURE 1
Effective Protection Factor.

Respiratory Protection Program Status

OSHA regulation 29 CFR 1910.134 requires employers to develop and implement a written program to maximize the effectiveness of all respiratory protective devices.[15] The program must include work site-specific procedures governing all aspects of respirator use, and be overseen by a suitably trained program administrator. The program must include the following provisions, as applicable to the devices in use: (1) selection procedures; (2) medical evaluations of employees required to use respirators; (3) fit testing procedures for tight-fitting respirators; (4) procedures for proper use of respirators in routine and reasonably foreseeable emergency situations; (5) procedures and schedules for cleaning, disinfecting, storing, inspecting, repairing, discarding, and otherwise maintaining respirators; (6) procedures to ensure adequate breathing air quality, quantity, and flow (for atmosphere-supplying respirators); (7) employee training on the respiratory hazards to which they are potentially exposed during routine and emergency situations; (8) training of employees in the proper use of respirators, including putting on and removing them, and any limitations on their use and their maintenance; and (9) procedures for regularly evaluating the effectiveness of the program.

FFR and other certified half-facepiece respirators can reliably reduce particle exposures at least 10-fold when used in the context of a proper respiratory protection program.[34–37] This pattern holds true for non-infectious bioaerosols. Cho et al.[38] determined that geometric mean exposures to endotoxins, fungal spores, and (1→3)-β-D-glucan were all reduced by a factor of 18 or more using half-facepiece respirators. Conversely, when one or more program elements are missing, protection can be significantly compromised.[40]

PERFORMANCE STUDIES

Controlled laboratory studies with human test subjects wearing different types of protective devices have measured higher C_o:C_i ratios for FFR compared to SM.[41–43] These studies used an inert particle challenge. Similar data are necessary to demonstrate that FFR are providing protection from inhaled infectious aerosols superior to that provided by SM.

However, none of the clinical and field studies attempting to estimate the effects of SM and FFR on disease transmission have measured C_o:C_i ratios and have, instead, used widely disparate methods.[3,4,12,13, 20–25] Most are epidemiological studies that use FFR or SM as an intervention, alone, or in combination with other interventions. Performance of the respiratory device is evaluated based on changes in clinical outcomes (e.g., infection rate of the group using the device). No study to date has adequately taken into account the five confounding factors listed above. Until this is done, definitive conclusions about the ability of either FFR or SM to reduce disease transmission cannot be drawn.

The reliance on subjects' self-reporting and/or inconsistent monitoring of the compliance of subjects' use of the device under evaluation are the deficiencies common to nearly every study to date addressing the effects of FFR or SM use on disease transmission. *Estimated* compliance rates in the range of 50–75% are commonly reported.[21,23] However, it is important to note that self-reported compliance is not a reliable indicator of actual

compliance. For example, one study on hand hygiene compliance among health care workers (HCW) reported low correlation between self-reported adherence and observed adherence, with statistically higher levels of self-reported compliance compared to observed compliance.[44] For FFR/SM, no study was found to report 100% wear time during all exposure periods, verified by continuous, direct observation of test subjects. As shown in Figure 1 , non-wear during exposure rapidly reduces the superior respiratory protection expected of FFR as compared to SM.

Studies in which compliance is optional are evaluating the impact of *subject behavior* rather than the capability of the FFR or SM to reduce inhalation of infectious aerosols. Several recent studies illustrate this and other deficiencies that make it impossible to judge the performance of a properly used respiratory device:

- Loeb et al.[3] found no statistically significant difference in influenza infection rates of HCW wearing either a fit tested N95 FFR or an SM. Subject compliance (wear) rates were not known, as only periodic audits of device usage were done. In addition, use of gloves, gowns, and hand washing were not monitored, and the possibility of community exposure to influenza was acknowledged.

- Another study of the efficacy of SM and N95 FFR (both fit tested and not fit tested) in HCW found respiratory illness/influenza infection rates in workers in either FFR group were roughly half the rate of those wearing SM.[22] Interestingly, both groups of FFR performed equivalently, i.e., fit testing showed no beneficial effect. While laboratory studies show that FFR are expected to provide more protection from inhaled aerosols than SM, it is not certain that they were actually responsible for the lower infection rates in the two groups who wore them. First, the authors defined compliance as wearing the device as ≥80% of the work shift, and 68–76% of subjects were said to comply (Figure 1). Compliance was determined by head nurses' observations and subjects' self-reporting. Additionally, the devices were evaluated in different groups of hospitals, and no air samples were taken to ensure exposures were equivalent for all the groups. Again, it is plausible that exposures and behaviors (including non-compliance) at individual sites were dissimilar and could account for the differences in infection rates.

- Studies of health care facilities that used both N95 FFR and SM for workers potentially exposed to H1N1 influenza or severe acute respiratory syndrome (SARS) have been reported by Seto et al.[4] and Ang et al.,[45] respectively. The Seto study also included unspecified "paper masks." The two investigations suggested that both FFR and SM controlled infection, but the "paper masks" in the Seto et al. study did not. However, both studies were retrospective and relied on participants' self-reporting on the use of the devices, other PPE, and hand washing. As such, no valid conclusions regarding the performance of a properly used FFR or SM can be drawn.

- Community studies using SM and N95 FFR (or a European P2 FFR) on influenza patients and/or household members have also been conducted.[20,21,23,24] In some cases, hand washing was used as an additional intervention. Because these studies typically describe subject-reported compliance rates of ~50%, they are of essentially no value for assessing respiratory device performance.

Several literature reviews [46–48] identify these and additional deficiencies of studies conducted to date. These authors described most studies as underpowered, too small, and/or poorly designed. In concert with the discussion above, bin-Reza et al.[48] call for objective exposure data and *objective monitoring of compliance* and examination of other confounders to determine if FFR or SM have any beneficial effect on disease transmission. In spite of limited data on the benefit of any specific intervention, bin-Reza et al. suggest "masks" would best be used in combination with other interventions, especially hand washing in both health care and home settings. Few studies or literature reviews acknowledge the need for a comprehensive respiratory protection program to manage the use of FFR or SM.

DISCUSSION AND CONCLUSIONS

The "respirators versus surgical mask" debate is complex and remains hotly debated.[31] Advocates of SM note the accessibility and lower costs of these devices and the lack of a need for fit testing.[49] Thus, some have argued for the need for comparative effectiveness in clinical trials to better address performance. Although many laboratory studies in controlled environments using manikins and human subjects exist, there have been no properly designed field studies to assess the ability of FFR and SM to reduce disease transmission rates. The difficulty of conducting such studies is compounded by lack of exposure limits, knowledge of an inhaled infectious dose, multiple exposure venues, and the interactions of several interventions used simultaneously. Current studies[50] may properly address these difficulties, but it is unlikely a true FFR or SM clinical efficacy study will be completed in the near future. Thus, their role in reducing disease transmission must be based on inference and laboratory studies for the time being.

Because biological particles have repeatedly been shown to be filtered in the same manner as other particles,[7–13,37,38] the same level of FFR performance can be expected when they are used against biological aerosols: that is, if properly fitted and worn during all periods of exposure to an infectious aerosol of concern, inhalation of that aerosol will be reduced 10-fold. Because there are no requirements for small particle filtration efficiency or fit for SM, they should not be expected to provide respiratory protection.

A similar finding was provided in a 2009 report by an Institute of Medicine (IOM) committee tasked with providing recommendations on respiratory

protection for HCW in the workplace during the novel H1N1 influenza pandemic.[51] That committee concluded that HCW in close contact with individuals with novel H1N1 influenza or influenza-like illnesses should use fit tested N95 FFR in accordance with OSHA respiratory protection standards and not SM. Similar to this article, the IOM committee based its findings on the evidence of possible airborne transmission of novel H1N1 influenza and the superior filtering and fit characteristics of FFR compared to SM.

As discussed previously, noncompliance with FFR use is a major detriment to effective respiratory protection. A recent study by Nichol et al.[52] concluded that adherence to the use of FFR in a health care setting could be improved with the ready availability of equipment, training and fit testing, organizational support for worker health and safety, and good communication practices. These recommendations are consistent with the elements of an effective respiratory protection program described by 1910.134. It is likely that facilities that implement these practices will achieve FFR performance equivalent to that shown in industrial studies. If particle inhalation is a significant route of exposure for that aerosol, FFR are far more likely to reduce infection via this route than are SM.

Furthermore, no evidence suggests that significant respiratory protection from biological aerosols can be achieved in *any* exposure venue without addressing respirator program elements. Unlike health care workplaces, members of the general public or casual (voluntary) workplace users will not have identified where and when exposures to infectious aerosols might occur; it is therefore likely that FFR would not be in use when an exposure does occur. Secondly, the benefits of individual fit testing have been well documented,[34–38, 53,54] and general public FFR users generally do not make the effort to be fit tested properly. Thus, these users may or may not achieve meaningful inhalation exposure reduction, even if the FFR is properly donned during an exposure episode. These limitations hold for *all* FFR, including those cleared by the FDA as N95/surgical masks or for general public use.[55]

As is the case with any respiratory hazard, the industrial hygiene hierarchy of controls should be applied to control infectious aerosols; the hazard should be reduced through engineering and administrative methods to the extent possible. Infection control practices and the use of other personal protective equipment as described Siegel et al.[56] should also be implemented.

Acknowledgments

This manuscript was prepared by a working group of the American Industrial Hygiene Association's Respiratory Protection Committee (RPC). David Abrams was a member of the working group, and his input is acknowledged. The authors would also like to thank the members of the RPC for their thoughtful comments on previous drafts of the manuscript. The findings and conclusions of this article are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health, U.S. Army Institute of Public Health, or the American Industrial Hygiene Association.

Article information

J Occup Environ Hyg. Author manuscript; available in PMC 2015 Dec 4.

Published in final edited form as:

J Occup Environ Hyg. 2013; 10(8): D97–D103.

doi: 10.1080/15459624.2013.799964

PMCID: PMC4670234

NIHMSID: NIHMS738710

PMID: 23767796

Larry Janssen,[1] Harry Ettinger,[2] Stephan Graham,[3] Ronald Shaffer,[4] and Ziqing Zhuang[4]

[1]Larry Janssen Consulting, LLC, Stillwater, Minnesota

[2]Harry Ettinger and Associates, Los Alamos, New Mexico

[3]U.S. Army Institute of Public Health, Aberdeen Proving Ground, Maryland

[4]National Institute for Occupational Safety and Health, Pittsburgh, Pennsylvania

Address correspondence to: Larry Janssen, Larry Janssen Consulting, LLC, 12097 87th St. North, Stillwater, MN 55082; LLJanssen@visi.com

References

1. Pandemic Influenza Preparedness and Response Guidance for Healthcare Workers and Healthcare Employers. Available at http://www.osha.gov/Publications/3328-05-2007-English.html (accessed August 4, 2012)

2. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. Available at http://www.cdc.gov/hicpac/2007IP/2007isolationPrecautions.html (accessed August 4, 2012) [PubMed]

3. Loeb M, Dafoe N, Mahony J, et al. Surgical mask vs. N95 respirator for preventing influenza among health care workers: A randomized trial. JAMA. 2009;302(17):1865–1871. [PubMed] [Google Scholar]

4. Seto WH, Tsang D, Yung RWH, et al. Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS) Lancet. 2003;361(9368):1519–1520. [PubMed] [Google Scholar]

5. U.S. Department of Health and Human Services (DHHS), Food and Drug Administration (FDA), Center for Devices and Radiological Health (CDRH) Guidance for Industry and FDA staff. Surgical Masks—Premarket Notification [510(k)] Submissions. Washington, D.C: DHHS, FDA, and CDRH; 2004. [Google Scholar]

6. ACGIH. Threshold Limit Values for Chemical Substances and Physical Agents. Cincinnati, Ohio: ACGIH; 2011. [Google Scholar]

7. Chen SK, Vesley D, Brosseau LM, Vincent JH. Evaluation of single-use masks and respirators for protection of health care workers against mycobacterial aerosols. Am J Infect Control. 1994;22:65–74. [PubMed] [Google Scholar]

8. Brosseau LM, McCullough NV, Vesley D. Mycobacterial aerosol collection efficiency of respirator and surgical mask filters under varying conditions of flow and humidity. Appl Occup Environ Hyg. 1997;12:435–445. [PubMed] [Google Scholar]

9. McCullough NV, Brosseau LM, Vesley D. Collection of three bacterial aerosols by respirator and surgical mask filters under varying conditions of flow and relative humidity. Ann Occup Hyg. 1997;41(6):677–690. [PubMed] [Google Scholar]

10. Qian Y, Willeke K, Grinshpun SA, Donnelly J, Coffey CC. Performance of N95 respirators: Filtration efficiency for airborne microbial and inert particles. AIHA J. 1998;59:128–132. [PubMed] [Google Scholar]

11. Willeke K, Qian Y, Donnelly J, Grinshpun SA, Ulevicius V. Penetration of airborne microorganisms through a surgical mask and a dust/mist respirator. AIHA J. 1996;57:348–355. [PubMed] [Google Scholar]

12. Zhili Z, Kuehn TH, Pui DYH. Performance evaluation of filtering facepiece respirators using virus aerosols. Am J Infect Control. 2013;41(1):80–82. [PubMed] [Google Scholar]

13. Noti JD, Lindsley WG, Blachere FM, et al. Detection of infectious influenza virus in cough aerosols generated in a simulated patient examination room. Clin Infect Dis. 2012;54(11):1569–1577. [PMC free article] [PubMed] [Google Scholar]

14. Approval of Respiratory Protective Devices. Code of Federal Regulations Title. 2010;42 Part 84. [Google Scholar]

15. Respiratory Protection. Code of Federal Regulations Title. 2005;29:419–444. Part 1910.134. [Google Scholar]

16. Occupational Safety and Health Act of 1970. Available at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=2743&p_table=OSHACT. (accessed August 4, 2012)

17. Oberg T, Brosseau LM. Surgical mask filter and fit performance. Am J Infect Control. 2008;36(4):276–282. [PubMed] [Google Scholar]

18. Rengasamy S, Eimer BC, Shaffer RE. Filtration performance of FDA-cleared surgical masks. J Int Soc Resp Prot. 2009;26(I–II):54–70. [Google Scholar]

19. Bischoff WE, Reid T, Russell GB, Peters TR. Transocular entry of seasonal influenza-attenuated virus aerosols and the efficacy of N95 respirators, surgical masks, and eye protection in humans. J Infect Dis. 2011;204:193–199. [PMC free article] [PubMed] [Google Scholar]

20. Cowling BJ, Chan KH, Fang VJ, et al. Facemasks and hand hygiene to prevent influenza transmission in households. Ann Intern Med. 2009;151:437–446. [PubMed] [Google Scholar]

21. MacIntyre CR, Cauchemez CS, Dwyer DE, et al. Face mask use and control of respiratory virus transmission in households. Emerg Infect Dis. 2009;15(2):233–241. [PMC free article] [PubMed] [Google Scholar]

22. MacIntyre CR, Wang Q, Cauchemez CS, et al. A cluster randomized clinical trial comparing fit-tested and non-fit-tested N95 respirators to medical masks to prevent respiratory virus infection in health care workers. Influenza Other Respir Viruses. 2011;5(3):170–179. [PMC free article] [PubMed] [Google Scholar]

23. Suess T, Remschmidt C, Schink SB, et al. The role of facemasks and hand hygiene in the prevention of influenza transmission in households: Results from a cluster randomised trial; Berlin, Germany, 2009–2011. BMC Infect Dis. 2012;12:26. [PMC free article] [PubMed] [Google Scholar]

24. Aiello AE, Perez V, Coulborn RM, et al. Facemasks, hand hygiene, and influenza among young adults: A randomized intervention trial. PLoS One. 2012;7(1):e29744. [PMC free article] [PubMed] [Google Scholar]

25. Dharmadhikari AS, Mphahlele M, Stoltz A, et al. Surgical face masks worn by patients with multidrug-resistant tuberculosis: Impact on infectivity of air on a hospital ward. Am J Respir Crit Care Med. 2012;185(10):1104–1109. [PMC free article] [PubMed] [Google Scholar]

26. Lindsley WG, Blachere FM, Davis KA, et al. Distribution of airborne influenza virus and respiratory syncytial virus in an urgent care medical clinic. Clin Infec Dis. 2010;50:693–698. [PubMed] [Google Scholar]

27. Yang W, Elankumaran S, Marr LC. Concentrations and size distributions of airborne influenza A viruses measured indoors at a health centre, a day-care centre and on aeroplanes. J R Soc Interface. 2011;8(61):1176–1184. [PMC free article] [PubMed] [Google Scholar]

28. Han K, Zhu X, He F, et al. Lack of Airborne Transmission During Outbreak of Pandemic (H1N1) 2009 among Tour Group Members, China, June 2009. Emerg Infect Dis. 2009;15(10):1578–1581. [PMC free article] [PubMed] [Google Scholar]

29. Baker MG, Thornley CN, Mills C, et al. Transmission of Pandemic A/H1N1 2009 Influenza on Passenger Aircraft: Retrospective Cohort Study. BMJ. 2010;340:c2424. [PMC free article] [PubMed] [Google Scholar]

30. Snider DE, Bridges CB, Weissman DN. Meeting Summary of the Workshop 'Approaches to Better Understand Human Influenza Transmission' Available at http://www.cdc.gov/influenzatransmission_workshop2010/pdf/Influenza_Transmission_Workshop_Summary_508.pdf (accessed September 12, 2012)

31. Killingley B. Respiratory versus medical masks: Evidence accumulates but the jury remains out. Influenza Other Respir Viruses. 2011;5(3):143–145. [PMC free article] [PubMed] [Google Scholar]

32. Henle W, Henle G, Stokes J, Jr, Maris EP. Experimental exposure of human subjects to viruses of influenza. J Immunol. 1946;52:145–165. [PubMed] [Google Scholar]

33. Alford RH, Kasel JA, Gerone PJ, Knight V. Human influenza resulting from aerosol inhalation. Proc Soc Exp Biol Med. 1966;122(3):800–804. [PubMed] [Google Scholar]

34. Bidwell JO, Janssen LL. Workplace performance of an N95 respirator in a concrete block manufacturing plant. J Int Soc Resp Prot. 2004;21(III–IV):94–102. [Google Scholar]

35. Janssen LL, Nelson TJ, Cuta KT. Workplace protection factors for an N95 filtering facepiece respirator. J Occup Environ Hyg. 2007;4:698–707. [PubMed] [Google Scholar]

36. Janssen LL, Bidwell JO, McCullough NV, Nelson TJ. Performance of an N95 filtering facepiece respirator in a grinding operation. J Int Soc Respir Prot. 2007;24:21–31. [Google Scholar]

37. Cho KJ, Jones S, Jones G, et al. Effect of particle size on respiratory protection provided by two types of N95 respirators used in agricultural settings. J Occup Environ Hyg. 2010;(7):622–627. [PubMed] [Google Scholar]

38. Cho KJ, Reponen T, McKay R, et al. Comparison of workplace protection factors for different biological contaminants. J Occup Environ Hyg. 2011;(8):417–425. [PubMed] [Google Scholar]

39. American Industrial Hygiene Association (AIHA) Respiratory Protection Committee. Respirator performance terminology. Am Ind Hyg Assoc J. 2002;(63):132. [Letter to the Editor] [Google Scholar]

40. Hewson GS, Ralph MI. Determination of program protection factors for half-mask respirators used at a mineral sands separation plant. Am Ind Hyg Assoc J. 1992;(53):713–720. [PubMed] [Google Scholar]

41. Duling MG, Lawrence RB, Slaven JE, Coffey CC. Simulated workplace protection factors for half-facepiece respiratory protective devices. J Occup Environ Hyg. 2007;(4):420–431. [PubMed] [Google Scholar]

42. Grinsphun SA, Haruta H, Eninger RM, Reponen T, McKay RT, Lee SA. Performance of an N95 filtering facepiece particulate respirator and a surgical mask during human breathing: Two pathways for particle penetration. J Occup Environ Hyg. 2009;(6):593–603. [PubMed] [Google Scholar]

43. Van der Sande M, Teunis P, Sabel R. Professional and homemade face masks reduce exposure to respiratory infections among the general population. PLoS ONE. 2008;3(7):e2618. [PMC free article] [PubMed] [Google Scholar]

44. O'Boyle CA, Henly SJ, Larson E. Understanding adherence to hand hygiene recommendations: The theory of planned behavior. Am J Infect Control. 2001;(29):352–360. [PubMed] [Google Scholar]

45. Ang B, Poh BF, Win MK, Chow A. Surgical masks for protection of health care personnel against pandemic novel swine-origin influenza A (H1N1)-2009: Results from an observational study. Clin Infect Dis. 2010;50(7):1011–1014. [PubMed] [Google Scholar]

46. Gralton J, McLaws ML. Protecting health care workers from pandemic influenza: N95 or surgical masks? Crit Care Med. 2010;38:657–667. [PubMed] [Google Scholar]

47. Jefferson T, Foxlee R, Del Mar C, et al. Physical interventions to interrupt or reduce the spread of respiratory viruses: Systematic review. BMJ. 2009;339:b3675. [PMC free article] [PubMed] [Google Scholar]

48. Bin-Reza F, Chavarrias VL, Nicoll A, Chamberland ME. The use of masks and respirators to prevent transmission of influenza: A systematic review of the scientific evidence. Influenza Other Respir Viruses. 2012;6(4):257–267. [PMC free article] [PubMed] [Google Scholar]

49. Radonovich LJ, Perl TM, Davey V, Cohen H. Preventing the soldiers of health care from becoming victims on the pandemic battlefield: Respirators or surgical masks as the armor of choice. Disaster Med Public Health Prep. 2009;3(Suppl 2):S203–S210. [PubMed] [Google Scholar]

50. Johns Hopkins University (Sponsor) The Respiratory Protection Effectiveness Clinical Trial (ResPECT) Available at http://www.clinicaltrials.gov/ct2/show/NCT01249625 (accessed September 10, 2012) [PMC free article] [PubMed]

51. Liverman CT, Harris TA, Rogers MEB, Shine Kenneth I, editors. Institute of Medicine Committee on Respiratory Protection for Health Care Workers in the Workplace Against Novel H1N1 Influenza A. Respiratory Protection for Health Care Workers in the Workplace Against Novel H1N1 Influenza A: A Letter Report. Washington, D.C: The National Academies Press; 2009. [Google Scholar]

52. Nichol K, McGeer A, Bigelow P, et al. Behind the mask: Determinants of nurse's adherence to facial protective equipment. Am J Infect Control. 2013;41(1):8–13. [PubMed] [Google Scholar]

53. Coffey CC, Campbell DL, Zhuang Z. Simulated workplace performance of N95 respirators. Am Ind Hyg Assoc J. 1999;(60):618–624. [PubMed] [Google Scholar]

54. Danyluk Q, Hon C, Neudorf M, et al. Health care workers and respiratory protection: Is the user seal check a surrogate for respirator fit-testing? J Occup Environ Hyg. 2011;8:267–270. [PubMed] [Google Scholar]

55. AIHA. ([Letter to the Food and Drug Administration (FDA)]).Regarding the FDA Attempt to Regulate Respirators. Available at http://www.aiha.org/news-pubs/govtaffairs/Pages/PublicPolicyComments.aspx (accessed August 6, 2012)

56. Siegel JD, Rhinehart E, Jackson M, et al. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. Available at http://www.cdc.gov/hicpac/2007IP/2007isolationPrecautions.html (accessed August 6, 2012) [PubMed]

11

Ann. Occup. Hyg., Vol. 56, No. 1, pp. 92–101, 2012

doi:10.1093/annhyg/mer054

Effectiveness of Three Decontamination Treatments against Influenza Virus Applied to Filtering Facepiece Respirators

MICHAEL B. LORE[1], BRIAN K. HEIMBUCH[2], TEANNE L. BROWN[1], JOSEPH D. WANDER[3] and STEVEN H. HINRICHS[1]*

[1]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198, USA; [2]Engineering and Science Division, Applied Research Associates, Panama City, FL 32401, USA; [3]Airbase Sciences Branch, Air Force Research Laboratory, Tyndall Air Force Base, FL 32403, USA

Received 2 December 2010; in final form 21 June 2011; published online 22 August 2011

Filtering facepiece respirators (FFRs) are recommended for use as precautions against airborne pathogenic microorganisms; however, during pandemics demand for FFRs may far exceed availability. Reuse of FFRs following decontamination has been proposed but few reported studies have addressed the feasibility. Concerns regarding biocidal efficacy, respirator performance post decontamination, decontamination cost, and user safety have impeded adoption of reuse measures. This study examined the effectiveness of three energetic decontamination methods [ultraviolet germicidal irradiation (UVGI), microwave-generated steam, and moist heat] on two National Institute for Occupational Safety and Health-certified N95 FFRs (3M models 1860s and 1870) contaminated with H5N1. An aerosol settling chamber was used to apply virus-laden droplets to FFRs in a method designed to simulate respiratory deposition of droplets onto surfaces. When FFRs were examined post decontamination by viral culture, all three decontamination methods were effective, reducing virus load by >4 log median tissue culture infective dose. Analysis of treated FFRs using a quantitative molecular amplification assay (quantitative real-time polymerase chain reaction) indicated that UVGI decontamination resulted in lower levels of detectable viral RNA than the other two methods. Filter performance was evaluated before and after decontamination using a 1% NaCl aerosol. As all FFRs displayed <5% penetration by 300-nm particles, no profound reduction in filtration performance was caused in the FFRs tested by exposure to virus and subsequent decontamination by the methods used. These findings indicate that, when properly implemented, these methods effectively decontaminate H5N1 on the two FFR models tested and do not drastically affect their filtering function; however, other considerations may influence decisions to reuse FFRs.

Keywords: bioaerosol; decontamination; healthcare workers; influenza virus; N95 respirator; respirator reuse

INTRODUCTION

The recent emergence of novel strains of influenza virus has renewed public health interest in the transmission and control of infectious agents. Significant attention has been placed on the avian influenza virus H5N1 and 2009 H1N1 (California) influenza virus type A. The communicable nature of these pathogens has created demand for inexpensive and efficient respiratory protection. Disposable filtering facepiece respirators (FFRs) are commonly used to reduce the exposure to airborne particles. FFRs have been recommended for use as part of a comprehensive infection control strategy by the Centers for Disease Control and Prevention (CDC). Manufacturers of respirators [those that are both approved by the

*Author to whom correspondence should be addressed.
Tel: +1-402-559-9415; fax: +1-402-559-7799;
e-mail: mlore@unmc.edu

National Institute for Occupational Safety and Health (NIOSH) as respirators and approved by the Food and Drug Administration (FDA) for medical uses] recommend that they be discarded if soiled or contaminated. However, the epidemic potential of influenza raises concerns that the manufacturing supply of FFRs would be unable to meet a sudden surge in demand. The Institute of Medicine (IOM) estimated that the healthcare sector would require 90 million FFRs for a 6-week influenza pandemic outbreak (Bailar *et al.*, 2006). This estimate, combined with the Occupational Safety and Health Administration's (OSHA) prediction that an influenza pandemic would last 24 weeks (OSHA, 2009), suggests that an outbreak could require as many as 360 million FFRs. The likelihood of widespread FFR shortages has prompted the consideration of reuse of FFRs during pandemics, when supply is short and the device has not been visibly soiled or damaged (Bailar *et al.*, 2006; Siegel *et al.*, 2007; APIC, 2009; OSHA, 2009; CDC, 2010). Although reuse may increase the potential for cross-contamination, FFR shortages may impose a far greater burden on the ability to control an outbreak. Therefore, a need exists for objective experimental information upon which decisions about the safety and practicality of decontamination for reuse of FFRs can be based.

FFRs remove pathogenic microorganisms from aerosols generated by infected individuals and are thus potentially fomites. Viability of influenza virus on inanimate surfaces is well-recognized even though it may be highly variable (Bean *et al.*, 1982; Brady *et al.*, 1990; Tiwari *et al.*, 2006; Boone and Gerba, 2007; Weber and Stilianakis, 2008). Since previously worn FFRs may serve as a reservoir for the spread of virus, the reuse of filters exposed to microorganisms requires careful consideration. If respirator shortages are to be mitigated through reuse, rapid, low-cost, and efficient decontamination methods must be established.

This study evaluated the virucidal capability of three energetic decontamination methods: ultraviolet germicidal irradiation (UVGI), microwave-generated steam (MGS), and moist heat (MH). These methods were utilized to two models of commercially available NIOSH-approved N95 FFRs, on which standardized quantities of influenza (A/H5N1) virus were applied as aerosolized droplets.

METHODS

Experimental design

FFR models 1860s and 1870 (3M Company, St Paul, MN, USA) were selected for study and exposed to influenza virus-containing aerosol using an aerosol test system. Influenza virus type A of the low-pathogenicity H5N1 strain was selected for use. Table 1 shows the study design, whereby a total of 108 FFRs were exposed and studied. 'Exposure' refers to the application of virus and 'treatment' refers to application of any one of the three decontamination procedures. The treated respirators were subjected to one of three decontamination methods while the non-treated respirators served as controls and were exposed to virus but received no disinfection treatment. All untreated FFRs were incubated for the same duration of time and at the same environmental conditions (temperature and humidity) as the treated respirators.

Treatment and extraction times were held constant between FFRs exposed to virus and the controls. Virus was applied using the droplet method described below.

Respirator descriptions. Two models of N95 FFRs common in healthcare settings were chosen for this study: 3M models 1860s (small size)—a ridged, cup-shaped design—and 1870, a flat-fold/three-panel design. Both designs are multi-layered and use a filtration medium of electrostatically charged, polypropylene microfibers. These FFRs are commercially available and carry a NIOSH N95 filter efficiency rating (NIOSH Federal Respiratory Regulations 42 CFR Part 84). The N95 designation certifies that respirators are $\geq$95% efficient at capturing oil-free airborne particles and aerosols with an aerodynamic mass median diameter of 300 nm when evaluated at the NIOSH-specified test conditions. These respirators have also been approved by FDA as medical devices.

Virus stock. Influenza A/H5N1 (VNH5N1) was acquired from the CDC and transferred with authorization to a commercial laboratory for production in eggs. Virus was produced and recovered from allantoic fluid and quantified. After receipt, the virus stock was re-titered in house. The viral titer was $\sim$5.5 $\log_{10}$ median tissue culture infective dose assay (TCID-50) ml-1_{50} ml^{-1}.

Droplet chamber. The aerosol test system (Fig. 1) used in this study was designed to mimic respiratory droplet transmission of viruses onto surfaces (ASTM: E2721-10, ASTM, 2010). The chamber was composed of a stainless steel box measuring

Table 1. FFR sample sets exposed to H5N1 virus.

FFR models	Control	UVGI	Control	MGS	Control	MH	# Tests
3M 1860s	9	9	9	9	9	9	54
3M 1870	9	9	9	9	9	9	54
					Total		108

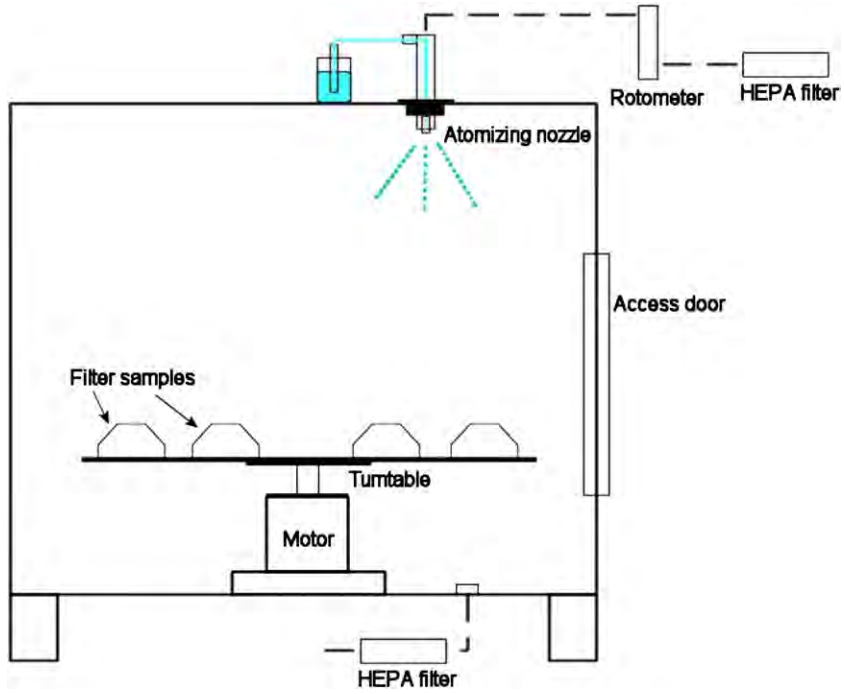


Fig. 1. Schematic of the aerosol test system.

61 × 61 × 76 cm with an approximate volume of 283 l. A pneumatic atomizing nozzle mounted vertically in the top of the chamber generated large, virus-laden droplets as described below. Airflow inside the chamber was designed to allow direct settling onto the FFRs. FFRs were rotated slowly on a turntable inside the chamber to achieve uniform deposition. Air containing excess aerosol flowed out of the chamber through a high efficiency particulate air (HEPA) filter at the bottom. Relative humidity (RH) and temperature within the test chamber were measured and maintained at constant levels throughout the testing. The aerosol test chamber was operated within a BSL3 laboratory facility and housed inside a Purair 20 ductless fume hood (Air Science LLC, Fort Myers, FL, USA).

Droplet size characterization. Droplet size distribution of the nozzle was characterized using a Spraytec droplet analyzer (Malvern, Worcestershire, UK) using standard conditions specified by the manufacturer. The Spraytec could not be directly linked with the chamber so the nozzle was analyzed external to the chamber, which may have produced smaller droplets due to an overall reduction in RH. The nozzle was operated at the same conditions used in this study (see below) and droplets were analyzed at five locations downstream of the nozzle (5, 15, 30, 45, and 60 cm).

Viral droplet loading protocol. Respirators were arranged in two equally spaced, concentric circles on the rotating platform inside the aerosol chamber. The door was sealed and rotation of the platform was adjusted to three revolutions per minute. As the table revolved, the pneumatic atomizing nozzle (model 2000VL; Paasche, Chicago, IL, USA) delivered 25 ml of influenza A/H5N1 virus suspension at a concentration of 5.5 $\log_{10}$ $TCID_{50}$ ml^{-1}.

A flowmeter (Cole–Parmer, Vernon Hills, IL, USA) regulated delivery of ∼4 l min^{-1} of HEPA-filtered air to the nebulizer, which atomized ∼5 ml of viral suspension per minute until the reservoir was depleted. The average time of exposure ranged between 5 and 8 min. After exposure, the airflow was shut off and aerosol remaining within the chamber was allowed to settle for 3 min before the respirators were removed. Treated filters were exposed to one of the three decontamination methods while the non-treated filters were used as controls. All aerosol tests were conducted using an undiluted virus concentration of 5.5 $\log_{10}$ $TCID_{50}$ ml^{-1}. Under test conditions, the RH within the chamber was >60% and the temperature range was 22 ± 2°C.

Virus extraction efficiency. To determine the extraction efficiency from the test material, a single circular coupon was cut from each of the four quadrants of the FFRs using a 3.8-cm diameter (11.3 cm^2) arch

punch (C.S. Osborne & Co., Harrison, NJ, USA). A volume of 250 µl of virus stock (1 ml total per set) was pipetted onto each coupon and allowed to dry for 20 min inside the biosafety cabinet. The coupons (four total) were placed in a 50-ml conical tube containing 15 ml of serum-free Eagle's minimum essential medium (sf-EMEM) supplemented with 1% penicillin–streptomycin and 10% L-glutamine. Conical tubes were mixed for 20 min to liberate virus particles from the coupons using a multi-tube vortex mixer (VWR Scientific, West Chester, PA, USA) at maximum speed. The supernatants were then removed from the tubes and aliquots assayed for viable virus particles through log serial dilutions in sf-EMEM. Results were expressed as virus titers in units of $\log_{10}$ $TCID_{50}$ ml^{-1}. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on the recovered supernatant and mean cycle threshold (Ct) values were compared to the stock concentration. Each experiment was performed in triplicate.

Virus culture quantification. Virus was quantified using Diagnostic HYBRIDS FreshCells™ cell cultures (Athens, OH, USA). The Madin–Darby canine kidney cells were maintained using standard methods, plates were incubated, and wells were monitored daily for presence or absence of cytopathic effect (CPE) by observation with an inverted light microscope. On day five, adherent cells were fixed with 400 µl of a 0.07% (w/v) crystal violet and 10% (v/v) glutaraldehyde solution for 1 h. Plate-well monolayers were scored for CPE and virus titers quantified according to the Spearman–Karber formula (Finney, 1978). The cell culture limit of detection was empirically set at >0.5 $\log_{10}$ $TCID_{50}$. Therefore, any data resulting in values below the detection limit (BDL) were expressed as zero in log reduction calculations (Tables 2 and 3).

Decontamination procedures. Virus-laden respirators were subjected to one of three decontamination procedures as described below. The orientation of the filter was convex panel facing the droplet or treatment source. Controls were subjected to the same decontamination procedures as the treated FFRs but were not exposed to virus.

Ultraviolet germicidal irradiation. A 126- (L) × 15.2- (W) × 10.8-cm (H), dual-bulb, 15-W UV-C (254-nm wavelength) lamp (Ultraviolet Products, Upland, CA, USA) was placed in a Labgard class II, type A2, laminar flow cabinet (NuAire, Inc., Plymouth, MN, USA) set to a height 25 cm above the cabinet's working surface. Measured by a UVX digital radiometer (UVP Inc., Upland, CA, USA), the lamp's UV-C wavelength irradiance ranged between 1.6 mW cm^{-2} and 2.2 mW cm^{-2}. Virus-laden respirators were placed inside the cabinet, directly under the ultraviolet lamp with the convex panel facing the treatment, and exposed for a total of 15 min at a UV-C wavelength dose of 18 kJ m^{-2}.

Microwave-generated steam. A 1250-W (2450 MHz) commercially available microwave oven (Panasonic Corp., Secaucus, NJ, USA) with a rotating glass plate was used to irradiate a single respirator per treatment. Samples were placed above

Table 2. Mean $\log_{10}$ $TCID_{50}$ virus concentrations recovered from 3M 1860s FFRs.

3M 1860s	UVGI		MGS		MH	
	Non-treated	Treated	Non-treated	Treated	Non-treated	Treated
Replicate 1	4.51	BDL[a]	4.76	BDL[a]	4.68	BDL[a]
Replicate 2	4.68	BDL[a]	4.84	BDL[a]	4.68	BDL[a]
Replicate 3	4.43	BDL[a]	4.84	BDL[a]	4.51	BDL[a]
ΔLog_{10} $TCID_{50}$	>4.54		>4.81		>4.62	

Non-treated, No decontamination procedure was performed (control group).
[a]BDL < 0.5 $\log_{10}$ $TCID_{50}$.

Table 3. Mean $\log_{10}$ $TCID_{50}$ virus concentrations recovered from 3M 1870 FFRs.

3M 1870	UVGI		MGS		MH	
	Non-treated	Treated	Non-treated	Treated	Non-treated	Treated
Replicate 1	4.93	BDL[a]	4.76	BDL[a]	4.68	BDL[a]
Replicate 2	4.68	BDL[a]	4.76	BDL[a]	4.68	BDL[a]
Replicate 3	4.34	BDL[a]	4.84	BDL[a]	4.59	BDL[a]
ΔLog_{10} $TCID_{50}$	>4.65		>4.79		>4.65	

[a]BDL < 0.5 $\log_{10}$ $TCID_{50}$.

a plastic box filled with 50 ml of room temperature tap water. The top of the box was perforated with 96 holes (7 mm diameter) evenly distributed over the entire surface to allow MGS to vent through the respirator. The virus-contaminated respirator was placed with the convex surface pointed toward the steam source and the FFR was then irradiated for 2 min at full power.

Moist heat. A 6-l sealable container (19 × 19 × 17 cm) was filled with 1 l of tap water, placed in an oven (Thermo Fisher Scientific Inc., Marietta, OH, USA), and heated to 65 ± 5°C for 3 h. This allowed the liquid to reach the desired temperature prior to any decontamination tests. For testing, the container was removed from the oven and a single virus-contaminated respirator was placed on the rack. For each decontamination procedure, the container was opened and the FFR placed onto the rack with the convex surface pointed toward the water layer. The container was then sealed and returned to the oven for the 20-min treatment.

Physical penetration measurements. To determine whether the decontamination methods had any effect on the filter performance, each of the three decontamination methods described above was applied to five separate samples of each model of FFR listed in Table 1. Physical penetration measurements were then conducted with a 1% NaCl aerosol challenge as described in Lore *et al.* (2010). Filter testing was performed at a flow rate of 85 l min^{-1}, the flow rate specified by NIOSH for FFR certification testing.

Data analysis

Determination of viable virus concentrations. Viable virus particles were quantified by median $TCID_{50}$ using the Spearman–Karber formula (Finney, 1978). This widely used methodology utilizes dilutions of pathogenic agents to demonstrate absolute thresholds of infectivity (0 or 100%). Infectivity is defined as the concentration capable of producing an observable CPE in the cell culture monolayer. Sample inocula were performed over a range of geometric series dilutions that bracketed the infectivity threshold. This allowed for positive identification of infectivity and proper application of the Spearman–Karber formula. The $\Delta\log_{10}$ $TCID_{50}$ between the control samples and the treated samples was calculated as $\Delta\log_{10}$ $TCID_{50}$ = $\log_{10}$ $TCID_{50}$ (control) − $\log_{10}$ $TCID_{50}$ (test). Results were expressed as virus titers in units of $\log_{10}$ $TCID_{50}$ ml^{-1}.

Virus quantification by qRT-PCR. Viral RNA (vRNA) was extracted using a QIAamp vRNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The vRNA was recovered in 15 µl (final volume) of elution buffer and quantitated spectrophotometrically (in triplicate) using NanoDrop ND-1000 (Saveen Werner, Limhamn, Sweden). vRNA amplification of the hemagglutinin viral protein target (H5a) was carried out according to the CDC protocol using Invitrogen's SuperScript III Platinum One-Step qRT-PCR System (cat no. 11732-088), which combines the reverse transcription and amplification steps.

Assay conditions for quantification of extracted vRNA were optimized in a Roche LightCycler 480 Real-Time PCR System (Roche Diagnostics). The Superscript III Platinum mastermix reaction components were prepared to the indicated end concentration: 5.5 µl nuclease-free water, 0.5 µl H5a-F forward primer (SO3307; CDC), 0.5 µl H5a-R reverse primer (SO3308; CDC), 0.5 µl H5a-P probe (SO3294; CDC), SuperScript III RT/Platinum *Taq* mix (Invitrogen), and 12.5 µl ×2 PCR master mix. For each sample, 20 µl of the complete LightCycler mastermix and 5 µl of extracted vRNA (25 µl total) were loaded into each well of a 96-well plate. The plate was then loaded into the LightCycler. Samples were run in triplicate for each dilution and presented as the Ct value.

The qRT-PCR thermocycling parameters were as follows: initial complementary DNA synthesis at 50°C for 30 min and then denaturation at 95°C for 2 min, followed by 45 cycles of 15 s of denaturing at 95°C, 30 s of annealing at 55°C, and 30 s of extension at 72°C with a final holding step at 4°C. Total run time was ∼2 h. The cut-off for determining a negative sample was 37 Ct units.

A standard curve was generated from a dilution series constructed from an extracted stock virus sample. RT-PCR was performed on serial log dilutions of the stock in triplicate using the Roche LightCycler 480. These data provided the reference standard by which experimental samples could be extrapolated. Efficiency of the RT-PCR reaction was estimated through linear regression analysis of the dilution curve. This was performed using the LINEST function of Microsoft Excel v2007 (Microsoft Corporation, Redmond, WA, USA), which draws the best-fit line using the least-squares method of regression analysis.

The limit of quantification (LoQ) was determined for the qRT-PCR reaction by serial log dilution of extracted vRNA to the lowest dilutions reliably detectable (−7 and −8). Twenty replicate series of both dilutions were prepared and run simultaneously in triplicate. A confidence interval (CI) was then calculated by multiplying the non-amplified samples by 5 and subtracting from 100. This number was then reported as a percentage. Only threshold cycle values

<40 were included in the data set. A CI of ≥95% was utilized. Sample dilutions with Ct values above 36.5 were considered negative.

RESULTS

The consideration for reuse of FFRs following decontamination must address two major issues: first, whether the FFR retains full function and provides a similar level of protection after treatment and second whether the decontamination treatment is effective at reducing the infectious capability of the targeted organism. This study focused primarily on the second point and the development of methods for accurate assessment of the amount of virus contaminating the FFR and the amount removed by the decontamination method. To achieve this goal, the first challenge was to demonstrate uniform application of virus-laden droplets and recovery of virus from FFRs.

Droplet size variation

Droplets at the source were 5 μm count median diameter (CDM) and they grew to ~12 μm CMD at the 30-cm mark, presumably due to coalescence of the droplets. The droplet size at the 60-cm mark was back down to 5 μm again, which is most likely due to evaporation.

Virus recovery and extraction efficiency

FFRs are intended to protect the user by capturing infectious particles within the composite materials of the device, either the surface covering or the filtering medium. Due to uncertainty about the depth to which virus would penetrate the cover web, all layers were sampled. Following application of virus to the FFRs, a total of four, circular, full-thickness cuttings (coupons) were taken comprising a total area of 45.36 cm^2. Because this study focused on vertical settling of large droplets, only the panel facing the droplet source was sampled. The total estimated area of the convex surface of 1860s and 1870 was 129.46 and 202.79 cm^2, respectively. The efficiency of recovery of vRNA from all coupon replicate sets was 70 ± 5% (data not shown) as determined by qRT-PCR.

qRT-PCR limit of detection, LoQ, and calibration

The limit of detection of the PCR method was determined as summarized in the methods section using serial dilutions of vRNA. Using a CI limit of 95%, the Roche LightCycler 480 reliably detected a 7-log serial dilution of the H5N1 virus stock. The slope of the standard curve used for calibration purposes was −3.48 with an R^2 value of 0.998.

Decontamination assessed by viral culture

The effect of the three decontamination treatments was assessed on two different models of FFRs (Table 1). Viral recovery from FFRs with and without treatment was assessed by the tissue culture method. Results comparing the recovery of virus expressed as $\log_{10}$ $TCID_{50}$ ml^{-1} are shown in Tables 2 and 3 for 1860s and 1870 respirators, respectively. Each value reported within the table was the mean of three FFRs tested under identical conditions. In total, 27 treated and 27 untreated FFRs were tested for each model of FFR. The decrease in virus titer was calculated by subtracting the average of the treated group from the untreated (control) group. Results were reported as the mean log reduction in virus titer. In all cases, decontamination procedures resulted in viable counts below the cell culture assay's detection limit (BDL).

The mean concentration of influenza A/H5N1 virus recovered from all untreated (control) 1860s samples was 4.66 $\log_{10}$ $TCID_{50}$. Within each treatment, the virus titer replicates were within ±0.25 SD $\log_{10}$ $TCID_{50}$ of each other and between groups ±0.27 SD $\log_{10}$ $TCID_{50}$ (Table 2).

The mean concentration of influenza A/H5N1 virus recovered from all untreated (control) 1870 samples was 4.70 $\log_{10}$ $TCID_{50}$. Within each treatment group, the virus titer replicates were within ±0.59 $\log_{10}$ $TCID_{50}$ of each other and between groups ±0.14 $\log_{10}$ $TCID_{50}$ (Table 3).

The average log recovery of virus obtained for all untreated controls with the 1860s was 4.66 $\log_{10}$ $TCID_{50}$, similar to the 3M 1870, from which recovery was 4.70 $\log_{10}$ $TCID_{50}$. The $\log_{10}$ $TCID_{50}$ reduction for the decontaminated 1860s respirators was ≥4.54 (UVGI), ≥4.81(MGS), and ≥4.62 (MH). The $\log_{10}$ $TCID_{50}$ reduction for the 1870 respirators exposed to decontamination methods was ≥4.65 (UVGI), ≥4.79 (MGS), and ≥4.65 (MH). All three decontamination methods achieved an absolute log reduction of >4.0 logs for both respirator models.

Decontamination assessed by qRT-PCR

vRNA extraction was performed on an aliquot of the same eluate used to recover viable virus, and qRT-PCR was performed as described. All values are reported as Ct units representing the number of amplification cycles. Testing of the coupons from non-treated FFRs (control) showed an average Ct value of 16.72 ± 0.7, demonstrating the reproducibility of the application and recovery process over multiple days. Within-run coefficient of variation was <7%.

Each value within the table represented a mean of nine replicates. Analysis of the treated samples showed a difference in Ct cycles between the UVGI decontamination method and the other two methods, MGS and MH, for both FFR models. Material recovered from UVGI-treated samples required more amplification cycles to detect vRNA than from either of the other methods. Consistent with the $TCID_{50}$ determinations, there was no significant difference between MGS and MH as determined by qRT-PCR (Table 4). These results demonstrated that although no viable virus was detectable following decontamination as measured by culture, none of the methods disrupted the viral genome to a level that could not be amplified by PCR.

Post-decontamination filter performance

Results indicate that the mean penetration at 300 nm was <5% for all FFR models tested (Table 5). These data demonstrate that the decontamination methods did not significantly degrade the filter performance at 300-nm particle size.

DISCUSSION

During pandemics, shortages of FFRs may lead to their reuse to extend supplies. This is currently a potential strategy being considered by several government agencies (e.g. CDC) during times of shortage—provided the respirator is not visibly soiled or damaged and does not impede breathing. However, bioaerosol contamination, which often does not display gross soiling, may pose a risk to the user if viable pathogenic microorganisms are still present. Furthermore, without proper guidance and procedures, any non-standardized decontamination method may prove to be inadequate. The functionality of the device must also be considered, as decontamination methods may damage straps, nosefoam, and other components that affect the fit.

Table 4. qRT-PCR Ct and coefficient of variation (CV) values of FFRs exposed to H5N1 aerosol.

	UVGI		MGS		MH	
Ct	CV (%)	Ct	CV (%)	Ct	CV (%)	
3M 1860s						
Control	16.39	4.42	16.14	6.63	16.88	2.71
Treated	30.67	2.97	21.8	5.87	22.98	5.62
3M 1870						
Control	16.59	1.67	16.98	5.66	17.36	3.95
Treated	29.1	5.60	19.81	4.01	20.05	2.45

Table 5. Mean penetration ($n = 5$) of 1% NaCl aerosol at 300-nm particle size.

FFR	Control	UVGI	MGS	MH
1860s (%)	1.08	0.99	1.51	1.04
1870 (%)	0.39	0.37	0.99	0.99

The objective of this study was to evaluate the virucidal effects of three energetic decontamination methods—UVGI, MGS, and MH—on FFRs exposed to large droplets delivered as aerosols containing influenza A/H5N1 virus. The viral load deposition in this study represented a 'worst-case scenario', in which the viral load onto the FFRs was in probable excess of what a user in a healthcare setting would realistically expect to encounter. These methods were selected based on several factors including common availability, low cost of the technology, and ease of use. For example, high-energy irradiation is present in many hospitals but would not be available to the general public. Efficacy guidelines have yet to be established for FFR fomite decontamination and pathogenic viruses. However, recommendations have been issued by the IOM regarding reusability of face masks during an influenza pandemic. The IOM suggests that any decontamination method applied to an N95 FFR must eliminate the viral threat, be harmless to the user, and have no deleterious effects on filter performance (Bailar *et al.*, 2006). This recommendation is in agreement with previously published guidance on the virucidal test effectiveness on inanimate surfaces set forth by the Environmental Protection Agency (EPA) (EPA, 1982). Therefore, this study used the EPA criterion of complete inactivation of the virus at all dilutions, quantified using the Spearman–Karber method expressed as $\log_{10}$ $TCID_{50}$, to demonstrate successful decontamination.

In addition to $TCID_{50}$ assays, a molecular-based analysis of the samples was performed using qRT-PCR. This non-culture technique, which detects vRNA with sensitivity several orders of magnitude greater than culture assays, can provide additional insight into the extent of biocidal effects beyond infectivity assays. However, molecular amplification assays for the assessment of decontamination effectiveness have not been addressed by regulatory agencies.

UVGI is a highly energetic short-wave (254 nm) ultraviolet light shown to be a useful sterilization technique in a variety of applications. The virucidal mechanism of UVGI is derived from the energy contained within the electromagnetic wave. Single-stranded RNA viruses have been shown to be especially susceptible to this type of radiation (Rauth, 1965; Tseng and Li, 2005). Miller and Plagemann (1974) demonstrated these effects on mengovirus, an RNA virus analogous to influenza virus. Their results demonstrated

progressively increasing changes in the viral proteins, which are the determinants of infectivity.

In this study, a 15-min exposure to high-intensity UVGI was found to be an effective virucidal treatment. A reduction of >4 $\log_{10}$ $TCID_{50}$ ml^{-1} was observed for both FFRs, indicating functionally complete removal of detectable virus by culture assay. In comparison, results of the qRT-PCR assay indicated a significant reduction in amplified RNA ($\sim$14 cycles, Table 4) following decontamination procedures; however, amplifiable vRNA remained. These results suggest that the energy input of the UVGI decontamination method was capable of eliminating viral infectivity as seen by the cell culture (TCID) assay. Our data indicate that the virus' infectivity is destroyed but its RNA genetic signature remains, as shown by the reduction in viral genome amplified. These data are in good agreement with previous observations of the effectiveness of UVGI on FFRs as measured by plaque assay made by Vo *et al.* (2009) and Fisher and Shaffer (2011) using surrogate viruses.

Microwave radiation is a form of radio frequency energy that, as when used in household ovens (2450 MHz), excites water molecules, generating heat. Microwave heating has been shown to inactivate or reduce several species of pathogenic microorganisms (Woo *et al.*, 2000). Despite many well-documented studies on microbial damage by microwave irradiation, the mechanism of action is not entirely understood. However, the presence of moisture appears to be a key factor influencing the biocidal effect (Vela and Wu, 1979; Jeng *et al.*, 1987). Therefore, the combined mechanisms of radiation and steam heat were applied in this study (MGS) where the FFRs were supported above a water reservoir during the decontamination process. This allowed steam generated from the water to pass through the FFR, further enhancing the biocidal effects of the microwave radiation treatment.

The MGS decontamination procedure relied solely on steam for disinfection. The biocidal action of steam is believed to be derived from the liquid phase's latent heat of vaporization, which is released upon contact, allowing the exotherm to denature enzymes and other essential cell constituents of the organism. The MH decontamination method is mechanistically analogous to the MGS decontamination, in which warm moisture acts as the main component of biocidal action. This method was chosen over dry heat sterilization because MH is more effective than dry heat for killing microorganisms and lower heat input is less likely to have deleterious results on filter performance (Hutten, 2007; Viscusi *et al.*, 2007, 2009).

As tested, each method (MGS and MH) was fully effective in inactivating influenza A/H5N1 virus particles for both FFR models. The EPA criterion for virucidal test effectiveness was met in this study, using the virus culture method, by the demonstration of results below detectable limits. Although the virus was completely inactivated by the MGS and MH treatments as assessed by culture methods, qRT-PCR methods were able to detect viral genomic material. More of the viral genomic material remained amplifiable following MGS and MH than after the UVGI treatment (Table 4). This was expected given that the mode of action of both methods is denaturation of proteins. Some vRNA was also denatured, which accounted for the reduction in amplified RNA. The results from treatment by MGS or MH were comparable even though the time of treatment differed from 2 to 20 min, respectively.

One challenge related to decontamination using a microwave-based method is the concern that the metal noseband of FFRs would generate combustion. Previous studies have demonstrated that dry microwave irradiation caused the filtration medium around metallic nosebands to melt (Viscusi *et al.*, 2007, 2009). In this study, the microwave-based decontamination method used steam as the dominant biocidal mechanism. Much of the energy is absorbed by water—reducing the potential for damage to the filtration medium. Gross observation of the FFRs post-MGS treatment showed no signs of filter damage. Furthermore, results of the penetration tests indicated that the decontamination methods did not significantly alter the filter performance at the 300-nm particle diameter (Table 5). It is important to note that, although the physical penetration was measured under conditions similar to those of the NIOSH certification test, these results are not equivalent to official NIOSH certification testing. The filtration measurements reported herein should not be interpreted as having any bearing on whether a particular FFR sample meets its designated NIOSH certification.

Any factor that contributes to nonuniform distribution of steam across the face of the respirator can alter its effectiveness. For MGS, the reservoir's surface area, liquid volume, and microwave power level are significant in relation to the exposure time. Smaller liquid surface areas, larger liquid volumes, or a microwave delivering <1250 W will require longer exposure times to generate sufficient amounts of steam. This decontamination method was the least time intensive and utilized commonly available items found in most households. The low throughput might restrict its use in large healthcare settings but this technology

would be practicable for home use or small organizations. This study was limited to evaluating the effect of three decontamination methods on the mask surface and did not examine the straps or nose clip. Additional evaluation must be considered regarding other components of the FFR prior to general adoption of these decontamination methods. Further research is appropriate to standardize this process and confirm its effectiveness for use by healthcare workers, first responders, and the general public.

CONCLUSION

This study showed that three decontamination methods (ultraviolet germicidal irradiation, MGS, and MH) satisfactorily decontaminated the 3M 1860s and 1870 FFRs as measured by a virus culture method. Within the constraints of the experiment, the three methods were all completely effective for the decontamination of FFRs as assessed by a culture method. These conclusions are further supported by data reported by Heimbuch *et al.* (2011) in which H1N1 droplets and droplet nuclei applied to six models of FFRs were decontaminated using the same three energetic methods described here. The construction or type of FFR did not result in any measured difference among the decontamination methods applied.

These findings suggest that, when properly implemented, these decontamination methods could suppress cross-contamination through contact with FFRs during situations in which reusing FFRs is necessary. However, these conclusions apply only to the models tested in this study—other FFR models may show different effects. Although this study did not investigate the effect of these treatments on fit, Viscusi *et al.* (2011) reported no significant decrease in the protective capability of FFRs following decontamination.

FUNDING

Air Force Research Laboratory Contract Number FA8650-07-C-5911 to S.H.H.

Acknowledgements—We thank Prof. C.-Y. Wu for the original design of the aerosol droplet chamber.

Disclaimer—publication of this work does not indicate endorsement or approval of this work by the Department of Defense.

REFERENCES

ASTM. (2010) ASTM International: E2721-10. Standard test method of evaluation of the effectiveness of decontamination procedures for surfaces when challenged with droplets containing human pathogenic viruses. West Conshohocken, PA: ASTM International.

APIC. (2009) Reuse of respiratory protection in prevention and control of epidemic- and pandemic-prone acute respiratory diseases (ARD) in healthcare. [serial online] 2009 http://www.apic.org/Content/NavigationMenu/GovernmentAdvocacy/PublicPolicyLibrary/Reuse_of_Respiratory_Proctection_archive_1209.pdf. Accessed 16 July 2011.

Bailar JC, Brosseau LQ, Cohen HJ, *et al*. (2006) Reusability of facemasks during an influenza pandemic: facing the flu. Washington, DC: National Academies Press.

Bean B, Moore BM, Sterner B *et al*. (1982) Survival of influenza viruses on environmental surfaces. J Infect Dis; 146: 47–51.

Boone SA, Gerba CP. (2007) Significance of fomites in the spread of respiratory and enteric viral disease. Appl Environ Microbiol; 73: 1687–96.

Brady MT, Evans J, Cuartas J. (1990) Survival and disinfection of parainfluenza viruses on environmental surfaces. Am J Infect Control; 18: 18–23.

CDC. (2010) Questions and answers regarding respiratory protection for preventing 2009 H1N1 influenza among healthcare personnel. [serial online] 2010 Available at http://www.cdc.gov/h1n1flu/guidelines_infection_control_qa.htm. Accessed 16 July 2011.

EPA. (1982) Pesticide assessment guidelines. Subdivision G: product performance, Section 91-30. Washington, DC: Environmental Protection Agency. pp. 72–6.

Finney DJ. (1978) Statistical method in biological assay. London, UK: Griffin.

Fisher EM, Shaffer RE. (2011) A method to determine the available UV-C dose for the decontamination of filtering facepiece respirators. J Appl Microbiol; 110: 287–95.

Heimbuch BK, Wallace WH, Kinney K *et al*. (2011) A pandemic influenza preparedness study: use of energetic methods to decontaminate filtering facepiece respirators contaminated with H1N1 aerosols and droplets. Am J Infect Control; 39: 1–9.

Hutten IM. (2007) Handbook of nonwoven filter media. Oxford, UK: Butterworth-Heinemann.

Jeng DK, Kaczmarek KA, Woodworth AG, *et al*. (1987) Mechanism of microwave sterilization in the dry state. Appl Environ Microbiol; 53: 2133–7.

Lore M, Sambol A, Japuntich D, *et al*. (2010) Inter-laboratory performance between two nanoparticle air filtration systems using scanning mobility particle analyzers. J Nanopart Res; 13: 1581–91.

Miller RL, Plagemann PG. (1974) Effect of ultraviolet light on mengovirus: formation of uracil dimers, instability and degradation of capsid, and covalent linkage of protein to viral RNA. J Virol; 13: 729–39.

OSHA. (2009) Pandemic influenza preparedness and response guidance for healthcare workers and healthcare employers. [serial online] 2009. Available at http://www.osha.gov/Publications/OSHA_pandemic_health.pdf. Accessed 16 July 2011.

Rauth AM. (1965) The physical state of viral nucleic acid and the sensitivity of viruses to ultraviolet light. Biophys J; 5: 257–73.

Siegel JD, Rhinehart E, Jackson M *et al*. (2007) 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. Am J Infect Control; 35: S65–164.

Tiwari A, Patnayak DP, Chander Y, *et al*. (2006) Survival of two avian respiratory viruses on porous and nonporous surfaces. Avian Dis; 50: 284–7.

Tseng C-C, Li C-S. (2005) Inactivation of virus-containing aerosols by ultraviolet germicidal irradiation. Aerosol Sci Technol; 39: 1136–42.

Vela GR, Wu JF. (1979) Mechanism of lethal action of 2,450-MHz radiation on microorganisms. Appl Environ Microbiol; 37: 550–3.

Viscusi D, King W, Shaffer R. (2007) Effect of decontamination on the filtration efficiency of two filtering facepiece respirator models. J Int Soc Respir Protect; 24: 93–107.

Viscusi DJ, Bergman MS, Eimer BC, *et al*. (2009) Evaluation of five decontamination methods for filtering facepiece respirators. Ann Occup Hyg; 53: 815–27.

Viscusi DJ, Bergman MS, Novak DA, *et al*. (2011) Impact of three biological decontamination methods on filtering facepiece respirator fit, odor, comfort, and donning ease. J Occup Environ Hyg; 8: 426–36.

Vo E, Rengasamy S, Shaffer R. (2009) Development of a test system to evaluate procedures for decontamination of respirators containing viral droplets. Appl Environ Microbiol; 75: 7303–9.

Weber TP, Stilianakis NI. (2008) Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. J Infect; 57: 361–73.

Woo IS, Rhee IK, Park HD. (2000) Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure. Appl Environ Microbiol; 66: 2243–7.

12

Preventive Medicine 62 (2014) 1–7

Contents lists available at ScienceDirect

Preventive Medicine

journal homepage: www.elsevier.com/locate/ypmed

Efficacy of face masks and respirators in preventing upper respiratory tract bacterial colonization and co-infection in hospital healthcare workers

C. Raina MacIntyre [a,b], Quanyi Wang [c], Bayzidur Rahman [a], Holly Seale [a], Iman Ridda [a,b], Zhanhai Gao [a], Peng Yang [c], Weixian Shi [c], Xinghuo Pang [c], Yi Zhang [c], Aye Moa [a,*], Dominic E. Dwyer [d]

[a] School of Public Health and Community Medicine, UNSW Medicine, University of New South Wales, Australia
[b] National Centre for Immunization Research and Surveillance, The Children's Hospital at Westmead, Sydney, Australia
[c] The Beijing Center for Disease Prevention and Control, Beijing, China
[d] Centre for Infectious Diseases and Microbiology Laboratory Services, Institute for Clinical Pathology and Medical Research, Westmead Hospital and University of Sydney, Australia

ARTICLE INFO

Available online 25 January 2014

Keywords:
N95 respirators and medical masks
Healthcare workers
Hospitals
Bacterial colonization

ABSTRACT

Objective. We compared the efficacy of medical masks (MM) and N95 respirators (N95) in preventing bacterial colonization/infection in healthcare workers (HCWs).

Methods. A cluster randomized clinical trial (RCT) of 1441 hospital HCWs randomized to medical masks or N95 respirators, and compared to 481 control HCWs, was performed in Beijing, China, during the winter season of 2008–2009. Participants were followed for development of clinical respiratory illness (CRI). Symptomatic subjects were tested for *Streptococcus pneumoniae*, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* or *Haemophilus influenza* type B by multiplex polymerase chain reaction (PCR).

Results. The rate of bacterial colonization was 2.8% in the N95 group ($p = 0.02$), 5.3% among medical mask users ($p < 0.01$) and 7.5% among the controls ($p = 0.16$). N95 respirators were significantly protective (adjusted RR 0.34, 95% CI: 0.21–0.56) against bacterial colonization. Co-infections of two bacteria or a virus and bacteria occurred in up to 3.7% of HCWs, and were significantly lower in the N95 arm.

Conclusions. N95 respirators were significantly protective against bacterial colonization, co-colonization and viral-bacterial co-infection. We showed that dual respiratory virus or bacterial-viral co-infections can be reduced by the use of N95 respirators. This study has occupational health and safety implications for health workers.

Introduction

Healthcare workers (HCWs) are at a significantly increased occupational risk for a range of infections. These include infections that cause substantial illness and occasional deaths in HCWs (Decker and Schaffner, 1996; Eriksen et al., 2005; Klevens et al., 2007), or are associated with healthcare associated infections (the majority of which are caused by bacteria). Various infectious agents can be transmitted from patients to HCWs and vice versa (Weber et al., 2010). As droplet transmission is a major mode of transmission of some pathogens, standard infection control measures like hand washing alone may not be enough to prevent HCW transmission or outbreaks. HCWs can transmit infections such as tuberculosis, varicella, and influenza by the airborne route (Weber et al., 2010); it is less well appreciated that airborne and other routes of transmission of certain bacterial pathogens may occur.

There is a low awareness of bacterial infections as an occupational health risk for HCWs. In addition, antibiotic resistant bacteria are a very significant problem facing hospitals, and HCWs play a role in their transmission. Bacterial respiratory tract infections are generally not considered a major occupational problem for HCWs. A growing body of evidence suggests that the risk of bacterial respiratory infections is increased by co-infection with viruses and vice-versa, and this has been studied mostly around the relationship between influenza and pneumococcus (Klugman et al., 2009; Madhi and Klugman, 2004; MMWR, 2009; Zhou et al., 2012). Bacterial load in the nasopharynx is also thought to be related to risk of invasive disease or bacterial–viral co-infection (Klugman et al., 2009). A meta-analysis showed frequent bacterial co-infections during influenza outbreaks (Wang et al., 2011). *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus* spp. and other *Streptococcus* spp. are the commoner causes of bacterial secondary infection following an influenza-like illness (ILI) (Wang et al., 2011).

Case studies documenting the role of HCWs in transmission of *S. pneumoniae* are absent, possibly because this is usually not an outbreak-associated disease, and because the pathogenesis of invasive

* Corresponding author at: School of Public Health and Community Medicine, UNSW Medicine, University of New South Wales, Gate 11, Botany Street, Randwick, NSW 2052, Australia.
E-mail address: a.moa@unsw.edu.au (A. Moa).

http://dx.doi.org/10.1016/j.ypmed.2014.01.015

disease is complex (including the relationship with prior colonization). Further, HCWs with invasive pneumococcal disease may go unreported in the occupational context (Sherertz et al., 2001). On the other hand, *Bordetella pertussis* outbreaks among HCWs have been widely reported (Addiss et al., 1991; Gehanno et al., 1999; Pascual et al., 2006), with such outbreaks attributed to airborne transmission through droplets (Nouvellon et al., 1999). In another study, evidence of acute infection with *Chlamydia pneumoniae* was detected in 2% of HCWs (Hyman et al., 1995). Outbreaks of *Mycoplasma pneumoniae* among HCWs have been observed in Finland, where 44% (n = 97) of HCWs tested positive for the pathogen without detectable *M. pneumoniae*-specific antibody, suggesting acute infection (Kleemola and Jokinen, 1992). *Legionella* has also been described as an occupational risk factor for HCWs (Borella et al., 2008; Rudbeck et al., 2009). In contrast to these outbreaks, there are few prospective studies of bacterial respiratory infections or colonization and the clinical implications for HCWs.

There has been recent interest in the role of medical masks and respirators in preventing respiratory infections in HCWs and the general community (MacIntyre et al., 2009, 2011, 2013). Medical masks (MMs) are unfitted devices worn by an infected person, HCW, or member of the public to reduce transfer of potentially infectious body fluids between individuals. They were originally designed for surgeons in order to attenuate wound contamination, but have not been demonstrated to have their intended efficacy (Mitchell and Hunt, 1991; Orr, 1981; Tunevall, 1991). Of note, MMs have not been shown to clearly provide respiratory protection in the community or HCW setting (Aiello et al., 2012; Cowling et al., 2009; MacIntyre et al., 2009, 2011). This may be attributed to lower filtration efficiency and poorer fit than respirators which, in contrast, are specifically designed to provide respiratory protection (Balazy et al., 2006; Lawrence et al., 2006; Weber et al., 1993). We have previously shown that a N95 respirator provides significantly better protection against clinical respiratory infection than medical masks in HCWs (MacIntyre et al., 2011, 2013). Although our previous work tested clinical efficacy in preventing infection, the relative importance of different routes of transmission (airborne, aerosol, and direct hand-to-mouth contact) in the clinical efficacy of respiratory protection is unknown. That is, a mask may provide protection against more than one mode of transmission. The only bacterial infection for which respirators are considered and recommended for HCWs is tuberculosis (Chen et al., 1994; Nicas, 1995). In this study, our aim was to determine the efficacy of respiratory protection in preventing bacterial colonization and co-infections or co-colonization in HCWs.

Methods

A prospective, cluster randomized trial of N95 respirators (fit tested and non-fit tested) and medical masks compared to each other and to controls who did not routinely wear masks was conducted in frontline HCWs during the winter of 2008–2009 (December to January) in Beijing, China. The methodology and consort diagram used in the study and the primary clinical and viral infection outcomes have been previously described (MacIntyre et al., 2011). We also measured bacterial colonization/infection and co-infections in symptomatic trial subjects, which has not been previously reported. This study describes the efficacy of the interventions (N95 respirators and medical masks) in preventing bacterial colonization and co-infection in HCWs.

Recruitment commenced on December 1, 2008 and final follow-up completed on January 15, 2009. 1441 HCWs in 15 hospitals were randomized to one of three intervention arms: (1) Medical masks (3M™ medical mask, catalog number 1820); (2) N95 fit tested mask (3M™ flat-fold N95 respirator, catalog number 9132); (3) N95 non-fit tested mask (3M™ flat-fold N95 respirator, catalog number 9132) (MacIntyre et al., 2011). A secure computerized randomization program was used to randomize the hospitals to each intervention. A convenience control group of 481 HCW who did not routinely wear masks were recruited and prospectively followed up in the same way as the trial participants for the development of symptoms. The study protocol was approved by the Institutional Review Board (IRB), Human Research Ethics Committee of the Beijing Ministry for Health. Staff who agreed to participate provided informed consent.

The primary study endpoint was the presence of laboratory-confirmed bacterial colonization of the respiratory tract in subjects who were symptomatic. We tested for *S. pneumoniae*, *Legionella* spp., *B. pertussis*, *Chlamydia*, *M. pneumoniae* or *H. influenzae* type B by multiplex PCR. These organisms have been reported in the HCW setting (Kurt et al., 1972; Rudbeck et al., 2009; Wang et al., 2011). We also looked at co-colonization with more than one bacteria, and co-infection with a laboratory-confirmed viral infection and bacterial colonization. Laboratory-confirmed viral respiratory infection was defined as detection of adenoviruses, human metapneumovirus, coronaviruses 229E/NL63 and OC43/HKU1, parainfluenza viruses 1, 2 and 3, influenza viruses A and B, respiratory syncytial viruses A and B, or rhinovirus A/B by nucleic acid testing (NAT) (MacIntyre et al., 2011).

Eligibility

Nurses or doctors who worked full time in the emergency or respiratory wards at the participating hospitals were eligible. HCWs were excluded if they: (1) were unable or refused to consent; (2) had beards, long mustaches or long facial hair stubble; (3) had a current respiratory illness, rhinitis and/or allergy; and (4) worked part-time or did not work in the selected wards/departments (MacIntyre et al., 2011).

Intervention

Subjects were randomized to masks or respirators, and wore the mask or respirator on every shift (8–12 h) for four consecutive weeks and were shown how to wear it and fit it correctly. Participants were supplied daily with three masks for the medical mask group or two N95 respirators. They were asked to store the mask in a paper bag every time they removed it (for toilet breaks, tea /lunch breaks and at the end of every shift) and place the bagged mask or respirator in their locker. All participants were instructed on the importance of hand hygiene prior to/ after the removal of medical masks and respirators, as described (MacIntyre et al., 2011). Participants in the fitted N95 arm underwent a fit testing procedure using a 3M™ FT-30 Bitrex Fit Test Kit according to the manufacturers' instructions (3M™, St Paul, MN, USA) (MacIntyre et al., 2011).

Follow-up

All participants were followed up for four weeks for development of respiratory symptoms, and for an additional week after mask wearing had ceased (to account for incubation of infections acquired in week 4). Validated diary cards were provided for the four-week period to record daily the (1) number of hours worked; (2) mask/respirator usage; and (3) recognized CRI (MacIntyre et al., 2011).

Participants were contacted daily by the study team either by phone or face-to-face contact to actively identify incident cases of viral respiratory infection. CRI was defined as at least two respiratory symptoms (cough, sneezing, runny nose, shortness of breath, sore throat) or one respiratory symptom and one systemic symptom (including fever, headache, and lethargy). If any respiratory symptom was present, subjects were tested, following collection of a nose and throat swab, for bacterial and viral pathogens.

Sample collection and laboratory testing

Subjects with respiratory symptoms had two pharyngeal swabs collected by a trained nurse or doctor. Double rayon-tipped, plastic-shafted swabs were used to scratch both tonsil areas and the posterior pharyngeal wall. These were transported immediately after collection to the laboratory, or at 4 °C if transport was delayed within 48 h. Pharyngeal swabs were tested at the Laboratories of the Beijing Centers for Disease Control and Prevention. A multiplex PCR (Seegen Inc., Seoul, Korea) was used to detect *S. pneumoniae*, *M. pneumoniae*, *B. pertussis*, *Legionella* spp., *Chlamydia* and *H. influenza* type B. After preheating at 95 °C for 15 min, 40 amplification cycles were carried out under the following conditions in a thermal cycler (GeneAmp PCR system 9700, Foster City, CA, USA): 94 °C for 30 s, 60 °C for 1.5 min, and 72 °C for 1.5 min. Amplification was completed at the final extension step at 72 °C for 10 min. The multiplex PCR products were visualized by electrophoresis on an ethidium bromide-stained 2% agarose gel. Laboratory-confirmed viral respiratory infection, defined as detection of adenoviruses, human metapneumovirus, coronaviruses 229E/NL63 and OC43/HKU1, parainfluenza viruses 1, 2 and 3, influenza viruses A and B, respiratory syncytial viruses A and B, or rhinovirus A/B by nucleic acid testing (NAT)

using a commercial multiplex polymerase chain reaction (PCR) (Seegen, Inc., Seoul, Korea) as previously described (MacIntyre et al., 2011).

Analysis

The endpoint of interest, bacterial colonization and co-infection with two bacteria or virus and bacteria were analyzed by intention-to-treat analysis. The two N95 arms (fit-tested and non-fit-tested) were combined for analysis, given that there was no significant difference between them and because rates of fit test failure were extremely low in the fit tested arm (5/461 fit test failures — in other words, the majority of HCWs who underwent fit-testing were wearing the mask correctly prior to fit testing, and fit testing did not add a significant benefit, allowing us to combine data from the fit tested and non-fit tested arms) (MacIntyre et al., 2011). We calculated the relative risk and efficacy of the N95 arms using medical mask group as the reference category, and also the efficacy of N95 and medical mask group using control as the reference category.

We fitted a multivariable log binomial model, using generalized estimating equation (GEE) to account for clustering by hospital, to estimate relative risk (RR) after adjusting for potential confounders. In the initial model, we included all the variables along with the main exposure variable (randomization arm) that were significant ($p < 0.25$) in the univariable analysis. A backward elimination method was used to remove the variables that did not have any confounding effect, that is, could not make meaningful change ($\pm 10\%$) in the RR of the N95 arms (Kleinbaum et al., 2007, 2010; Vittinghoff et al., 2012). In the multivariable analysis we estimated RR for N95 and medical mask arms compared to the control arm.

Results

A total of 1441 nurses and doctors in 15 hospitals were recruited into the intervention arms, and 481 nurses and doctors in 9 hospitals were recruited into the control group (Fig. 1). The distribution of socio-demographic variables was generally similar between arms, as previously reported (MacIntyre et al., 2011).

Fig. 2 illustrates the rates of bacterial detection in symptomatic HCWs by trial arm, and shows increasing rates with decreasing level of respiratory protection. Table 1 shows bacterial and viral infections, as well as co-infections or co-colonization with multiple pathogens, including co-infection with bacteria and virus. The rates of bacterial detection were lower for N95 respirators compared to MM (2.8% and 5.3% respectively), and was highest (7.5%) among the controls. By intention to treat analysis, N95 respirators were significantly more protective than MM against the laboratory-confirmed presence of bacteria, with an efficacy of 46% against medical masks and 62% against control. MMs had no significant efficacy against any outcome compared to control (Table 1).

Rates of all types of co-infection were significantly lower in the N95 group. N95 (but not MM) demonstrated efficacy against multiple bacterial pathogen colonization as well as co-infection with a virus and bacteria, and against dual virus infection (Table 1). There were no dual virus infections in controls (0/481), 2/949 in the N95 group and 5/492 in MM group. The MM arm had a higher rate of dual virus infection than controls, but the difference between MM and control did not reach statistical significance. The most common bacteria identified was *S. pneumoniae*; 2.5% for N95; 4.7% for MM, and 6.2% for control arm, followed by *H. influenzae* type B; 2%, 3.7%, and 5% respectively (data not shown). These differences were statistically significant across all three arms. *B. pertussis* was also detected in three HCWs.

In a multivariable cluster adjusted log binomial model, when compared to the control group, the N95 group was significantly protective against bacterial colonization (Table 2). We demonstrated 59% efficacy of N95 respirators against any co-infection (Table 3), and 67% against bacterial and viral co-infection (Table 4) in adjusted multivariate analyses. The only other significant variable for bacterial infection and

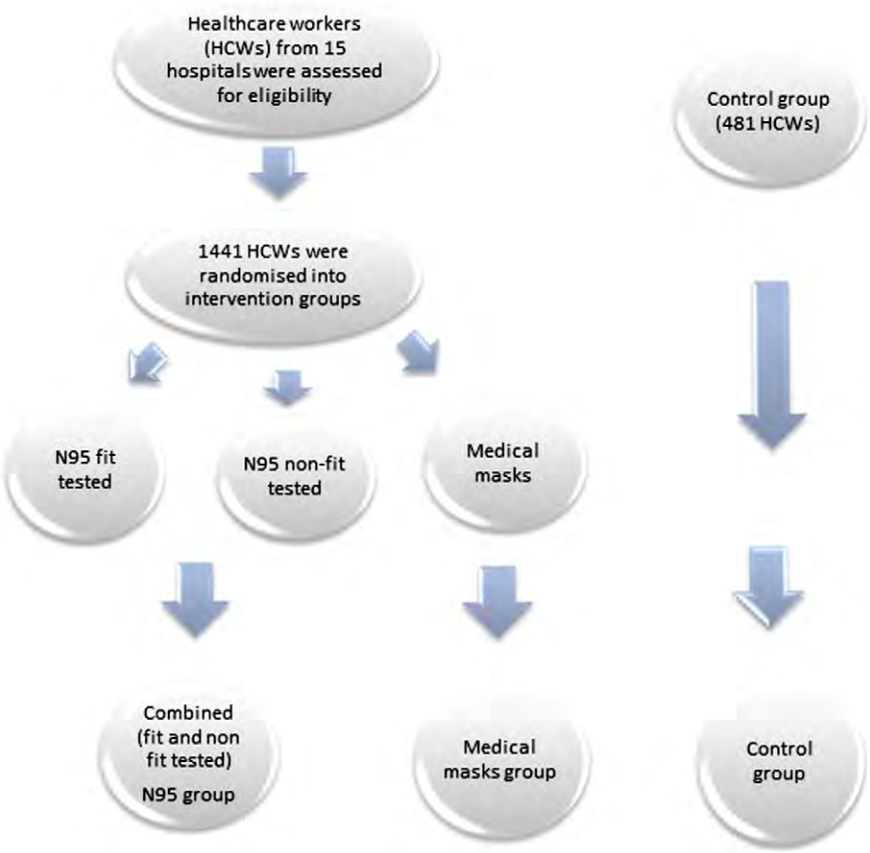
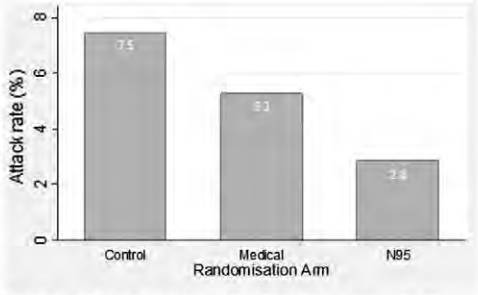


Fig. 1. A consort diagram for the study selection.



[1] P = 0.02, from cluster adjusted chi-squared test for differences in rates between arms

Fig. 2. Bacterial colonization by trial arm.[1].

Table 2
Multivariable cluster adjusted log binomial model of bacterial infection compared with control group.

Variables in the model	Relative risk (95% CI)
N95	0.34 (0.21–0.56)[a,†]
Medical mask	0.67 (0.38–1.18)
Hospital level	1.48 (0.91–2.42)
High-risk procedure	1.34 (0.84–2.13)
Influenza vaccine	1.03 (0.58–1.83)
Hand washing	0.82 (0.47–1.43)
Respiratory ward vs other	2.15 (1.39–3.31)[†]

[a] Efficacy 66%.
[†] Significant p values ($p < 0.01$).

bacterial and viral co-infection was the respiratory ward, which significantly increased the risk of colonization or co-infection compared to other wards (Tables 2 and 4).

In addition, univariable analyses of infection and co-infection rates by other factors, such as, smoking (current vs non-smoker), staff type (doctor vs nurses) and ward type (respiratory vs other) were conducted in the analysis. For bacterial infection, HCWs working in a respiratory ward were significantly at higher risk of infection than HCWs in other wards (7.3% vs 3.5%, $p < 0.001$). For bacterial co-infection, nurses had a significantly higher risk than doctors (3.2% vs 1.4%, $p = 0.02$) and the rate was also significantly higher in respiratory wards (4.4% vs 1.8%, $p = 0.001$). Respiratory wards had a higher rate of bacteria–virus co-infection than other wards (2.5% vs 1%, $p = 0.02$).

Discussion

We have previously shown that N95 respirators protect against clinical respiratory illness (MacIntyre et al., 2011, 2013). N95 respirators, but not medical masks, were significantly protective against bacterial colonization, co-colonization, viral-bacterial co-infection and dual virus infection in HCWs. We also showed a statistically significant decrease in rates of bacterial respiratory colonization with increasing levels of respiratory protection. The lowest rates were in the N95 group, followed by the medical mask group, and the highest rates were in HCWs who did not wear a mask. Although the clinical significance of this finding is unknown in terms of the implications for HCWs, we have shown that such colonization can be prevented by the use of N95 respirators. These findings are consistent with other work we have published, which shows a reduction in bacterial colonization following use of N95 respirators (MacIntyre et al., 2013).

While the role of nosocomial viral respiratory infections is accepted, bacterial infections are less well understood. Our findings suggest that bacterial respiratory tract colonization or infection in HCWs should be studied further. Bacterial colonization may be a precursor to viral and bacterial co-infections and invasive bacterial infections in individuals with influenza or other respiratory viral infections. It is possible that the onset of upper respiratory tract bacterial colonization may itself cause mild respiratory tract symptoms, given that only symptomatic HCWs were swabbed in our study. This requires further investigation, in particular comparison with an asymptomatic HCW group. We believe that these results may have occupational health implications for HCWs, given the body of evidence that supports a complex, synergistic and poorly understood pathogenic relationship between bacterial and viral respiratory infection (Klugman et al., 2009; Madhi and Klugman, 2004; MMWR, 2009; Zhou et al., 2012). The finding that bacterial colonization and co-infections were a greater risk on respiratory wards than

Table 1
Intention to treat analysis of bacterial, viral and bacterial–viral co-infections. Bold indicates "significant p value".

	N95 (n = 949)		Medical (n = 492)		Control (n = 481)	
All infections		Efficacy of N95 vs medical masks % (95% CI)[a]		Efficacy of N95 vs control % (95% CI)[b]		Efficacy of medical mask vs control % (95% CI)[b]
Bacteria	2.8% (27/949)	**46.2 (8.8–68.2) p = 0.02**	5.3% (26/492)	**62.0 (38.0–77.0) p = 0.001**	7.5% (36/481)	29.0 (0.0–57.0) p = 0.16
Virus	1.4% (13/949)	48.2 (0.0–75.8) p = 0.085	2.6% (13/492)	**56.1 (8.4–78.9) p = 0.024**	3.1% (15/481)	15.3 (0.0–59.2) p = 0.657
Bacteria or virus	3.3% (31/949)	**49.8 (18.7–69.0) p = 0.004**	6.3% (32/492)	**59.7 (36.3–74.5) p < 0.001**	8.1% (39/481)	19.8 (0.0–48.9) p = 0.336
Co-infections						
≥2 bacteria	1.7% (16/949)	48.2 (0.0–74.4) p = 0.064	3.1% (15/492)	**57.8 (16.9–78.5) p = 0.010**	3.7% (18/481)	18.5 (0.0–58.5) p = 0.550
Virus and bacteria	1.0% (9/949)	33.3 (0.0–75.0) p = 0.415	1.4% (7/492)	**62.0 (10.4–83.9) p = 0.022**	2.5% (12/481)	43.0 (0.0–77.4) p = 0.227
Co-infection						
≥2 viruses	0.1% (2/949)	**72.3 (0.0–96.0) p = 0.05**[d]	1.0% (5/492)	Incalculable[c] p = 0.553[d]	0.0% (0/481)	Incalculable[c] p = 0.062[d]

[a] Efficacy and p-values were calculated using medical group as the referent category.
[b] Efficacy and p-values were calculated using control group as the referent category.
[c] Efficacy could not be calculated because zero events in the control group.
[d] Fisher's exact test was used to calculate the p-value because of small expected cell frequencies.

Table 3
Multivariable cluster adjusted log binomial model of any co-infections compared with control group.

Variables in the model	Relative risk (95% CI)
N95	0.41 (0.23–0.75)[a,†]
Medical mask	0.87 (0.44–1.73)
Hospital level	1.41 (0.77–2.56)
High-risk procedure	1.45 (0.84–2.50)
Influenza vaccine	0.90 (0.46–1.78)
Hand washing	1.07 (0.51–1.23)

[a] Efficacy 59%.
[†] Significant p values ($p < 0.01$).

other clinical settings also supports the fact that occupational transmission is occurring in HCWs. Interestingly, smoking was not a risk factor for colonization or co-infection. We also found that nurses had significantly higher rate of bacterial co-infection than doctors. This may be due to higher patient contact or differences in use of infection control measures and personal protection (Chan, 2010; Chan et al., 2002).

The clinical significance of bacterial colonization in HCWs is uncertain, and this is an under-studied and unrecognized risk in HCWs. The significant protection against this afforded by N95 respirators mirrors the same trend seen in our previous study for clinical outcomes (MacIntyre et al., 2011, 2013). Outbreaks of bacterial respiratory infection do occur in HCWs (Kleemola and Jokinen, 1992; Ong et al., 2006; Pascual et al., 2006). Therefore, the observed reduction in bacterial colonization may translate to clinical protection against infection. *S. pneumoniae* was the most common bacteria identified in the upper respiratory tract. Invasive pneumococcal disease is thought to occur shortly after acquisition of colonization (Boulnois, 1992; Gray et al., 1980), and the infection can be transmitted by a colonized, asymptomatic individual. The rate of pneumococcal colonization demonstrated in our study was 6% (30/481 in controls), which is within the range described in adults (who have lower rates of colonization than children) (Austrian, 1986; Kadioglu et al., 2008; Obaro et al., 1996; Ridda et al., 2011). In an earlier study of frail elderly adults, only 1/315 subjects carried *S. pneumonia* (Ridda et al., 2011), although rates of adult carriage in the pre-vaccine era of up to 28% have been described (Hammitt et al., 2006). Bacterial load in the nasopharynx, not measured in this study, may be important in predicting the risk of invasive disease or viral co-infection and warrants further study (Klugman et al., 2009). We demonstrated that N95 respirators prevent carriage with *S. pneumoniae*. Although *S. pneumoniae* is not typically associated with outbreaks, nosocomial transmission and invasive disease in hospital patients from a carrier HCW have been reported (Guillet et al., 2012). In addition, transmission of bacterial pathogens from patients to HCWs during high-risk procedures has been described (Baba et al., 2009).

Table 4
Multivariable cluster adjusted log binomial model of bacterial and viral co-infection compared with control group.

Variables in the model	Relative risk (95% CI)
N95	0.33 (0.14–0.78)[a,†]
Medical mask	0.59 (0.20–1.73)
Hospital level	1.93 (0.80–4.62)
High-risk procedure	1.22 (0.52–2.86)
Influenza vaccine	1.60 (0.64–4.01)
Hand washing	1.24 (0.37–4.11)
Respiratory ward vs other	2.85 (1.30–6.26)[†]

[a] Efficacy 67%.
[†] Significant p values ($p = 0.01$).

The issue of co-infection is not well studied in HCWs, therefore our findings are quite novel. We have shown that all combinations of co-infection or co-colonization, with bacteria, viruses and both bacteria and virus, occur in symptomatic HCWs. These co-infections also display the same trend of decreasing frequency with increasing respiratory protection. Whatever their clinical significance, co-infection can be reduced by respiratory protection, and this may have implications for both patient safety, control of outbreaks and occupational health and safety of HCWs in hospitals. Co-infections, particularly bacterial–viral co-infection and dual viral infections can be more clearly implicated in causing disease in HCWs than colonization with a single bacterial species. This aspect of our findings, as well as the increased risk for staff in respiratory wards, therefore, has more direct clinical implications.

We demonstrated 59% efficacy against control of N95 respirators against any co-infection, and 67% against bacterial/viral co-infection. Medical masks were not protective and may in fact increase the risk of viral co-infections (5/492 compared to 0/481 in controls and 2/949 in N95). This finding, while not reaching statistical significance, may be due to chance, but is concerning and should certainly be investigated further. It is possible that the physical conditions of a medical mask may increase moisture or other parameters to increase risk of co-infection.

The limitations of this study include the fact that we did not test asymptomatic subjects, and therefore cannot examine the relationship of bacterial colonization to symptoms. Quantitative data on bacterial load would also have strengthened the study. Finally, the mechanisms of protection of a mask against respiratory tract colonization may be multi-modal. A mask may protect against respiratory transmission of pathogens, but may also act as a barrier to reduce hand to nose or hand to face contact, and may reduce infection in this way. Barrier precautions have been shown to reduce the rate of nasopharyngeal bacterial colonization (Safdar et al., 2006), so it would be expected that the barrier provided by a mask may have the same effect. A limitation of this study is that we cannot differentiate the relative contributions of prevention of airborne, droplet or direct contact transmission, but the study provided clinical efficacy estimates regardless of the different potential mechanisms of protection. If masks act by preventing multiple modes of transmission, they could have utility in preventing multidrug-resistant bacteria colonization of the nasopharynx of HCWs. Organisms such as methicillin-resistant *S. aureus* (MRSA) are a serious hospital infection control problem for HCWs (Morgan et al., 2012). Rates of clinical infections in HCWs with MRSA of 5.1% have been described, as has transmission of MRSA from HCWs to patients (Elie-Turenne et al., 2010; Sherertz et al., 2001; Verwer et al., 2012; Wang et al., 2011). A future research question could be the role of masks in preventing MRSA colonization in HCWs.

In summary, we have described novel data on bacterial infection and co-infections in HCWs, something which has not widely been documented or accepted previously, and shown that N95 respirators consistently provide protection against bacterial colonization and co-infections of the respiratory tract of hospital HCWs. The risk of such colonization is higher in ward types where more respiratory infections are expected (such as respiratory wards). The documented nosocomial outbreaks of bacterial infections such as pertussis and even *S. pneumoniae* in HCWs (Guillet et al., 2012; Pascual et al., 2006), as well as the efficacy against co-infections suggest there may be occupational safety benefits to HCWs in high-risk settings using a respirator, and that more studies are needed to better understand potential bacterial nosocomial respiratory pathogens.

Conflict of interest statement

The masks/respirators used in this study were provided by mask manufacturer 3M. The investigators have also partnered with 3M on an Australian Research Council Linkage Grant on masks. Prof MacIntyre also receives funding from influenza vaccine manufacturers GSK and CSL Biotherapies for investigator-driven research. Dr Holly Seale holds an NHMRC Australian based Public Health Training Fellowship (1012631) and has received funding for investigator-driven research/invitations to present from GSK, CSL and Sanofi-Pasteur.

Dr Iman Ridda holds an NHMRC Early career (630739) and has received funding for Investigator initiated research from GSK and for consultation from Merck. The remaining authors declare that they have no competing interests.

Contribution of each author

Professor C Raina MacIntyre: As a lead investigator Prof. MacIntyre was responsible for conception and design of the trial, overseeing the whole study, analyzing data, writing the report.

Professor Quanyi Wang: Study implementation, contribution to design, analysis and drafting of paper.

Dr. Bayzidur Rahman: Statistical analysis and drafting of paper.

Dr. Holly Seale: Study design, form/database development, monitoring, review and drafting of paper.

Dr. Iman Ridda: Literature review and drafting of manuscript.

Dr. Zhanhai Gao: Statistical analysis and drafting of paper.

Dr. Peng Yang: Study design, acquisition of data and drafting of paper.

Dr. Weixian Shi: Study design, Laboratory testing, review of the paper.

Dr. Xinghuo Pang: Study implementation, acquisition of data and review of the paper.

Dr. Yi Zhang: Database management and analysis.

Ms Aye Moa: Literature review and drafting of manuscript.

Professor Dominic E Dwyer: Study design, clinical and laboratory technical assistance and drafting of paper.

Financial disclosure

This study was funded by Strategic Research Funding from UNSW Medicine, The University of New South Wales, Australia. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics statement

The study protocol was approved by the Institutional Review Board (IRB), Human Research Ethics Committee of the Beijing Ministry for Health, and National Ethics Application Form (NEAF), National Health and Medical Research Council (NHMRC), Australia.

References

Addiss, D.G., Davis, J.P., Meade, B.D., et al., 1991. A pertussis outbreak in a Wisconsin nursing home. J. Infect. Dis. 164, 704–710.

Aiello, A.E., Perez, V., Coulborn, R.M., Davis, B.M., Uddin, M., Monto, A.S., 2012. Facemasks, hand hygiene, and influenza among young adults: a randomized intervention trial. PLoS ONE 7, e29744.

Austrian, R., 1986. Some aspects of the pneumococcal carrier state. J. Antimicrob. Chemother. 18, 35–45.

Baba, H., Iinuma, Y., Imaizumi, K., et al., 2009. Transmission of bacterial infections to healthcare workers during intubation and respiratory care of patients with severe pneumonia. Infect. Control Hosp. Epidemiol. 30, 1019–1021.

Balazy, A., Toivola, M., Adhikari, A., Sivasubramani, S.K., Reponen, T., Grinshpun, S.A., 2006. Do N95 respirators provide 95% protection level against airborne viruses, and how adequate are surgical masks? Am. J. Infect. Control 34, 51–57.

Borella, P., Bargellini, A., Marchesi, I., et al., 2008. Prevalence of anti-*Legionella* antibodies among Italian hospital workers. J. Hosp. Infect. 69, 148–155.

Boulnois, G.J., 1992. Pneumococcal proteins and the pathogenesis of disease caused by *Streptococcus pneumoniae*. J. Gen. Microbiol. 138, 249–259.

Chan, M., 2010. Factors affecting the compliance of operating room nursing staff toward standard and transmission-based precautions in an acute care hospital (Letter to the Editor). Am. J. Infect. Control 38, 666–667.

Chan, R., Molassiotis, A., Eunice, C., et al., 2002. Nurses' knowledge of and compliance with universal precautions in an acute care hospital. Int. J. Nurs. Stud. 39, 157–163.

Chen, S.K., Vesley, D., Brosseau, L.M., Vincent, J.H., 1994. Evaluation of single-use masks and respirators for protection of health care workers against mycobacterial aerosols. Am. J. Infect. Control 22, 65–74.

Cowling, B.J., Chan, K.-H., Fang, V.J., et al., 2009. Facemasks and hand hygiene to prevent influenza transmission in households. Ann. Intern. Med. 151, 437–446.

Decker, M., Schaffner, W., 1996. Nosocomial diseases in healthcare workers spread by the airborne or contact routes (other than tuberculosis). In: Mayhall, C.G. (Ed.), Hospital Epidemiology and Infection Control. Williams & Wilkins, Baltimore, pp. 859–882.

Elie-Turenne, M., Fernandes, H., Mediavilla, J., et al., 2010. Prevalence and characteristics of *Staphylococcus aureus* colonization among healthcare professionals in an urban teaching hospital. Infect. Control Hosp. Epidemiol. 31, 574–580.

Eriksen, H.M., Iversen, B.G., Aavitsland, P., 2005. Prevalence of nosocomial infections in hospitals in Norway, 2002 and 2003. J. Hosp. Infect. 60, 40–45.

Gehanno, J.F., Pestel-Caron, M., Nouvellon, M., Caillard, J.F., 1999. Nosocomial pertussis in healthcare workers from a pediatric emergency unit in France. Infect. Control Hosp. Epidemiol. 20, 549–552.

Gray, B.M., Converse 3rd, G.M., Dillon Jr., H.C., 1980. Epidemiologic studies of Streptococcus pneumoniae in infants: acquisition, carriage, and infection during the first 24 months of life. J. Infect. Dis. 142, 923–933.

Guillet, M., Zahar, J.-R., Timsit, M.-O., et al., 2012. Horizontal transmission of *Streptococcus pneumoniae* in the surgical ward: a rare source of nosocomial wound infection. Am. J. Infect. Control 40, 71–72.

Hammitt, L., Bruden, D., Butler, J., et al., 2006. Indirect effect of conjugate vaccine on adult carriage of *Streptococcus pneumoniae*: an explanation of trends in invasive pneumococcal disease. J. Infect. Dis. 193, 1487–1494.

Hyman, C., Roblin, P., Gaydos, C., Quinn, T., Schachter, J., Hammerschlag, M., 1995. Prevalence of asymptomatic nasopharyngeal carriage of *Chlamydia pneumoniae* in subjectively healthy adults: assessment by polymerase chain reaction-enzyme immunoassay and culture. Clin. Infect. Dis. 20, 1174–1178.

Kadioglu, A., Weiser, J.N., Paton, J.C., Andrew, P.W., 2008. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. Nat. Rev. Microbiol. 6, 288–301.

Kleemola, M., Jokinen, C., 1992. Outbreak of *Mycoplasma pneumoniae* infection among hospital personnel studied by a nucleic acid hybridization test. J. Hosp. Infect. 21, 213–221.

Kleinbaum, D.G., Kupper, L.L., Muller, K.E., Nizam, A., 2007. Applied Regression Analysis and Other Multivariable Methods, 4th ed. Belmont, CA.

Kleinbaum, D.G., Klein, M., Pryor, E.R., 2010. Logistic Regression: A Self-learning Text, 3rd ed. Springer, New York.

Klevens, R.M., Edwards, J.R., Richards Jr., C.L., et al., 2007. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Rep. 122, 160–166.

Klugman, K., Chien, Y., Madhi, S., 2009. Pneumococcal pneumonia and influenza: a deadly combination. Vaccine 27, C9–C14.

Kurt, T.L., Yeager, A.S., Guenette, S., Dunlop, S., 1972. Spread of pertussis by hospital staff. JAMA 221, 264–267.

Lawrence, R.B., Duling, M.G., Calvert, C.A., Coffey, C.C., 2006. Comparison of performance of three different types of respiratory protection devices. J. Occup. Environ. Hyg. 3, 465–474.

MacIntyre, C.R., Cauchemez, S., Dwyer, D., et al., 2009. Effectiveness of face mask use to control respiratory virus transmission in households. Emerg. Infect. Dis. 15, 233–241.

MacIntyre, C.R., Wang, Q., Cauchemez, S., et al., 2011. A cluster randomized clinical trial comparing fit-tested and non-fit-tested N95 respirators to medical masks to prevent respiratory virus infection in health care workers. Influenza Other Respi Viruses 5, 170–179.

Macintyre, C.R., Wang, Q., Seale, H., et al., 2013. A randomised clinical trial of three options for N95 respirators and medical masks in health workers. Am. J. Respir. Crit. Care Med. 187, 960–966.

Madhi, S., Klugman, K., 2004. A role for *Streptococcus pneumoniae* in viral associated pneumonia. The Vaccine Trialist Group. Nat. Med. 10, 811–813.

Mitchell, N.J., Hunt, S., 1991. Surgical face masks in modern operating rooms — a costly and unnecessary ritual? J. Hosp. Infect. 18, 239–242.

MMWR, 2009. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) — United States, May–August 2009. Morb. Mortal. Wkly Rep. 58, 1071–1074.

Morgan, D.J., Rogawski, E., Thom, K.A., et al., 2012. Transfer of multidrug-resistant bacteria to healthcare workers' gloves and gowns after patient contact increases with environmental contamination. Crit. Care Med. 40, 1045–1051.

Nicas, M., 1995. Respiratory protection and the risk of *Mycobacterium tuberculosis* infection. Am. J. Ind. Med. 27, 317–333.

Nouvellon, M., Gehanno, J.F., Pestel-Caron, M., Weber, C., Lemeland, J.F., Guiso, N., 1999. Usefulness of pulsed-field gel electrophoresis in assessing nosocomial transmission of pertussis. Infect. Control Hosp. Epidemiol. 20, 758–760.

Obaro, S., Monteil, M., Henderson, D., 1996. The pneumococcal problem. Br. Med. J. 312, 1521–1525.

Ong, A., Rudoy, I., Gonzalez, L., Creasman, J., Kawamura, L., Daley, C., 2006. Tuberculosis in healthcare workers: a molecular epidemiologic study in San Francisco. Infect. Control Hosp. Epidemiol. 27, 453–458.

Orr, N., 1981. Is a mask necessary in the operating theatre? Ann. R. Coll. Surg. Engl. 63, 390–392.

Pascual, F.B., Mccall, C.L., Mcmurtray, A., Payton, T., Smith, F., Bisgard, K.M., 2006. Outbreak of pertussis among healthcare workers in a hospital surgical unit. Infect. Control Hosp. Epidemiol. 27, 546–552.

Ridda, I., Seale, H., Katelaris, A.L., et al., 2011. Pneumococcal colonisation following influenza infection. Vaccine 29, 6444–6445.

Rudbeck, M., Viskum, S., Molbak, K., Uldum, S.A., 2009. Legionella antibodies in a Danish hospital staff with known occupational exposure. J. environ. Public Health 2009, 812829.

Safdar, N., Marx, J., Meyer, N.A., Maki, D.G., 2006. Effectiveness of preemptive barrier precautions in controlling nosocomial colonization and infection by methicillin-resistant *Staphylococcus aureus* in a burn unit. Am. J. Infect. Control 34, 476–483.

Sherertz, R.J., Bassetti, S., Bassetti-Wyss, B., 2001. "Cloud" health-care workers. Emerg. Infect. Dis. 7, 241–244.

Tunevall, T.G., 1991. Postoperative wound infections and surgical face masks: a controlled study. World J. Surg. 15, 383–387 (discussion 387–8).
Verwer, P.E.B., Robinson, J.O., Coombs, G.W., et al., 2012. Prevalence of nasal methicillin-resistant *Staphylococcus aureus* colonization in healthcare workers in a Western Australian acute care hospital. Eur. J. Clin. Microbiol. Infect. Dis. 31, 1067–1072.
Vittinghoff, E., Glidden, D.V., Shiboski, S.C., McCulloch, C.E., 2012. Regression Methods in Biostatistics: Linear, Logistic, Survival, and Repeated Measures Models, 2nd ed. Springer, New York.
Wang, X.Y., Kilgore, P.E., Lim, K.A., et al., 2011. Influenza and bacterial pathogen coinfections in the 20th century. Interdiscip. Perspect. infect. Dis. 146376.
Weber, A., Willeke, K., Marchioni, R., et al., 1993. Aerosol penetration and leakage characteristics of masks used in the health care industry. Am. J. Infect. Control 21, 167–173.
Weber, D.J., Rutala, W.A., Schaffner, W., 2010. Lessons learned: protection of healthcare workers from infectious disease risks. Crit. Care Med. 38, S306–S314.
Zhou, H., Haber, M., Ray, S., Farley, M., Panozzo, C., Klugman, K., 2012. Invasive pneumococcal pneumonia and respiratory virus co-infections. Emerg. Infect. Dis. 18, 294–297.

13

Ann. Occup. Hyg., Vol. 53, No. 2, pp. 117–128, 2009
Published by Oxford University Press
on behalf of the British Occupational Hygiene Society
doi:10.1093/annhyg/men086

Comparison of Nanoparticle Filtration Performance of NIOSH-approved and CE-Marked Particulate Filtering Facepiece Respirators

SAMY RENGASAMY[1]*, BENJAMIN C. EIMER[2] and RONALD E. SHAFFER[1]

[1]National Personal Protective Technology Laboratory, National Institute for Occupational Safety and Health, 626 Cochrans Mill Road, PO Box 18070, Pittsburgh, PA 15236, USA; [2]EG&G Technical Services Inc., 626 Cochrans Mill Road, PO Box 18070, Pittsburgh, PA 15236, USA

Received 16 October 2008; in final form 16 December 2008

The National Institute for Occupational Safety and Health (NIOSH) and European Norms (ENs) employ different test protocols for evaluation of air-purifying particulate respirators commonly referred to as filtering facepiece respirators (FFR). The relative performance of the NIOSH-approved and EN-certified 'Conformité Européen' (CE)-marked FFR is not well studied. NIOSH requires a minimum of 95 and 99.97% efficiencies for N95 and P100 FFR, respectively; meanwhile, the EN requires 94 and 99% efficiencies for FFRs, class P2 (FFP2) and class P3 (FFP3), respectively. To better understand the filtration performance of NIOSH- and CE-marked FFRs, initial penetration levels of N95, P100, FFP2 and FFP3 respirators were measured using a series of polydisperse and monodisperse aerosol test methods and compared. Initial penetration levels of polydisperse NaCl aerosols [mass median diameter (MMD) of 238 nm] were measured using a method similar to the NIOSH respirator certification test method. Monodisperse aerosol penetrations were measured using silver particles for 4–30 nm and NaCl particles for 20–400 nm ranges. Two models for each FFR type were selected and five samples from each model were tested against charge neutralized aerosol particles at 85 l min^{-1} flow rate. Penetrations from the 238 nm MMD polydisperse aerosol test were <1% for N95 and FFP2 models and <0.03% for P100 and FFP3 models. Monodisperse aerosol penetration levels showed that the most penetrating particle size (MPPS) was in the 30–60 nm range for all models of FFRs tested in the study. Percentage penetrations at the MPPS were <4.28, <2.22, <0.009 and <0.164 for the N95, FFP2, P100 and FFP3 respirator models, respectively. The MPPS obtained for all four FFR types suggested particle capturing by electrostatic mechanism. Liquid isopropanol treatment of FFRs shifted the MPPS to 200–300 nm and dramatically increased polydisperse as well as monodisperse aerosol penetrations of all four FFR types indicating that all the four FFR types share filtration characteristics of electret filters. Electrostatic charge removal from all four FFR types also increased penetration levels of 400–1000 nm range particles. Particle penetration data obtained in this study showed that the eight models of NIOSH-approved N95 and P100 and CE-marked FFP2 and FFP3 respirators used in this study provided expected levels of laboratory filtration performance against nanoparticles.

Keywords: filtration; monodisperse aerosol; NaCl particles; nanoparticle; particle penetration; respirator; silver particles

INTRODUCTION

The rapid growth of nanotechnology industries has introduced engineered nanomaterials into the workplace. Engineered nanomaterials show unique properties different from the bulk materials. Workers handling or manipulating nanomaterials can generate aerosolized nanoparticles (Schulte *et al.*, 2008) which may be inhaled, ingested or absorbed through skin. Among the different routes of nanoparticle entry, inhalation is considered to be the primary mechanism. Once inhaled, nanoparticles with increased solubility can reach parts of a biological system which

*Author to whom correspondence should be addressed.
Tel: +412-386-6853; fax: +412-386-5852;
e-mail: arengasamy@cdc.gov

are not readily accessible by larger particles. Nanoparticle inhalation has been shown to cause adverse effects on pulmonary and systemic functions (Pope *et al.*, 2002; Elder *et al.*, 2006). Many organizations recommend the use of personal respiratory protection devices when engineering controls and other control technologies do not reduce the occupational exposure to nanoparticles to acceptable levels. Because of concerns regarding respirator performance, in particular the filtration of nanoparticles, the National Institute for Occupational and Safety and Health (NIOSH, 2008), Nanotechnology Environmental and Health Implications working group (NEHI, 2008), International Council on Nanotechnology (ICON, 2008) and other organizations have called for increased emphasis on research to better understand the effectiveness of respirators.

Respiratory protection devices throughout the world are often regulated nationally. In the US, NIOSH certifies N, R and P series particulate filtering respirator types 95, 99 and 100 with minimum filtration efficiencies of 95, 99 and 99.97%, respectively. Several countries including Canada, Mexico and Chile recognize NIOSH certification of respirators, while in Europe, respirators marked with 'Conformité Européen' (CE) such as FFP1, FFP2 and FFP3 types meet minimum filtration efficiencies of 80, 94 and 99%, respectively. NIOSH and European Norm (EN) certifications of particulate respirators employ different test protocols for approval. NIOSH conducts respirator certification testing according to 42 CFR Part 84 (Federal Register, 1995) and the approved products are required to be labeled with 'NIOSH' in capital letters and with other information including part and lot number and company name. The European Community (EC) legislation specifies that EN standards must be followed for testing respirators (European Directive, 1996). A CE mark on the product indicates EC conformity. Both NIOSH and EN respirator certification programs are widely known in different parts of the world.

For certification of particulate respirators, NIOSH and EC notified bodies or test houses conduct filtration tests using different protocols. NIOSH regulations for N-series respirator testing require a polydisperse distribution of NaCl particles with a count median diameter (CMD) of 0.075 ± 0.020 μm and a geometric standard deviation (GSD) of <1.86 (NIOSH, 2005a). The mass median diameter (MMD) of the target distribution of test particles is 238 nm with a mass median aerodynamic diameter (MMAD) of 347 nm. For R- and P-designated respirators, a polydisperse distribution of dioctyl phthalate (DOP) particles with a CMD of 0.185 ± 0.020 μm and a GSD of <1.60 is used (NIOSH, 2005b). The MMD of DOP aerosol corresponds to 356 nm with a MMAD of 359 nm. The NIOSH certification test is conducted using charge neutralized polydisperse aerosol particles (NaCl and DOP) at 85 l min^{-1} flow rate using a TSI 8130 Automated Filter Tester, which employs a forward light scattering photometer to measure the flux of light scattering from particles. A reported limitation of the photometer used in the TSI 8130 is that it has a higher measurement efficiency for particles >100 nm size (Eninger *et al.*, 2008b). On the other hand, CE-marked particulate respirators are tested with non-neutralized polydisperse NaCl as well as paraffin oil particles at 95 l min^{-1} according to EN standards (BS EN 2000, 2002). For NaCl aerosol, the diameter of the particles varies from 40 to 1200 nm with a MMD of 600 nm. NaCl aerosol particles upstream and downstream of respirators are passed through a hydrogen flame and vaporized. The intensity of light emitted at 589 nm is measured, which is proportional to sodium concentration. For polydisperse oil aerosol production, paraffin oil is atomized at 100°C and diluted with filtered air. The particle size distribution is a log-normal distribution with a number median Stokes diameter of 400 nm and a GSD of 1.82. The aerosol concentration is measured before and after the test filter by a light scattering photometer.

Laboratory filtration performance of air-purifying particulate filtering respirators which include filtering facepiece respirators (FFRs) is well characterized for a wide size range of aerosol particles most commonly found in workplaces (Moyer and Bergman, 2000; Lee *et al.*, 2005; Balazy *et al.*, 2006; Rengasamy *et al.*, 2007; Eninger *et al.*, 2008a). Moyer and Bergman (2000) reported $<5\%$ initial percentage penetration levels of NaCl aerosols for three models of N95 FFRs. In one study, initial penetration levels of 50 nm monodisperse NaCl particles (most penetrating particle size, MPPS) $>5\%$ was reported for one of two N95 FFRs tested at 85 l min^{-1} (Balazy *et al.*, 2006). Further studies with additional N95 FFR models showed that penetration levels at the MPPS for some FFR models slightly exceeded NIOSH allowed 5% level, but the increase was not significantly different from 5% (Rengasamy *et al.*, 2007). Some studies also reported the filtration performance of other types of FFRs and filter media including R and P types (Martin and Moyer, 2000; Richardson *et al.*, 2006; Eninger *et al.*, 2008a; Rengasamy *et al.*, 2008b). NIOSH-approved P100 FFRs showed penetration levels within approved levels ($<0.03\%$) at 85 l min^{-1} flow rate. The MPPS for P100 FFRs was found to be in the 40–50 nm range (Richardson *et al.*, 2006; Rengasamy *et al.*, 2008b). A recent study reported $>1\%$ penetration for size-fractioned NaCl (20–500 nm) and viral aerosols (100 nm) for two models of N99 FFRs at 85 l min^{-1} flow rate (Eninger *et al.*, 2008a).

Very few studies reported the filtration performance of CE-marked FFR against nanoparticles (Wake *et al.*, 1992; Wilkes, 2002; Checchi *et al.*,

2005; Golanski *et al.*, 2008). One study assessed the respirator performance against radon daughter aerosols by measuring the filtration efficiency of filtering facepieces and filters approved by the British Standard Institution and Health and Safety Executive of UK with monodisperse NaCl aerosols (Wake *et al.*, 1992). The results showed that penetration levels of neutralized aerosols were higher than that of charged aerosols.

Recent studies reported the penetration of a wide size range of particles through respirators and filters (Huang *et al.*, 2007; Golanski *et al.*, 2008). Huang *et al.* (2007) measured the filtration performance of respirators against nanoparticles by determining the penetration levels of 4.5 nm to 10 μm NaCl aerosols through one CE-marked FFP1 model and one NIOSH-approved N95 FFR model. The results showed that particles below 10 nm were effectively captured by the FFP1and N95 FFR models studied. Another study reported the penetration levels of graphite nanoparticles ranging from 5 to 100 nm for FFP3 and other filter media (Golanski *et al.*, 2008). FFP3 filter showed maximum penetration levels of ∼0.1% at the MPPS (30–40 nm) with varying penetration levels for high-efficiency particulate air (HEPA) and other filter media at a face velocity of 5.3 cm s^{-1}.

NIOSH and EN certification of particulate respirators employ different test protocols and a comparative performance of these FFR is not available for a wide range of particle sizes, in particular those particles <100 nm (i.e. nanoparticles). This study compared the filtration performance of two models each of NIOSH-approved N95 and P100 and CE-marked FFP2 and FFP3 FFRs using a polydisperse aerosol test (PAT) method similar to the method used in NIOSH certification and two monodisperse aerosol test methods. The relative filtration performances of the various respirators are discussed and data are presented on their filtration mechanisms.

MATERIALS AND METHODS

Filtering facepieces

Two models each of NIOSH-approved N95 and P100 and CE-marked FFP2 and FFP3 FFRs were purchased commercially. For comparison of filtration performance, class N95 and class FFP2 respirators as well as class P100 and class FFP3 respirators were selected. It could be argued that comparison of the filtration performances of NIOSH-approved class N99 respirator with the FFP3 respirator would be better because these two types are both certified to meet <1% particle penetration levels. However, a class P100 FFR was selected in this study to compare with FFP3 because it allows us to compare the results from this study with our previous work (Rengasamy *et al.*, 2008b). In addition, class P100 respirators are far more commonly used than class N99 respirators in the US. The manufacturers were randomly selected from the NIOSH- and CE-marked lists. A single respirator model was selected from each manufacturer, excepting FFP3. Two different models of FFP3 were selected from one manufacturer because of procurement difficulties.

Polydisperse NaCl aerosol penetration test (PAT)

Initial penetration levels of polydisperse NaCl aerosol were measured using a TSI 8130 Automated Filter Tester (TSI 8130) as described previously (Rengasamy *et al.*, 2007). Penetration levels were measured for 1 min of loading, instead of carrying out the entire NIOSH 42 CFR Part 84 test procedure (NIOSH, 2005a). Initial penetration levels were measured in order to be consistent with aerosol testing for various size monodisperse particles described below. Percentage particle penetration was measured at 85 l min^{-1} flow rate with the mask mounted in a Plexiglas box (20 × 20 × 10 cm). Five samples from each model were tested for particle penetration measurements.

Monodisperse 4–30 nm silver particle penetration test (MAT-1)

Silver nanoparticles were generated by an evaporation and condensation method and tested for penetration as described previously (Rengasamy *et al.*, 2008b). Briefly, pure metallic silver (Alfa Aesar, 99.99%) in a ceramic boat was placed inside a ceramic tube kept in a furnace (Lindberg/BlueM model: TF55035A-1) and heated at 1050°C (Figure 1). Polydisperse silver nanoparticles produced were transported by HEPA-filtered nitrogen gas at 2 l min^{-1} flow rate into a scanning mobility particle sizer (SMPS; TSI model 3080) equipped with a nano-differential mobility analyzer (Nano-DMA, TSI model 3085). Six different size (centered at 4, 8, 12, 16, 20 and 30 nm) monodisperse silver particles were produced based on electrical mobility. The size of the monodisperse aerosol particles generated by the test system was verified (Rengasamy *et al.*, 2008b). The exiting monodisperse particles were mixed with HEPA-filtered room air and passed through a ^{85}Kr neutralizer (TSI 3012). The charge neutralized monodisperse particles were passed into the Plexiglas respirator test box. Upstream and downstream particle numbers at 85 l min^{-1} flow rate were counted alternately using an ultrafine condensation particle counter (UCPC; TSI 3025A). Leakage of nanoparticles into the test system was checked by operating the nano-DMA at 0 V and measuring the counts by the UCPC. The absence of any leakage was ensured by measuring zero counts for 20 min. An equilibration time of ∼5 min was allowed between upstream

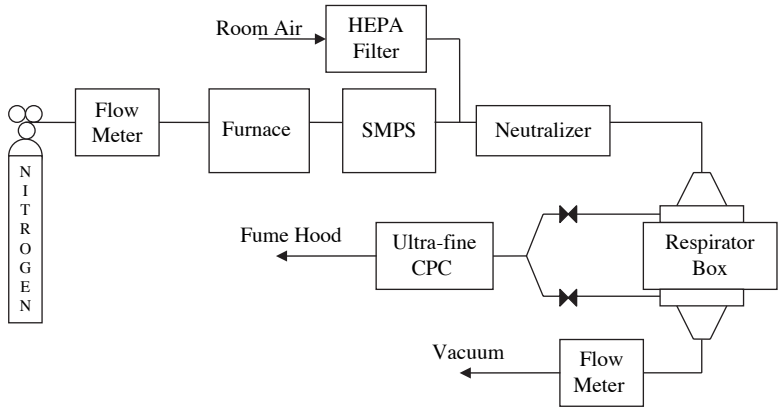


Fig. 1. Schematic diagram of the silver particle test system (Rengasamy *et al.*, 2008b. J. Occup. Env. Hyg. 5: 556–564, 2007).

and downstream sampling. Five samples from each model were tested for penetration of monodisperse silver particles.

For N95 and FFP2 respirator penetration studies, the furnace temperature was set at 1050°C to produce sufficient number of particles for measuring the penetration of the six different size monodisperse silver particles. For P100 and FFP3 respirators, furnace temperatures were kept at 950°C for 4 nm particles, 1050°C for 8 and 12 nm particles and at 1100°C for 16, 20 and 30 nm size particles to optimize the number of the test particles as described previously (Rengasamy *et al.*, 2008b).

Monodisperse 20–400 nm NaCl aerosol penetration test (MAT-2)

A different set of FFR samples from the same models that were employed for the PAT experiments were tested against monodisperse NaCl particles using a TSI 3160 Fractional Efficiency Tester (TSI 3160) equipped with a long DMA (TSI 3081) as described previously (Rengasamy *et al.*, 2007). Initial percentage penetration levels of 10 different size (centered at 20, 30, 40, 50, 60, 80, 100, 200, 300 and 400 nm) monodisperse particles were measured in one test run for each FFR at a flow rate of 85 l min^{-1}. Five samples from each model were tested for different size monodisperse particle penetrations.

Penetration of NaCl particles as a function of particle size from 30 to 1000 nm

To better understand the penetration of submicron size particle (<1000 nm), penetration was measured as a function of particle size from 30 to 1000 nm. NaCl aerosol was generated using a constant output atomizer (Model 3076, TSI) and the aerosol concentrations and size distributions (30–1000 nm range) were measured using a SMPS and a condensation particle counter (CPC) instead of using the TSI 3160 filter tester (Figure 2). Polydisperse NaCl particles were passed through a drier, a ^{85}Kr neutralizer and then into the Plexiglas box containing the test respirator. Particle number concentrations and size distributions upstream and downstream of the FFR were measured alternately using a SMPS in scanning mode. Percentage penetration was calculated from the ratio of the particle number concentration downstream to the concentration upstream.

Isopropanol treatment

Class N95 respirators typically capture particles by electrostatic and other mechanisms. It is unclear whether most P100, FFP2 and FFP3 respirators capture particles by mechanical or a combination of both mechanical and electrostatic mechanisms. The exact filtration mechanism of various respirator types is useful for filtration theory modeling and theoretical simulations (Balazy *et al.*, 2006) and for research to develop improved filters and air-purifying respirators. The physical interactions between particles and filter fibers can change dramatically when electrostatic charges on the fibers are introduced. To address this question for the models studied here, the FFR samples were subjected to isopropanol treatment, which is known to remove electrostatic charges on filter media and to increase particle penetration in laboratory tests (Chen *et al.*, 1993; Chen and Huang, 1998; Martin and Moyer, 2000; Kim *et al.*, 2007a). In the first set, five FFR samples were tested using the PAT method and then the FFRs were carefully removed from the test box and dipped into liquid isopropanol in a container for 1 min. FFR samples were removed from isopropanol solution, dried by evaporation overnight in a fume hood at room temperature and tested again using the PAT method with polydisperse NaCl aerosol particles. The second set consisting of five FFR samples was tested using the MAT-2 method using monodisperse NaCl aerosols (20–400 nm range) on the TSI 3160 and then treated with isopropanol and processed as described previously. The samples were tested again for particle penetration using the MAT-2 method. For the third

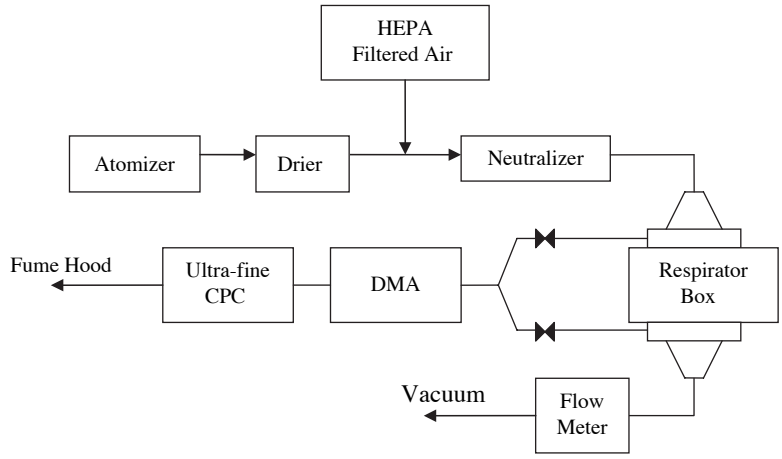


Fig. 2. Schematic diagram of test system for penetration of 30–1000 nm particles.

set, five FFR samples were tested for particle number concentrations and size distributions of NaCl particles from 30 to 1000 nm size using a SMPS in scanning mode and then removed from the test box, treated with isopropanol, processed as described previously and measured again the particle number concentrations and size distributions.

Data analysis

The data were analyzed using the SigmaStat computer program. Average, standard deviation and 95% confidence interval penetration levels were calculated for each model. Correlation coefficients between variable parameters were calculated using the Pearson's product-moment correlation method.

RESULTS

Table 1 shows the initial penetration levels of polydisperse NaCl aerosol and standard deviations for two models each of N95, FFP2, P100 and FFP3 FFR types at 85 l min^{-1} flow rate using the PAT method. Both N95 and FFP2 respirators showed average penetration levels of <1%. P100 and FFP3 respirators showed average penetration levels of <0.03%.

Percentage penetrations of six different size monodisperse silver particles in the 4–30 nm range were measured for the different FFR types using the MAT-1 method. Monodisperse particle penetration levels decreased with decreasing particle size for all N95, FFP2, P100 and FFP3 respirators tested at 85 l min^{-1} flow rate (Figure 3). Average penetration levels of the two N95 FFR models tested were similar to the two FFP2 models (top panel). Among the N95 and FFP2 respirator models tested, one FFP2 model showed no penetration for 4 nm particles. For P100 FFR models, the average penetration levels were one to two orders of magnitude less than the levels obtained for the two FFP3 respirator models (bottom panel).

Figure 4 shows average penetration levels of 10 different size monodisperse NaCl particles in the 20–400 nm range for N95 and FFP2 (top panels) and P100 and FFP3 respirators (bottom panels) at 85 l min^{-1} flow rate from the MAT-2 method. Average penetration levels increased from 20 to 30–60 nm and then decreased up to 400 nm particle size for all the respirator models tested. The MPPS for all the four FFR types was in the 30–60 nm range. Both N95 models showed penetration levels comparable to the FFP2 models for the different size particles in the 20–400 nm range (top panels). Penetration levels of both P100 models were approximately one order less than the FFP3 respirator models (bottom panels). Figure 5 shows the correlation of polydisperse (PAT) and monodisperse MPPS particle penetrations (MAT-2) for the NIOSH- and EN-certified FFRs. A significant correlation ($r = 0.97$; $P = 0.00006$) was obtained for each of two N95, FFP2, P100 and FFP3 respirator models.

Filter penetration was measured before and after isopropanol treatment of FFR to assess particle capturing by electrostatic mechanism. Penetration levels from the PAT method test were <1% for control N95 and FFP2 respirators (Figure 6, top panel). Isopropanol treatment increased the penetration levels by one to two orders of magnitude for both N95 and FFP2 respirator types. Figure 6 (bottom panel) shows polydisperse aerosol penetration levels of control and isopropanol-treated P100 and FFP3 respirators obtained using the PAT method. Average penetrations were <0.03% for the controls, which increased two to three orders of magnitude after isopropanol treatment.

Figure 7 shows the average penetration levels of monodisperse particles in the 20–400 nm range (MAT-2 method) for the four different FFR types before and after isopropanol treatment. The MPPS for the controls was ~30 to 60 nm, which shifted to

Table 1. Penetration levels from the PAT for the different FFR types

Respirator class	N95		FFP2		P100		FFP3	
Manufacturer	M1	M2	M1	M2	M1	M2	M1	M1
Mean penetration (%)	0.703	0.565	0.270	0.505	0.0034	0.0222	0.0098	0.0144
Standard deviation	0.200	0.525	0.096	0.275	0.002	0.036	0.004	0.011

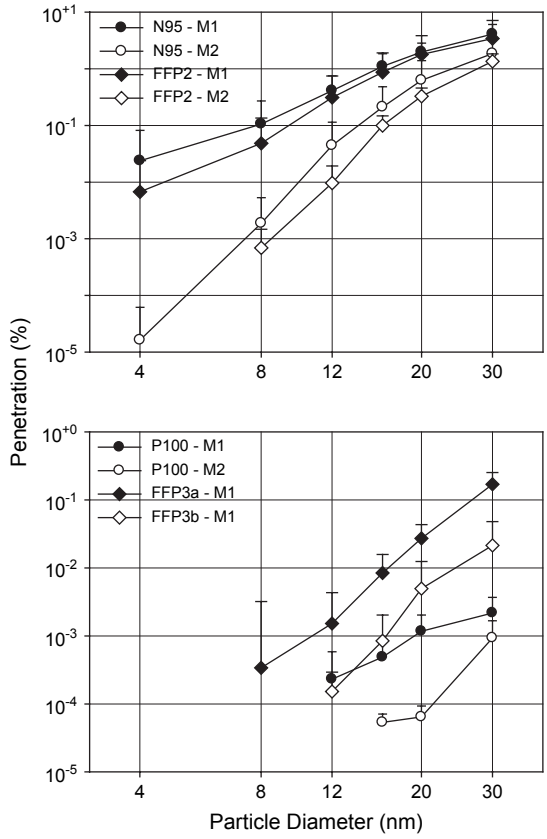


Fig. 3. Percentage penetrations of monodisperse silver particles (4–30 nm) through N95, FFP2, P100 and FFP3 FFR from two different manufacturers (M1 and M2) at 85 l min^{-1} flow rate (MAT-1 method). Error bar indicates the 95% confidence interval ($n = 5$).

the 200–300 nm range after isopropanol treatment. Isopropanol dramatically increased the penetration levels of different size monodisperse particles tested in the 20–400 nm range. The increase in penetration was greater for 200–300 nm particles compared to other size particles for all respirator models tested. The magnitude of increase in penetration was less than two orders for N95 and FFP2 respirators compared to more than four and more than two orders for P100 and FFP3 respirators, respectively. Figure 8 shows average penetration curves for NaCl aerosol particles as a function of particle size from 30 to 1000 nm range for control FFRs. Figure 8 (top panels) shows penetration levels of <3% for N95 and FFP2 respirators and <0.5% for P100 and FFP3 respirators for particles <100 nm. All respirator types showed negligible penetration levels for particles >400 nm. Figure 8 (bottom panels) shows the subsequent penetration levels for liquid isopropanol-treated FFRs. In general, the increase in penetration levels for 20–400 nm particles after isopropanol treatment agreed with the data obtained for individual monodisperse NaCl aerosols tested using the TSI 3160 filter tester (MAT-2 method). In addition, all the four respirator types showed a significant increase in penetration levels for 400–1000 nm particles after isopropanol treatment.

DISCUSSION

NIOSH and EN respirator programs employ different test protocols for certification of particulate FFR for respiratory protection. Penetration measurements and the test conditions used in this study are different from the penetration tests required by the NIOSH and EN certification protocols. The penetration results

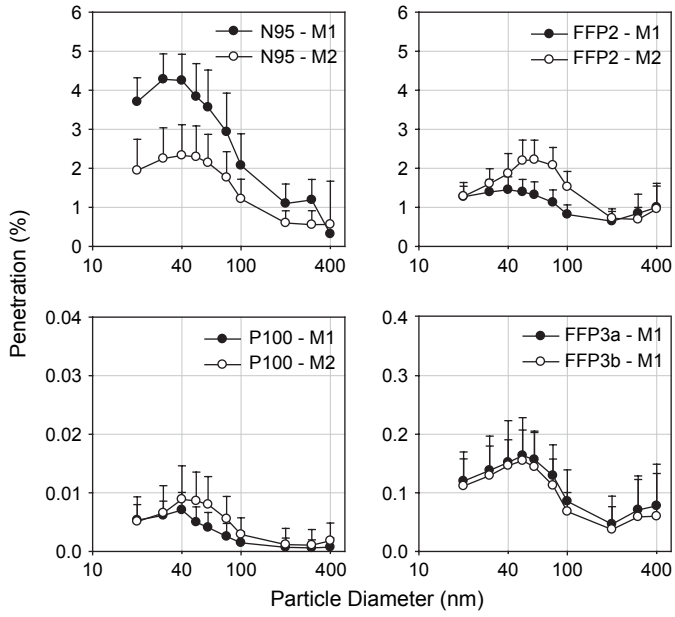


Fig. 4. Percentage penetrations of monodisperse NaCl (20–400 nm) particles through N95, FFP2, P100 and FFP3 FFR from two manufacturers (M1 and M2) at 85 l min^{-1} (MAT-2 method). Error bar represents the 95% confidence interval ($n = 5$).

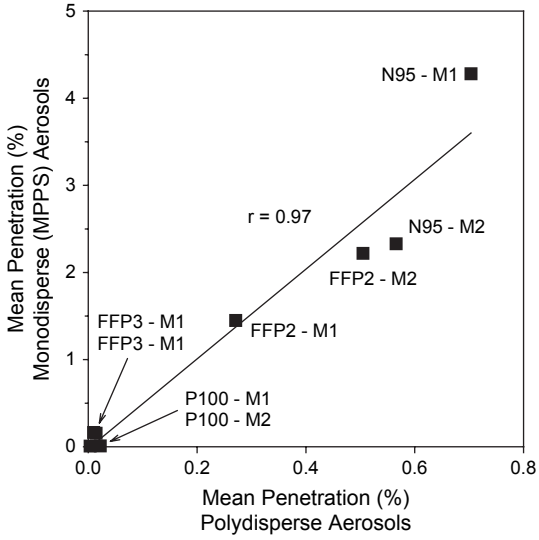


Fig. 5. Correlation of 238 nm MMD polydisperse (PAT method) and monodisperse (MAT-2 method) MPPS (30–60 nm) sodium chloride aerosol penetrations for N95, FFP2, P100 and FFP3 respirator models tested ($r = 0.97$, $P = <0.00006$) ($n = 5$).

obtained with the three test methods used in this study may not be predictive of the penetration results received using the respective certification test methods. For this reason, the results obtained in the study cannot be directly compared with the filtration performance of FFRs approved by the NIOSH and EN certification programs. Across all test methods employed, the penetration levels for N95 and P100 were within the NIOSH allowed <5 and <0.03% levels, respectively. Similarly, FFP2 and FFP3 respirators showed penetration levels <6 and <1%, respectively, as allowed by EN regulations. A comparison of the filtration performance from the PAT method showed that penetration levels were similar for N95 and FFP2 class respirators, as well as for P100 and FFP3 class respirators. Similar classifications of NIOSH and EN particulate respirators demonstrated similar penetration levels for polydisperse particles with a MMD of 238 nm. This observation is consistent with a previous report which compared the penetration levels of different breathing system filters using a TSI 8130 used in NIOSH certification tests and a Moore's Test Rig (CEN Bench Rig) (SPF Services, Christchurch, UK) (Wilkes, 2002) approved for CE marking. NIOSH respirator certification tests are conducted at 85 l min^{-1} with the TSI 8130 which uses charge neutralized polydisperse NaCl aerosols having a MMD of 238 nm. Particle penetration was measured using forward light scattering as described previously (Johnson and Smith, 1988). On the other hand, Moore's Test Rig uses non-neutralized NaCl particles of 40–1200 nm range with a MMD of 600 nm and the filter test is conducted at 95 l min^{-1}. NaCl particle penetration was measured using a neutral hydrogen flame photometer for different filter media (Wilkes, 2002). Their results showed no significant difference in the penetration values for the two methods. Although the NIOSH and EN FFR test methods employ polydisperse aerosol particles in the 22–259 nm (~95%) and 40–1200 nm ranges, respectively, the vast majority of particles that penetrate through the FFR are <300 nm size. Particle penetration results for N95 and FFP2 respirators are expected to be similar because of their expected

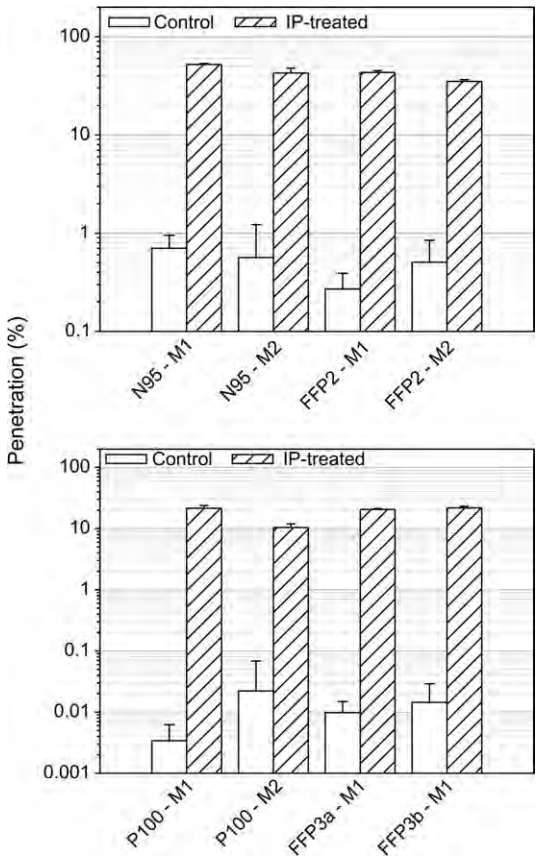


Fig. 6. Percentage penetration levels of 238 nm MMD polydisperse aerosols (PAT method) for different FFR types and manufacturers (M1 and M2) before (control) and after isopropanol treatment (IP-treated) at 85 l min^{-1} flow rate. Error bar indicates the 95% confidence interval ($n = 5$).

penetration levels <5 and <6%, respectively. Similarly, P100 and FFP3 class respirators allowed for <0.03 and <1.0% penetrations, respectively, are expected to show comparable penetration levels.

Monodisperse aerosol penetration results from this study showed that particle capture increased with decreasing size from 30 nm down to 4 nm for NIOSH-approved class N95 and P100 and CE-marked FFP2 and FFP3 FFR as expected by the single-fiber theory. The results are consistent with previous reports on the filtration performance of respirator filter media (Kim *et al.*, 2007a) and NIOSH-approved and CE-marked FFR (Huang *et al.*, 2007; Rengasamy *et al.*, 2008b). No measurable penetration levels for particles below 10 nm were obtained for one N95 and one FFP1 FFR models tested (Huang *et al.*, 2007). This is partly due to the particle generation method that produced fewer particles in the <10 nm range for penetration measurements. Recently, five N95 FFR models were tested against a relatively high concentration of monodisperse particles in the 4–30 nm range and showed measurable penetration levels for all different size monodisperse particles (Rengasamy *et al.*, 2008b). At the same time, no penetration was obtained for 4 nm particles for all the P100 and FFP3 models tested which is attributed to higher filtration efficiency compared to N95 FFR.

Similar penetration levels were obtained for N95 and FFP2 respirators using the PAT method. This may be partly due to the design of N95 and FFP2 respirators by manufacturers to meet 5 and 6% penetrations required by NIOSH and EN regulations, respectively. On the other hand, the penetration levels of some monodisperse aerosols for P100 FFR were one to two orders of magnitude less compared to FFP3 respirators while no significant difference in penetration was obtained for the PAT. This suggests that only a test method that is based on particle number instead of mass can reveal differences in penetration levels between the different FFR types. The PATs provide an overall penetration of different size particles based on mass of the particles as in the case of TSI 8130 as well as the EN approved equipment. The mass of particles <100 nm is a small fraction compared to the larger size particles and photometric test methods based on particle mass may not adequately measure light scattering of particles in this size range (Eninger *et al.*, 2008b).

Interestingly, the MPPS for all the four FFR types tested in this study was found to be in the 30–60 nm range at 85 l min^{-1} aerosol flow rate. This is consistent with previously reported MPPS values for N95 and P100 FFR (Balazy *et al.*, 2006; Richardson *et al.*, 2006; Rengasamy *et al.*, 2008b). The four types of FFRs studied also agree on the relative filtration performance measured using the monodisperse (MAT-2) and PAT methods. A consistent rank ordering and statistically significant linear correlation (Figure 5) of filtration performance of all four FFR types was obtained. Similar correlations between submicron polydisperse aerosol and monodisperse aerosol tests have been reported for N95 FFRs (Rengasamy *et al.*, 2007), dust masks (Rengasamy *et al.*, 2008a) and HEPA filter media (Lifshutz and Pierce, 1996; Pierce, 1998).

Using the MAT-2 method, percentage penetrations at the MPPS were <4.28, <2.22, <0.009 and <0.164 for the N95, FFP2, P100 and FFP3 respirator models, respectively. These data suggest the eight models of NIOSH-approved N95 and P100 and CE-marked FFP2 and FFP3 respirators used in this study provide expected levels of laboratory filtration performance against a wide range of particles, including those <100 nm (i.e. nanoparticles). A limitation of this study is that only two models from each respirator type were tested. Thus, the laboratory filtration performances seen in this study may not be representative of all commercially available respirators within the four types studied here. Indeed, studies both in our laboratory (Rengasamy *et al.*, 2007) and by other laboratories (Balazy *et al.*, 2006; Eninger

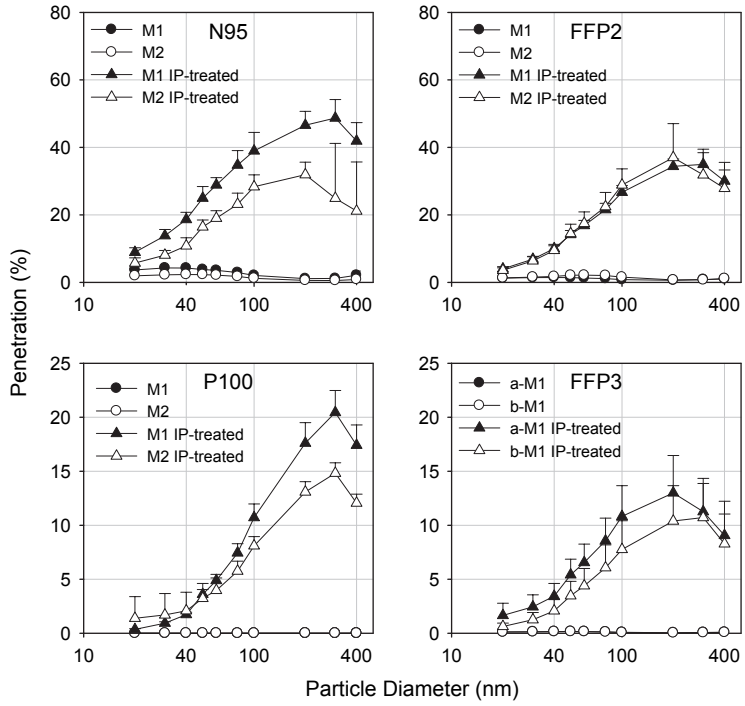


Fig. 7. Percentage penetrations obtained using the MAT-2 method of monodisperse NaCl (20–400 nm) particles for different FFR types and manufacturers (M1 and M2) before and after liquid isopropanol treatment (IP-treated) at 85 l min^{-1}. Error bar represents the 95% confidence interval ($n = 5$).

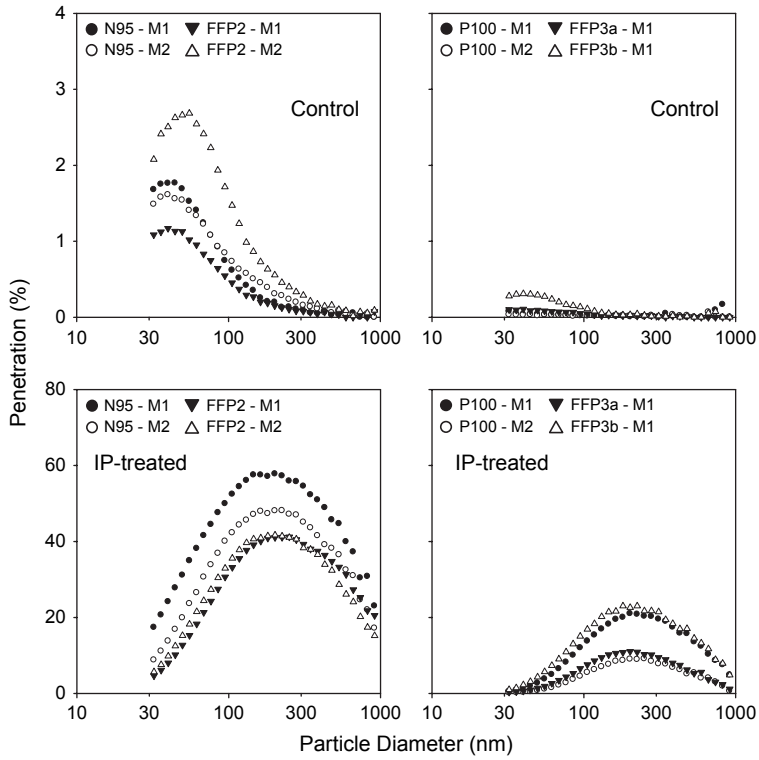


Fig. 8. Average penetration levels of NaCl particles as a function of particle size (30–1000 nm) particles through N95, FFP2, P100 and FFP3 respirators from two manufacturers (M1 and M2) before (control) and after isopropanol treatment (IP-treated) at 85 l min^{-1} ($n = 4$).

et al., 2008a) demonstrate that laboratory respirator filtration performance against nanoparticles in the MPPS range can vary widely, even within a specific respirator type.

The MPPS results obtained for the four different FFR types suggest that the NIOSH-approved as well as the CE-marked FFR models used in this study share filtration properties of electret filters. This was verified by exposing FFR to liquid isopropanol, which is known to remove the electric charge on filter media and to increase particle penetration in laboratory tests. Isopropanol treatment increased PAT penetration levels of N95 and FFP2 models by one to two orders of magnitude and P100 and FFP3 models by two to three orders of magnitude. Based on these results, one may speculate that the P100 and FFP3 FFR models used in this study have more electric charges on filter media fibers than that of the N95 and FFP2 models used in this study. The discrepancy can partly be explained by the filtration efficiency levels of FFR. The percentage penetration levels range from 0.270 to 0.703 for both N95 and FFP2 respirators and from 0.003 to 0.022 for both P100 and FFP3 respirators. Electret charge removal by isopropanol treatment can increase the percentage penetration levels up to only 100% even if the FFR is assumed to be fully electret. Based on the initial penetration levels for the control N95 and FFP2, electret charge removal can only increase penetration levels approximately two orders of magnitude (i.e. from 0.270–0.703 to 100%). At the same time, the penetration levels of P100 and FFP3 FFR can increase to three to four orders of magnitude (i.e. from 0.003–0.022 to 100%) after isopropanol treatment. Thus, isopropanol treatment of an electret filter with 10% penetration level can reach a maximum increase of 10-fold at the maximum. Indeed, two dust mask models with average penetration levels of 10–12% range showed 6- to 7-fold increase in penetration levels after isopropanol treatment (Rengasamy *et al.*, 2008a). Although, liquid isopropanol treatment is assumed to remove all the electret charge on the fiber, a small amount of residual electric charge might be expected to remain on the filter media (Chen *et al.*, 1993). The mechanism of removal of electric charge from filter fibers is not completely understood. Some studies suggested that isopropanol treatment did not remove electret effect from filter media, but caused swelling and dissolution of low-molecular weight polymers resulting in high penetration values (Myers and Arnold, 2003). On the contrary, a recent study employed electrostatic force microscopy and showed a significant removal of electric charges after isopropanol treatment (Kim *et al.*, 2007a). It is possible that isopropanol treatment may disrupt the bonding of non-woven fabric materials and release particles to produce increase in penetration levels. This was tested using HEPA-filtered air with no particles going through the isopropanol-treated respirators. The results showed no release of particles suggesting that the increase in particle penetration after isopropanol treatment may be due to removal of electric charges on filter medium.

Oil aerosols such as DOP decrease electret filter efficiency by mechanisms including neutralization of the charge on the fiber, masking the fiber charge by captured particles or disruption of the charge carrying fiber (Tennal *et al.*, 1991; Barrett and Rousseau, 1998). P-type NIOSH-approved FFRs are not degraded by oil aerosol particles, unlike the N-type electret FFRs. Based on this, NIOSH certification tests for P-type respirators use DOP liquid aerosol particles. This raises a question why a P-type respirator is resistant to oil particles and not an N-type, although both respirator types are electrostatic and susceptible to laboratory filter performance degradation via isopropanol treatment. This may be explained partly due to differences in the manufacturing process of the different types of FFRs (Barrett and Rousseau, 1998). The use of filter media with different chemical composition, different methods of introducing charge onto filter fibers and respirator design using hydrophilic and hydrophobic filter layers in some fashion may also contribute to this difference. Further studies are needed to better understand the mechanisms behind electret filter degradation of different types of respirators.

FFR upon exposure to liquid isopropanol showed a shift in the MPPS from 30 to 60 nm toward a larger size in the 250–300 nm. The results are consistent with the data obtained for filter media and FFR (Chen *et al.*, 1993; Chen and Huang 1998; Martin and Moyer, 2000). The increase in penetration levels of FFR after removal of electret charge by isopropanol treatment clearly shows that electrostatic mechanism plays a significant role in capturing particles of 250–300 nm size compared to particles outside the range as reported previously (Huang *et al.*, 2007). Particle penetration data obtained as a function of particle size after isopropanol treatment suggest that electrostatic forces also play a significant role in capturing particles at >400 nm size range.

The penetration levels measured using the three test methods for the eight models of FFRs were significantly less than the levels allowed by the NIOSH and EN certification test protocols. However, expected protection performance provided by these types of respirators is dependent upon both filtration performance and face seal leakage. Thus, worker protection levels are likely to be much less than the filtration levels seen in this study, which involved sealing the FFRs to the test system in the laboratory. Leakage is dependent upon several factors including proper respirator selection, fit and donning. Further research on leakage of nanoparticles is important to

better understand the effectiveness of FFRs in workplaces where nanoparticles are present.

CONCLUSIONS

Initial particle penetration data obtained in this study showed that the eight models of NIOSH-approved N95 and P100 and CE-marked FFP2 and FFP3 respirators provided expected levels of laboratory filtration performance against nanoparticles. Penetration levels of different size monodisperse particles from 4 to 400 nm showed that the MPPS was in the 30–60 nm range for all four FFR types tested in the study. Monodisperse aerosol particles below the MPPS showed a decrease in penetration levels with decreasing particle size as expected by the single-fiber filtration theory. The NIOSH approved and CE-marked FFR models tested in the study were found to share filtration characteristics of electret filters as shown by the shift in the MPPS from 30–60 to 200–300 nm range after the electric charges were removed.

FUNDING

NIOSH funding–CAN # 927 Z1NT.

Acknowledgements—We acknowledge NIOSH colleagues including William Newcomb, Heinz Ahlers, Liming Lo and William King for their critical review of the manuscript and suggestions.

Disclaimer—Mention of commercial product or trade name does not constitute endorsement by the NIOSH. The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the NIOSH.

REFERENCES

Balazy A, Toivola M, Reponen T *et al*. (2006) Manikin-based performance evaluation of N95 filtering-facepiece respirators challenged with nanoparticles. Ann Occup Hyg; 50: 259–69.

Barrett LW, Rousseau AD. (1998) Aerosol loading performance of electret filter media. Am Ind Hyg Assoc J; 59: 532–9.

BS EN. (2000) Respiratory protective devices. Particle filters—Requirements, testing, marking. London, UK: BSI British Standards. BS EN 143.

BS EN. (2002) Respiratory protective devices—Methods for test—Part 7: determination of particle filter penetration. London, UK: BSI British Standards. BS EN 13274-7.

Checchi L, Montevecchi M, Moreschi A *et al*. (2005) Efficacy of three face masks in preventing inhalation of airborne contaminants in dental practice. J Am Dent Assoc; 136: 877–82.

Chen CC, Huang SH. (1998) The effects of particle charge on the performance of a filtering facepiece. Am Ind Hyg Assoc J; 59: 227–33.

Chen CC, Lehtimaki M, Willeke K. (1993) Loading and filtration characteristics of filtering facepieces. Am Ind Hyg Assoc J; 54: 51–60.

Elder A, Gelein R, Silva V *et al*. (2006) Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect; 114: 1172–8.

Eninger RM, Honda T, Adhikari A *et al*. (2008a) Filter performance of N99 and N95 pacepiece respirators against viruses and ultrafine particles. Ann Occup Hyg; 52: 385–96.

Eninger RM, Honda T, Reponen T *et al*. (2008b) What does respirator certification tell us about filtration of ultrafine particles? J Occup Environ Hyg; 5: 286–95.

European Directive. (1996) Council Directive 89/686/EEC of 21 December 1989 on the approximation of the laws of the member states relating to personal protective equipment as amended by Directive 93/68/EEC, Directive 93/95/EEC and Directive 96/58/EC.

Federal Register. (1995) Respiratory protective devices. Final Rules and Notice; 60: 30335–98.

Golanski L, Guiot A, Tardif F. (2008) Are conventional protective devices such as fibrous filter media, respirator cartridges, protective clothing and gloves also efficient for nanoparticles? Efficiency of fibrous filters and personal protective equipments against nanoaerosols. Dissemination report. DR-325-326-200801-1. Project ID: NMP2-CT-2005-515843. European Strategy for Nanosafety; pp. 1–8.

Huang S-H, Chen C-W, Chang C-P *et al*. (2007) Penetration of 4.5 nm to 10 μm aerosol particles through fibrous filters. J Aero Sci; 38: 719–27.

ICON. (2008) Towards predicting nano-biointeractions: an international assessment of nanotechnology environment, health, and safety research needs. Available at http://cohesion.rice.edu/CentersAndInst/ICON/emplibrary/ICON_RNA_Report_Full2.pdf. Accessed 6 October 2008.

Johnson T, Smith S. (1998) Correlation of penetration results between filter testers that use different particle generators and detection methods. Proceedings of the 1998 Nonwovens Conference, *TAPPI*, St. Petersburg, Florida, #A104.

Kim J, Jasper W, Hinestroza J. (2007a) Direct probing of solvent-induced charge degradation in polypropylene electret fibers via electrostatic force microscopy. J Microsc; 225: 72–9.

Kim SC, Harrington MS, Pui DYH. (2007b) Experimental study of nanoparticles penetration through commercial filter media. J Nanopart Res; 9: 117–25.

Lee SA, Adhikari A, Grinshpun SA *et al*. (2005) Respiratory protection provided by N95 filtering facepiece respirators against airborne dust and microorganisms in agricultural farms. J Occup Environ Hyg; 2: 577–85.

Lifshutz N, Pierce M. (1996) A general correlation of MPPS penetration as a function of face velocity with the model 8140 using the CertiTest 8160. In: First MW, editor. Proceedings of the 24th DOE/NRC Nuclear Air Cleaning and Treatment Conference. The Harvard Air Cleaning Laboratory. United States Nuclear Regulatory Commission, Portland, OR. pp. 698–706.

Martin SB, Jr, Moyer ES. (2000) Electrostatic respirator filter media: filter efficiency and most penetrating particle size effects. Appl Occup Environ Hyg; 15: 609–17.

Moyer ES, Bergman MS. (2000) Electrostatic N-95 respirator filter media efficiency degradation resulting from intermittent sodium chloride aerosol exposure. Appl Occup Environ Hyg; 15: 600–8.

Myers DL, Arnold BD. (2003) Electret media for HVAC filtration applications. Int Nonwovens J; 12: 43–54.

NEHI. (2008) The National Nanotechnology Initiative: strategy for nanotechnology-related environmental, health, and safety research. Available at http://www.nano.gov/NNI_EHS_Research_Strategy.pdf. Accessed 6 October 2008.

NIOSH. (2005a) Procedure no. RCT-APR-STP-0057, 0058, 0059, Revision 1.1. Pittsburgh, PA: DHHS, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory. Available at http://www.cdc.gov/niosh/npptl/stps/pdfs/RCT-APR-0057%2058%2059.pdf. Accessed 6 October 2008.

NIOSH. (2005b) Procedure No. RCT-APR-STP-0051, 0052, 0053, 0054, 0055, 0056, Revision 1.1. Pittsburgh, PA: DHHS, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory. Available at http://www.cdc.gov/

niosh/npptl/stps/pdfs/RCT-APR-0051%2052%2053%2054%2055%2056.pdf. Accessed 6 October 2008.

NIOSH. (2008) NIOSH safety and health topic: nanotechnology. Strategic plan for NIOSH nanotechnology research: filling the knowledge gaps. Available at http://www.cdc.gov/niosh/topics/nanotech/strat_plan.html. Accessed 6 October 2008.

Pierce M. (1998) HEPA filter media testing: 1950–2000. In First MW, editor. Proceedings of the 25th DOE/NRC Nuclear air Cleaning and Treatment Conference, The Harvard Air Cleaning Laboratory. United States Nuclear Regulatory Commission, Minneapolis, MN. pp. 72–8.

Pope CA, Burenett RT, Thun MJ *et al*. (2002) Lung cancer, cardiopulmonary mortality and long term exposure to fine particulate air pollution. J Am Med Assoc; 287: 1132–41.

Rengasamy A, Verbofsky R, King WP *et al*. (2007) Nanoparticle penetration through NIOSH-approved N95 filtering-facepiece respirators. J Int Soc Res Prot; 24: 49–59.

Rengasamy S, Eimer B, Shaffer RE. (2008a) Nanoparticle filtration performance of commercially available dust masks. J Int Soc Res Prot; 25: 27–41.

Rengasamy S, King WP, Eimer B *et al*. (2008b) Filtration performance of NIOSH-approved N95 and P100 filtering-facepiece respirators against 4–30 nanometer size nanoparticles. J Occup Environ Hyg; 5: 556–64.

Richardson AW, Eshbaugh JP, Hofacre KC *et al*. (2006) Respirator filter efficiency against particulate and biological aerosols under moderate to high flow rates. Edgewood, MD: Edgewood Chemical and Biological Center, U.S. Army Research, Development and Engineering Command No. SP0700-00-D-3180, Task No. 335, ECBC-CR-085.

Schulte PA, Geraci C, Zumwalde R *et al*. (2008) Occupational risk management of engineered nanoparticles. J Occup Environ Hyg; 5: 239–49.

Tennal KB, Mazumder MK, Siag A *et al*. (1991) Effect of loading with an oil aerosol on the collection efficiency of an electret filter. Part Sci Technol; 9: 19–29.

Wake D, Brown RC, Trottier RA *et al*. (1992) Measurements of the efficiency of respirator filters and filtering facepieces against radon daughter aerosols. Ann Occup Hyg; 36: 629–36.

Wilkes AR. (2002) Comparison of two techniques for measuring penetration of sodium chloride particles through breathing system filters. Br J Anaesth; 89: 541–5.

14

AVIAN DISEASES 50:284–287, 2006

Research Note—

Survival of Two Avian Respiratory Viruses on Porous and Nonporous Surfaces

A. Tiwari, Devi P. Patnayak, Yogesh Chander, Minakshi Parsad, and Sagar M. Goyal[A]

Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1333 Gortner Avenue, St. Paul, MN 55108

Received 12 October 2005; Accepted 17 December 2005

SUMMARY. The transmission of pathogens from infected to susceptible hosts may occur through contaminated fomites and inanimate objects. This type of transmission depends on the ability of the pathogens to survive in the environment. In this report, we describe the survivability of two avian respiratory viruses, e.g., avian metapneumovirus and avian influenza virus on 12 different porous and nonporous surfaces. The viruses survived on some of the surfaces for up to 6 days postcontamination but not after 9 days. Both viruses survived longer on nonporous surfaces than on porous ones. One of the reasons for poor survival on porous surfaces could be inefficient elution of virus from these surfaces. These results should be helpful in determining how long the premises should be left vacant after an outbreak of these viruses has occurred in poultry houses.

RESUMEN. *Nota de Investigación*—Sobrevivencia de dos virus respiratorios aviares en superficies porosas y no porosas.

La transmisión de patógenos de huéspedes infectados a susceptibles puede ocurrir mediante fómites contaminados y objetos inanimados. Este tipo de transmisión depende de la capacidad de los patógenos de sobrevivir en el medio ambiente. En el presente reporte se describe la capacidad de supervivencia de dos virus respiratorios aviares (Metapneumovirus e influenza aviar) sobre 12 superficies porosas y no porosas. Los virus sobrevivieron sobre algunas de las superficies hasta por 6 días posteriores a la contaminación pero no después de 9 días. Ambos virus sobrevivieron por más tiempo en las superficies no porosas que en las porosas. Una de las razones que explica la baja supervivencia en las superficies porosas puede ser la liberación ineficiente del virus de este tipo de superficies. Estos resultados deben ser útiles para determinar el tiempo que deben dejarse vacías las instalaciones luego de que haya ocurrido un brote con estos virus en galpones avícolas.

Key words: survival, avian pneumovirus

Abbreviations: AIV = avian influenza virus; AMPV = avian metapneumovirus; ATCC = American Type Culture Collection; FCS = fetal calf serum; HBSS = Hanks' balanced salt solution; HPAIV = highly pathogenic AIV; IBV = infectious bronchitis virus; LPAIV = low pathogenic AIV; MDCK = Madin Darby canine kidney; NDV = Newcastle disease virus; PBS = phosphate buffer saline; $TCID_{50}$ = tissue culture infective dose

Transmission of pathogens from infected to susceptible hosts occurs by direct or indirect means. Indirect transmission may take place via water, food, air, or contaminated fomites. A critical factor in the indirect transmission of any pathogen is its ability to survive in the environment. Excretion of large amounts of respiratory viruses in excretions or secretions of infected birds may lead to environmental contamination. Virus survival for any length of time on environmental surfaces and fomites may help their transmission to susceptible populations.

Respiratory infections in poultry may lead to a severe drop in egg production as well as high mortality in poorly managed cases. These infections spread quickly and their etiology is complex, often involving more than one pathogen. In poultry, avian influenza virus (AIV), avian metapneumovirus (AMPV), Newcastle disease virus (NDV), and infectious bronchitis virus (IBV) are the most common viral causes of respiratory infections. These viruses may spread by infected aerosols or by contaminated surfaces. In the present study, survival of two avian respiratory viruses (AIV and AMPV) on porous and nonporous surfaces was determined.

Avian influenza, a highly contagious respiratory disease, is caused by type A AIV, which is an enveloped RNA virus belonging to the family *Orthomyxoviridae* (9). The extent of the disease caused by AIV may vary from mild respiratory illness to fatal pneumonia. The disease, which has been reported to affect all types of birds, including domestic, commercial, caged, and migratory birds, is characterized by sneezing, coughing, increased broodiness, reduced egg production and feed consumption, and sudden mortality in some cases. Severity of the disease may vary with virus type and with age, sex, and health status of the affected birds. This virus tends to lodge and reproduce in the digestive and respiratory tracts of birds and is shed from both sources during early stages of infection. The AIV can undergo genetic reassortment with human strains of influenza that coinfect pigs, which may lead to the emergence of new viral strains with a marked increase in virulence for humans (11). In addition, AIV can start out being low pathogenic but can mutate without warning to become highly pathogenic.

AMPV belongs to the *Metapneumovirus* genus of the family *Paramyxoviridae* and causes severe respiratory disease in turkeys and chickens of all ages (7). The disease, also known as turkey rhinotracheitis, is characterized by coughing, nasal discharge, swollen infraorbital sinuses, up to100% morbidity, and 3%–30% mortality (6). Of the four subtypes of AMPV, only subtype C is known to be present in the United States, while the other three subtypes (A, B, and D) are present in Europe.

Both of these diseases are airborne and have been reported to spread by infected aerosols. Contaminated fomites have also been reported to play a role in the spread of these viruses from one bird to the other and from one farm to the other. In addition, caretakers at poultry farms may inadvertently facilitate virus transmission if their

[A]Corresponding author.

Table 1. Survival of AIV on different fomites.

Fomite	Titer of the virus recovered ($TCID_{50}$/ml) after indicated time of storage[A,B,C]				
	0 hr	24 hr	48 hr	72 hr	6 days
Steel	8.7×10^3	1.9×10^3	0.8×10^2	0.9×10^2	<10
Latex	3.0×10^2	1.6×10^2	1.5×10^2	0.8×10^2	2.4×10^2
Tiles	4.4×10^3	9.7×10^2	1.5×10^2	1.0×10^2	<10
Wood	1.8×10^2	2.8×10^1	5.0×10^3	<10	<10
Gumboot	3.4×10^3	3.1×10^3	2.5×10^2	2.5×10^2	<10
Tire	7.8×10^3	1.6×10^2	5.2×10^2	1.0×10^2	<10
Egg tray	1.4×10^1	<10	<10	<10	<10
Egg shell	1.1×10^3	<10	1.5×10^2	1.4×10^1	<10
Cotton fabric	8.9×10^1	5.0×10^1	<10	<10	<10
Polyester fabric	2.8×10^1	<10	<10	<10	<10
Feather	1.5×10^4	2.9×10^3	3.0×10^2	2.3×10^2	2.8×10^1
Plastic	2.0×10^3	7.8×10^1	5.0×10^1	1.4×10^1	<10

[A]The amount of virus used to contaminate fomites was 10 μl each containing 6.3×10^4 $TCID_{50}$ (6.3×10^6 $TCID_{50}$/ml).

[B]The above values are the average of four experiments. The detection limit of the assay was 10 $TCID_{50}$. For the purpose of calculating averages, titers of <10 were considered as zero.

[C]No virus was detected on day 9 from any of the fomites tested.

hands, clothes, or other fomites are contaminated with secretions from infected birds. A number of studies have been carried out on the survival of many human viruses (polio, hepatitis A, enteric, adeno, and rhinoviruses) on various porous and nonporous surfaces, including apparel, glass, paper, aluminum foil, china, glazed tiles, latex, and counter tops, etc. (1,2,10,13). However, such studies are not available for avian respiratory viruses. It is imperative, therefore, to determine if avian respiratory viruses can persist long enough and in high enough numbers on fomites to pose an actual or potential risk to susceptible birds.

MATERIALS AND METHODS

Cells. Vero (ATCC CCL-81) and Madin Darby canine kidney (MDCK) cells (ATCC CRL-6253) were used to grow and titrate AMPV and AIV, respectively. The cells were grown in Eagle's minimum essential medium with Earle's salts (Media Tech, Herndon, VA) containing 150 IU/ml penicillin, 150 μg/per ml streptomycin, 50 μg/ml neomycin, 1 μg/per ml fungizone, 8% fetal calf serum (FCS), and Edamin S.

Viruses. Subtype C of AMPV (APV/MN-2a) adapted to grow in Vero cells at passage level 64 and AIV [Influenza A/Herring gull/Delaware 471/86 (H13N7)] at passage level four were used in this study. The AMPV grown in Vero cells was harvested when 80%–90% cells showed cytopathic changes. For growing AIV, MDCK monolayer was washed once with Hanks' balanced salt solution (HBSS). The maintenance medium consisted of MEM with antibiotics, 1.5 μg/per ml trypsin, and 0.3% bovine serum albumin. The virus was harvested when 90% cells showed cytopathic changes (usually 2–3 days). The virus stocks were stored in small aliquots at −70 C.

Experimental plan. Small pieces (1 cm^2) of 12 different materials (steel, wood, tile, tire, gumboot, feather, egg shell, egg tray, plastic, latex, cotton fabrics, and polyester fabric) were placed in 24-well tissue-culture plates. All pieces (except latex) were cleaned and sterilized by autoclaving before use. Each surface was contaminated with 10 μl of either AMPV or AIV. After air drying (approximately 30–40 min), these pieces were placed in 14-ml round-bottom tubes and stored in a drawer at room temperature. Twelve sets were made for each of the 12 materials. At regular intervals (0 hr, 24 hr, 48 hr, 72 hr, 6 days, and 9 days after contamination), one set was removed and virus from each material was eluted in 1 ml of 3% beef extract–0.05 M glycine (pH 8.5) (15) by intermittent vortexing for 40–60 sec. Immediately after elution, the virus present in the eluates was titrated. In another experiment, survivability of these two viruses (as infected cell-culture supernatant) was studied by storing them (contained in 4-ml tubes) at room temperature. Aliquots of both viruses were removed from these tubes at 0 hr, 24 hr, 48 hr, 72 hr, 6 days, 9 days, 12 days, 15 days, 18 days, and 21 days and titrated in their respective cells.

Virus titration. Virus titrations were done in Vero or MDCK cells grown in 96-well plates. The samples were diluted serially 10-fold, and 100 μl of each dilution was used to infect cells using four wells per dilution. For titration of AIV, MDCK cell monolayer was washed once with 100 μl of HBSS containing 1.5 μg per ml trypsin before infection. After infection, the plates were incubated at 37 C, examined for virus-specific cytopathic changes after 72 hr and expressed as tissue culture infective dose ($TCID_{50}$), and the titers calculated by the method of Reed and Muench (12). For AMPV, Vero cells were used and the end points were determined by staining the cells for immunofluorescence (6). Briefly, infected cells were fixed with chilled ethanol for 30 min at −20 C. After decanting ethanol, the plates were air dried in a laminar flow hood and washed once with phosphate buffer saline (PBS). A 1 : 150 dilution of turkey anti-APV hyperimmune serum was added (100 μl/well) followed by incubation for 1 hr at 37 C in a humid chamber. The cells were washed thrice with PBS, incubated with 1 : 150 dilution of fluorescein-labeled anti-turkey IgG conjugate for 45 min (KPL, Gaithersburg, MD) (100 μl/well), washed thrice with PBS, counter-stained with 0.01% Evans blue for 1 min, and observed under a fluorescent microscope.

RESULTS

Both AMPV and AIV survived for up to 72 hr on most of the surfaces tested (Tables 1 and 2). Survival of both viruses was similar on 9 of the 12 fomites tested, except on latex, wood, and egg tray. On latex, AIV survived for 6 days, while AMPV survived for 3 days. On wood, AIV and AMPV survived for 2 and 1 days, respectively. On egg tray, AIV survived for 1 day and AMPV for 6 days. In general, virus survival was less on porous surfaces than on nonporous ones. On cotton fabric, none of the viruses survived beyond 24 hr, while on polyester fabric, they were not detected even at 24 hr postcontamination. Both viruses in infected cell culture fluid survived for more than 15 days at room temperature (Table 3).

DISCUSSION

Severe economic losses to the poultry industry are caused by AMPV and AIV. Outbreaks of these viruses are contained either by rapid depopulation and/or quarantine of affected flocks. Rapid and stringent

Table 2. Survival of avian metapneumovirus on different fomites.

Fomite	Titer of the virus recovered ($TCID_{50}$/ml) after indicated time of storage[A,B,C]				
	0 hr	24 hr	48 hr	72 hr	6 days
Steel	4.0×10^2	6.3×10^2	6.3×10^2	0.6×10^2	<10
Latex	1.8×10^2	1.8×10^1	1.0×10^2	1.9×10^1	<10
Tiles	5.2×10^2	3.9×10^2	3.5×10^2	1.4×10^2	<10
Wood	3.3×10^2	1.8×10^1	<10	<10	<10
Gumboot	6.3×10^2	3.7×10^2	1.9×10^2	2.0×10^2	<10
Tire	1.0×10^3	6.6×10^1	1.1×10^3	5.9×10^2	<10
Egg tray	3.7×10^4	1.8×10^1	5.9×10^1	<10	3.3×10^1
Egg shell	3.3×10^2	9.7×10^1	3.3×10^1	1.0×10^2	<10
Cotton fabric	8.9×10^1	5.0×10^1	<10	<10	<10
Polyester fabric	3.3×10^2	<10	<10	<10	<10
Feather	1.1×10^3	5.2×10^1	$7.8. \times 10^1$	6.6×10^1	1.0×10^2
Plastic	2.2×10^3	1.9×10^1	<10	3.3×10^1	<10

[A]The amount of virus used to contaminate fomites was 10 µl each containing 3.1×10^4 $TCID_{50}$ (3.1×10^6 $TCID_{50}$/ml).
[B]Values are the average of four experiments. The detection limit was 10 $TCID_{50}$, which was considered as zero for the purposes of calculating averages.
[C] No virus was detected on day 9 from any of the fomites tested.

application of biosecurity measures and disinfection of contaminated premises and fomites can also help prevent viral spread. After the outbreak of a disease, the premises are left vacant for a period of time so that the virus can die out by natural processes. Unfortunately, no systematic studies are available on the survival of AMPV or AIV on different fomites. This study was conducted to fill that gap.

The results of our study indicated that both viruses survived for approximately 72 hr on most of the surfaces, although one or both viruses survived for as long as 6 days on latex, egg tray, and feather. Both viruses survived better on nonporous surfaces (steel, latex, ceramic tiles, gum boot, tire, and plastic) than on porous surfaces (cotton and polyester fabrics, wood, egg tray), which is in agreement with the results of Bean *et al.* (3), who found that human influenza virus types A and B survived for 24–48 hr on hard, nonporous surfaces (stainless steel and plastic) and for less than 8–12 hr on porous surfaces (cotton, paper, and tissues). Similarly, rotaviruses were recovered more readily from nonporous inanimate surfaces, such as stainless steel, plastic, and glass (14), and parainfluenza virus was recovered for 10 and 4 hr from nonabsorptive and absorptive surfaces, respectively (4). Human respiratory syncytial virus has also been shown to survive on various nonporous surfaces (including glass, aluminum foil, polyvinyl surfaces, stethoscope, and bed rails) for a considerable period of time, and these surfaces have been shown to act as vehicles for the spread of the virus (5).

Even at 0 hr (immediately after the application of virus), the overall virus recovery was less from porous surfaces than from nonporous surfaces, which may have been due to complete drying (desiccation) of the virus on these surfaces or to inefficient elution of viruses that may have entered the crevices. Earlier studies have also reported adverse effects of desiccation on virus survival (1,2,4). In certain cases, the virus was not detected at 24-hr postcontamination but was detectable at 3 or 6 days postcontamination. The reason for this was not immediately clear.

That survival rate of viruses varies with the type of inanimate surface has been reported previously (1,2,8,10,13). In the present study, egg trays made of cardboard had deleterious effect on the survival of AIV, as the virus could not be detected even at 24 hr postcontamination, whereas AMPV survived on egg tray for 6 days. Both viruses survived on feathers for 6 days, which may have implications in the transmission of virus from infected carriers or reservoir birds to healthy birds.

Mahl and Sadler (10) studied the survival of several different viruses (adeno, polio, vaccinia, coxsackie, and herpes simplex viruses) on nonporous inanimate surfaces (glass, ceramic tiles, steel, asbestos sheet) and found no difference in the rate of survival of different viruses. This is in agreement with the results of this study, in which not much difference was noticed in the survival of AMPV and AIV on nonporous surfaces. Abad *et al.* (1) studied survival of human enteric viruses on nonporous (aluminum, china, glazed tiles, latex) and porous (paper, cotton) environmental surfaces and found that these viruses survived for extended periods of time and that the stability was generally influenced by relative humidity, temperature, and type of surface contaminated. Survival of human astroviruses was reported to be better at 4 C than at 20 C on porous surfaces (2).

Both viruses, when suspended in a liquid cell-culture medium, survived for more than 15 days at room temperature. However, none of them survived for more than 6 days when dried on surfaces. One of the reasons for poor survival of viruses on different surfaces could be loss of virus during elution. The presence of protein material in the suspending medium (1% FCS in the case of AMPV) and 0.3% bovine serum albumin (in the case of AIV) may have had protective effects on virus survival. It should be noted that a low pathogenic strain of AIV (LPAIV) was used in this study. Whether these results will translate to the survival of highly pathogenic AIV (HPAIV) is

Table 3. Survival of AMPV and AIV in infected cell culture fluid at room temperature.[A]

Time	Titer of virus ($TCID_{50}$/ml)	
	AMPV	AIV
0 hr	3.0×10^7	3.0×10^7
24 hr	2.2×10^7	2.1×10^7
48 hr	1.5×10^7	7.2×10^6
72 hr	4.6×10^6	4.6×10^6
6 days	ND	1.5×10^5
9 days	6.8×10^3	3.0×10^4
12 days	4.5×10^2	5.6×10^2
15 days	1.5×10^2	3.1×10^2
18 days	<10	<10
21 days	<10	<10

[A]AMPV = avian metapneumovirus; AIV = avian influenza virus; ND = not done.

not known. Further studies are needed to compare the survival of LPAIV and HPAIV.

The persistence of viruses on inanimate surfaces for long periods of time constitutes an important epidemiologic factor in the spread of viral infections. The results of this study indicate that both AMPV and AIV would die off on most environmental surfaces after 6 days and that premises contaminated with these two viruses would be safe for the rehousing of birds after 6 days of being kept vacant.

REFERENCES

1. Abad, F. X., R. M. Pinto, and B. Albert. Survival of enteric viruses on environmental fomites. Food Environ. Microbiol. 60:3704–3710. 1994.

2. Abad, F. X., C. Villena, S. Guix, C. Santiago, R. M. Pinto, and B. Albert. Potential role of fomites in the vehicular transmission of human astroviruses. Appl. Environ. Microbiol. 67:3904–3907. 2001.

3. Bean, B., B. M. Moore, B. Sterner, L. R. Peterson, D. N. Gerding, and H. H. Balfour. Survival of influenza viruses on environmental surfaces. J. Infect. Dis. 146:47–51. 1982.

4. Brady, M. T., J. Evans, and J. Cuartas. Survival and disinfection of parainfluenza viruses on environmental surfaces. Am. J. Infect. Control 18: 18–23. 1990.

5. Hall, C. B., R. G. Douglas, and J. M. Geiman. Possible transmission by fomites of RSV (pneumovirus). J. Infect. Dis. 141:98–102. 1980.

6. Jirjis, F. F., S. L. Noll, D. A. Halvorson, K. V. Nagaraja, and D. P. Shaw. Pathogenesis of avian pneumovirus infection in turkeys. Vet. Pathol. 39:300–310. 2002.

7. Jones, R. C. Avian pneumovirus infection: question still unanswered. Avian Pathol. 25:639–648. 1996.

8. Keswick, B. H., L. K. Pickering, H. L. DuPont, and W. E. Woodward. Survival and detection of rotaviruses on environmental surfaces in day care centers. Appl. Environ. Microbiol. 46:813–816. 1983.

9. Lamb, R. A., and R. M. Krug. *Orthomyxoviridae*: the virus and their replication. In: Fields virology, 4th ed. D. M. Knipe and P. M. Howley, eds. Lippincott, Williams, and Wilkins, Philadelphia. pp. 1487–1532. 2001.

10. Mahl, M. C., and C. Sadler. Virus survival on inanimate surfaces. Can. J. Microbiol. 21:819–823. 1975.

11. Reed, K. D., J. K. Meece, J. S. Henkel, and S. K. Shukla. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. Clin. Med. Res. 1:5–12. 2003.

12. Reed, L. J., and H. Muench. A simple method of estimating fifty percent end points. Am. J. Hyg. 27:493–497. 1938.

13. Sattar, S. A., Y. G. Karim, V. S. Springthorpe, and C. M. Johnson-Lussenburg. Survival of human rhinovirus type 14 on to non porous inanimate surfaces: effect of relative humidity and suspending medium. Can. J. Microbiol. 33:802–806. 1987.

14. Sattar, S. A., N. Lloyd-Evans, V. S. Springthorpe, and R. C. Nair. Institutional outbreaks of rotavirus diarrhea: potential role of fomites and environmental surfaces as vehicles for virus transmission. J. Hyg. 96: 277–289. 1986.

15. Taku, A., B. R. Gulati, P. B. Alwood, K. Palazzi, C. W. Hedberg, and S. M. Goyal. Concentration and detection of caliciviruses from food contact surfaces. J. Food Prot. 65:999–1004. 2002.

ACKNOWLEDGMENTS

This work was supported in part by a grant from U.S. Poultry and Egg Association. The authors wish to thank Laura Bruner for excellent technical help.

15

Ann. Occup. Hyg., Vol. 53, No. 8, pp. 815–827, 2009

doi:10.1093/annhyg/mep070

Evaluation of Five Decontamination Methods for Filtering Facepiece Respirators

DENNIS J. VISCUSI[1], MICHAEL S. BERGMAN[2], BENJAMIN C. EIMER[2] and RONALD E. SHAFFER[1]*

[1]National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory, 626 Cochrans Mill Road, Pittsburgh, PA 15236, USA; [2]EG&G Technical Services, Inc., 626 Cochrans Mill Road, PO Box 18070, Pittsburgh, PA 15236, USA

Received 27 May 2009; in final form 2 September 2009; published online 4 October 2009

Concerns have been raised regarding the availability of National Institute for Occupational Safety and Health (NIOSH)-certified N95 filtering facepiece respirators (FFRs) during an influenza pandemic. One possible strategy to mitigate a respirator shortage is to reuse FFRs following a biological decontamination process to render infectious material on the FFR inactive. However, little data exist on the effects of decontamination methods on respirator integrity and performance. This study evaluated five decontamination methods [ultraviolet germicidal irradiation (UVGI), ethylene oxide, vaporized hydrogen peroxide (VHP), microwave oven irradiation, and bleach] using nine models of NIOSH-certified respirators (three models each of N95 FFRs, surgical N95 respirators, and P100 FFRs) to determine which methods should be considered for future research studies. Following treatment by each decontamination method, the FFRs were evaluated for changes in physical appearance, odor, and laboratory performance (filter aerosol penetration and filter airflow resistance). Additional experiments (dry heat laboratory oven exposures, off-gassing, and FFR hydrophobicity) were subsequently conducted to better understand material properties and possible health risks to the respirator user following decontamination. However, this study did not assess the efficiency of the decontamination methods to inactivate viable microorganisms. Microwave oven irradiation melted samples from two FFR models. The remainder of the FFR samples that had been decontaminated had expected levels of filter aerosol penetration and filter airflow resistance. The scent of bleach remained noticeable following overnight drying and low levels of chlorine gas were found to off-gas from bleach-decontaminated FFRs when rehydrated with deionized water. UVGI, ethylene oxide (EtO), and VHP were found to be the most promising decontamination methods; however, concerns remain about the throughput capabilities for EtO and VHP. Further research is needed before any specific decontamination methods can be recommended.

Keywords: decontamination; filtering facepiece respirator; healthcare workers; N95 respirator; pandemic influenza; respirator reuse

INTRODUCTION

During an influenza pandemic, a shortage of filtering facepiece respirators (FFRs) may occur if manufacturing production is unable to meet the demand or if FFR stockpiles become depleted. According to a 2006 report from the National Academies' Institute of Medicine, over 90 million N95 FFRs will be needed to protect workers in the healthcare sector during a 42-day influenza pandemic outbreak (Bailar *et al.*, 2006). Guidance provided by the Centers for Disease Control and Prevention (CDC) states that once an FFR is worn in the presence of an infected patient, it should be considered potentially contaminated and not be reused by the same person or a coworker (CDC, 2007). A contaminated FFR could potentially serve as a fomite and lead to self-inoculation or spread of the organism to patients and other healthcare workers. Guidance from the Occupational Safety and Health Administration (OSHA) considers

*Author to whom correspondence should be addressed.
Tel: +412-386-4001; fax: +412-386-6864;
e-mail: rshaffer@cdc.gov

FFRs to be one-time-use devices when used in the presence of infected patients and advises employers and employees to only reuse FFRs during a pandemic if FFRs are in short supply and the device has not been obviously soiled or damaged (e.g. creased or torn), and it retains its ability to function properly (OSHA, 2007).

One possible strategy to reduce the impact of a respirator shortage would be to apply a biological decontamination process (e.g. such as those used in hospital settings for infection control) to inactivate the influenza virus that may be on the FFR. If the treatment did not deteriorate the FFR or leave potentially toxic residues on the FFR, then it could be available for subsequent reuse by the original user. Until recently, no data were published on the effects of decontamination on FFR performance. Viscusi *et al.* (2007) measured the laboratory filtration performance of one N95 model and one P100 model FFR that were exposed to 20 different biological decontamination treatments. They found that filtration performance after one-time decontamination treatments using bleach, ethylene oxide (EtO), microwave oven irradiation, ultraviolet germicidal irradiation (UVGI), and hydrogen peroxide (vaporized and liquid forms) was observed to have filter aerosol penetration values that remained less than the National Institute for Occupational Safety and Health (NIOSH) certification criteria. It was also found that decontamination using an autoclave, 160°C dry heat, 70% isopropyl alcohol, and soap and water (20-min soak) caused significant degradation to filtration efficiency.

Expanding on that research, the goal of this study was to further evaluate five of the decontamination methods examined in the previous study using a more diverse set of nine models of NIOSH-certified FFRs to determine which decontamination methods should be considered for future research studies. The biological decontamination methods used in this study include: (i) UVGI, (ii) EtO, (iii) vaporized hydrogen peroxide (VHP), (iv) microwave oven irradiation, and (v) 0.6% aqueous solution of sodium hypochlorite (hereafter referred to as 'bleach'). Following treatment by each decontamination method, FFRs were evaluated for changes in physical appearance/odor (observational analysis) and laboratory performance (filter aerosol penetration and filter airflow resistance). Additional experiments were then conducted to examine the material properties of the FFRs in an attempt to rationalize some of the findings in the laboratory performance evaluation and observational analysis. The advantages and disadvantages of the various decontamination methods (including throughput capacity and possible health risk to the user) were also assessed.

METHODS

Respirator selection

Nine respirator models were used in this study, of which six models [three N95 FFR models (N95-A, N95-B, and N95-C) and three surgical N95 respirator models (SN95-D, SN95-E, and SN95-F)] constitute a random sampling from those N95 FFR models present in the US Strategic National Stockpile (SNS). Healthcare workers often use surgical N95 respirators, which are NIOSH-approved N95 FFRs that also have been cleared by the US Food and Drug Administration (FDA) for marketing as medical devices. Surgical N95 respirators are designed to be fluid resistant to splash and spatter of blood and other infectious materials and thus may respond differently to the decontamination processes than N95 FFRs. Three models of P100 FFRs (P100-G, P100-H, and P100-I) were randomly selected from models commercially available at the time of the study and included because they were considered likely to be more resistant to filtration efficiency degradation and thus offer a more rigorous basis of comparison. All respirators were purchased and verified to be from the same respective manufacturing lot at the beginning of the study to minimize any lot-to-lot variation as well as to ensure consistency during FFR filtration performance testing. FFRs used in this study consisted of electrostatically charged polypropylene filters (electret filter media).

Decontamination methods

The experimental conditions and parameters for the five decontamination methods and the 'as-received' (control) method are summarized in Table 1. All laboratory experiments were conducted under standard laboratory conditions (21 ± 2°C and relative humidity of 50 ± 10%) on triplicate sets of FFRs.

Respirator test methods

Observational analysis. All post-decontamination and control FFR samples were inspected and scrutinized carefully for any visible sign of degradation or changes that could be noted in texture or 'feel' of the respirator (softness, pliability, coarseness, roughness, etc.). All samples were sniffed for any discernible odor or smell.

Filter aerosol penetration. A Model 8130 Automated Filter Tester (AFT) (TSI, Inc., St Paul, MN, USA) was used to measure initial filter aerosol penetration for all post-decontamination and control FFR samples. All tests were conducted at room temperature with a continuous airflow of 85 ± 4 l min^{-1} in accordance with NIOSH certification test procedures (NIOSH, 2007) for challenging N-series filters, with two exceptions:

Table 1. FFR treatments

Treatment	Experimental conditions and parameters
As-received	No decontamination treatment was performed (control group).
UVGI	FFRs placed on the working surface of a Sterilgard III laminar flow cabinet (The Baker Company, Sanford, ME, USA) fitted with a 40-W UV-C light (average UV intensity experimentally measured to range from 0.18 to 0.20 mW cm^{-2}). Fifteen-minute exposure to each side (outer and inner), 176–181 mJ cm^{-2} exposure to each side of FFR.
EtO	Steri-Vac 5XL sterilizer (3M, St Paul, MN, USA). Single warm cycle (55°C and 725 mg l^{-1} 100% EtO gas). FFRs and a chemical indicator placed in an individual standard poly/paper pouch. EtO exposure for 1 h followed by 4 h of aeration. FFRs were shipped to and from a commercial facility specializing in low-temperature sterilization methods and were tested within 72 h of receipt.
VHP	STERRAD® 100S H_2O_2 Gas Plasma Sterilizer (Advanced Sterilization Products, Irvine, CA, USA), single 55-min standard cycle. FFRs and a chemical indicator placed in an individual Mylar/Tyvek™ self-seal pouch. FFRs were shipped to and from a commercial facility specializing in low-temperature sterilization methods and were tested within 72 h of receipt.
Microwave oven irradiation	Commercially available 2450 MHz, Sharp Model R-305KS (Sharp Electronics, Mahwah, NJ, USA) microwave oven with revolving glass carousel, 1100 W (manufacturer rated); 750 W ft^{-3} experimentally measured; 2-min total exposure (1 min each side of FFR). A paper towel was placed on the revolving glass plate for insulation to protect the FFRs from melting onto the glass plate. Using a power setting of 10 (maximum power), FFRs were placed faceseal-side down, initially, to reduce the risk of faceseal component materials melting onto the paper towel due to elevated temperatures reached by the glass plate when microwaved for 2 min. Ambient cooling of the glass plate was maintained between trials.
Bleach	Thirty minutes submersion in 0.6% (one part bleach to nine parts of deionized water) aqueous solution of sodium hypochlorite (original concentration = 6% available as Cl_2). Manufacturing specification: 6.00 ± 0.06% (w/w) available chlorine; Cat no. 7495.7-1, CAS no. 7732-18-5 (Ricca Chemical Company, Pequannock, NJ, USA). After treatment, FFRs were hung on a laboratory pegboard and allowed to air-dry overnight with assistance from a freestanding fan.

all filters were tested for filter aerosol penetration without any relative humidity pretreatment or NaCl aerosol loading. Collecting the data in this manner allows consistency with previous work (Viscusi *et al.*, 2007). Filter aerosol penetration levels were determined using a Plexiglas test box as previously used and described by Viscusi *et al.* (2007) or an appropriately sized test fixture supplied by the respective FFR manufacturer, as was the case for models N95-C, SN95-D, and P100-H.

Filter airflow resistance. For all control and post-decontamination FFR samples, a TSI Model 8130 AFT was also used to measure initial filter airflow resistance in millimeters of water column height pressure (mmH_2O). It must be clarified that the NIOSH certification test for inhalation airflow resistance for FFRs is not performed using the TSI 8130 AFT but is executed in accordance with NIOSH Standard Test Procedure RCT-APR-STP-0007, which specifies the use of a different calibrated apparatus incorporating a vacuum source and manometer (NIOSH, 2005). For this evaluation, it was convenient to report the filter airflow resistance obtained from the TSI Model 8130 AFT because filter aerosol penetration and filter airflow resistance results are generated simultaneously and the intent is to determine changes in filter airflow resistance. This methodology was used previously by the National Personal Protective Technology Laboratory (NPPTL) (Viscusi *et al.*, 2009).

Experimental design

The primary experimental design called for 162 FFRs (nine different FFR models × six test conditions × three samples per test condition) to be tested by observational analysis, for filter airflow resistance and for filter aerosol penetration. The 162 FFRs in the design included 135 post-decontamination FFRs and 27 control FFRs (no decontamination).

Statistical analysis

For statistical analysis, the six test conditions (see Table 1) comprised one control group and five decontamination treatments. A one-way analysis of variance (ANOVA) test was performed for each of the nine FFR models for filter aerosol penetration and filter airflow resistance (for 18 total tests). Thus, each model was treated independently due to its inherent uniqueness (difference in number of filter layers, hydrophobicity, materials of construction, etc.). Results were considered statistically significant if the *P*-value was <0.05. Statistical analyses were performed using Microsoft Excel (Microsoft Corporation, part of Microsoft Office Professional Edition 2003). No statistical analysis of the subjective observational analysis data was done.

Additional testing

Additional secondary experiments were subsequently conducted on the FFRs to understand better their material properties. This information can be

used to further optimize the decontamination methods and/or explain some of the findings from the observational analyses or laboratory performance evaluation experiments.

Dry oven experiments. To investigate the effects on filter aerosol penetration at various dry heat temperatures and to determine if these effects were similar to those of FFRs that underwent microwave oven irradiation, new FFRs were placed in a Fisher Scientific Isotemp 500 Series laboratory oven (Fisher Scientific, Pittsburgh, PA, USA) for 1 h at temperatures ranging from 80 to 120°C. Filter aerosol penetration was measured after samples cooled to ambient temperature.

Hydrophobicity testing. A qualitative assessment of water affinity for each FFR filter media layer was performed to determine the hydrophobic/hydrophilic nature of the various layers for the nine different FFR models. For this experiment, it was hypothesized that the number of layers and the nature of the outer layer (surface of the FFR most distant from the wearer) and the inner layer (surface of the FFR closest to the breathing zone of the wearer) would provide insight into any model-specific effects associated with liquid chemical-based decontamination methods. A circular swatch (~5 cm in diameter) was cut from additional, new as-received samples of each FFR model. Following layer separations, a 100 µl aliquot of deionized water was pipetted onto the surface of each side of each layer (front and back). Two FFR models incorporated layers of plastic webbing, presumably to support shape; these layers were not tested because they are not filtering layers. A layer was noted as hydrophilic when it absorbed the water droplet. A layer was noted as hydrophobic when the water droplet beaded on the layer's surface.

Chlorine off-gassing experiments. To quantify observations of discernable odor from FFRs following bleach decontamination, a series of off-gassing experiments was conducted using a Model 4340 Chlorine Gas Analyzer (Interscan Corp., Chatsworth, CA, USA). Chlorine off-gassing was measured from FFRs after bleach treatment as described in Table 1. A subset of four FFR models was chosen for testing based on the various combinations of water repellency discerned from the hydrophobicity experiments described previously: N95-A (outer hydrophobic layer/inner hydrophilic layer), N95-B (outer and inner hydrophilic layers), SN95-E (outer and inner hydrophobic layers), and SN95-F (outer hydrophobic layer/inner hydrophilic layer). Bleach off-gassing tests were conducted after a bleach decontamination treatment by immediately placing the FFR face up inside a plastic bag which was open to room air on one side. This setup was designed to minimize air fluctuation within the bag. The detector's sample tube inlet was positioned under the inside of the FFR and all tests were conducted at a flow rate of 0.5 l min^{-1}. FFRs were tested under four conditions: (i) immediately after a 30-min submersion in bleach, (ii) dried overnight after a 30-min submersion in bleach, (iii) a 30-min submersion in bleach, immediately rinsed (under a flowing stream of deionized water for ~1 min) and then dried overnight, and (iv) a 30-min submersion in bleach, then dried overnight followed by rinsing with deionized water.

RESULTS

Observational analysis

Changes to the FFR materials of construction caused by each decontamination treatment are summarized in Table 2. Respirator component materials melted on all six FFRs from two models (SN95-E and P100-I) during microwave oven irradiation. EtO and UVGI were the only methods that did not cause any observable physical changes to the FFRs.

Filter aerosol penetration

For each 'FFR model/decontamination treatment' combination, the average initial filter aerosol penetrations are summarized in Table 3. Not all the 135

Table 2. Discernible observations caused by FFR decontamination treatments

Decontamination treatment	Discernible observations
Bleach	Metallic nosebands were slightly tarnished and visibly not as shiny when compared with their as-received counterparts. SN95-E inner nose comfort cushion was discolored. Following air-drying overnight (16 h), all FFRs were dry to the touch and all still had a characteristic smell of bleach.
UVGI	No visible changes were observed for all samples.
EtO	No visible changes were observed for all samples.
VHP	Metallic nosebands were slightly tarnished and visibly not as shiny when compared with their as-received counterparts.
Microwave oven irradiation	All three physical samples of two different models (SN95-E and P100-I) melted partially. SN95-E filtration material melted in areas adjacent to the metallic nosebands. P100-I melted in various locations of the inner foam faceseal comfort lining. Both models were considered unwearable following treatment and subsequently were not evaluated for filter aerosol penetration or filter airflow resistance.

Table 3. Summary data of filter aerosol penetration and filter airflow resistance for FFRs following various decontamination treatments[a]

FFR model	Treatment	Average initial sodium chloride penetration (%P)	Standard deviation of penetration	Average initial resistance (mmH_2O)	Standard deviation of resistance
N95 FFRs					
N95-A	As-received	0.121	0.08	7.6	0.83
	UVGI	0.072	0.04	7.6	0.29
	EtO	0.101	0.06	7.3	0.10
	VHP	0.071	0.04	7.8	0.21
	Microwave	0.105	0.07	7.9	0.06
	Bleach	0.262	0.18	8.1	0.47
N95-B	As-received	1.00	0.64	9.4	0.68
	UVGI	0.76	0.43	10.3	0.12
	EtO	0.667	0.39	9.7	0.10
	VHP	0.659	0.34	9.6	0.50
	Microwave	1.06	0.74	9.0	0.40
	Bleach	0.629	0.34	9.8	0.30
N95-C	As-received	1.48	0.94	6.9	1.61
	UVGI	1.77	0.96	7.1	1.68
	EtO	1.82	1.12	6.9	1.47
	VHP	1.47	0.91	6.5	2.37
	Microwave	1.46	0.82	6.2	0.61
	Bleach	1.13	0.79	8.0	3.06
Surgical N95 respirators					
SN95-D	As-received	1.57	0.83	8.4	0.50
	UVGI	1.86	0.97	9.2	0.44
	EtO	0.90	0.49	8.1	0.32
	VHP	0.71	0.50	8.6	1.04
	Microwave	0.711	0.44	8.7	0.64
	Bleach	0.561	0.38	9.6	0.29
SN95-E	As-received	0.335	0.19	6.1	0.15
	UVGI	0.371	0.21	7.1	0.61
	EtO	0.498	0.32	6.7	0.40
	VHP	0.542	0.32	7.1	1.28
	Microwave	Melted	Melted	Melted	Melted
	Bleach	0.233	0.12	6.6	0.56
SN95-F	As-received	0.716	0.37	6.7	0.17
	UVGI	0.720	0.37	6.6	0.26
	EtO	0.687	0.35	6.3	0.25
	VHP	0.727	0.37	6.5	0.29
	Microwave	0.652	0.33	5.4	0.72
	Bleach	0.692	0.35	5.9	0.46
P100 FFRs					
P100-G	As-received	0.009	0.01	13.1	0.79
	UVGI	0.005	0.00	13.1	1.21
	EtO	0.003	0.00	12.8	0.57
	VHP	0.006	0.01	13.4	1.23
	Microwave	0.002	0.00	13.1	0.62
	Bleach	0.006	0.00	13.6	0.92

Table 3. *Continued*

FFR model	Treatment	Average initial sodium chloride penetration (%*P*)	Standard deviation of penetration	Average initial resistance (mmH_2O)	Standard deviation of resistance
P100-H	As-received	0.007	0.01	15.8	0.87
	UVGI	0.007	0.01	16.0	1.82
	EtO	0.003	0.00	15.2	0.64
	VHP	0.010	0.01	15.0	1.27
	Microwave	0.000	0.00	15.8	0.30
	Bleach	0.010	0.01	15.1	0.81
P100-I	As-received	0.008	0.00	16.4	0.85
	UVGI	0.012	0.01	16.5	0.10
	EtO	0.006	0.00	15.9	0.76
	VHP	0.007	0.00	16.2	0.93
	Microwave	Melted	Melted	Melted	Melted
	Bleach	0.004	0.00	17.0	0.98

[a]Filter aerosol penetration and filter airflow resistance testing performed using a TSI 8130 AFT ($n = 3$).

post-decontamination FFR samples in the experimental design were tested for filter aerosol penetration as planned; the six FFRs that exhibited melting after microwave irradiation could not undergo laboratory performance evaluation. The remaining 129 post-decontamination FFRs were tested and demonstrated expected levels of filtration efficiency performance. These results indicate that for all tested FFR samples that did not melt, FFR filtration performance was not adversely affected by the decontamination process. Most of the ANOVA tests for initial filter aerosol penetration were non-significant ($P > 0.05$), (Table 4). In terms of average initial filter aerosol penetration, only P100-I yielded a significant difference by treatment ($P = 0.0438$), which appeared to be primarily driven by the increased filter aerosol penetration levels for the UVGI treatment (0.012 versus 0.008% for the control). Although statistically significant, this difference in levels of filter aerosol penetration is practically insignificant because the penetration levels still are far less than expected levels for this class of FFRs ($<0.03\%$).

Table 4. One-way ANOVA test results for each FFR model

FFR model	Penetration (*P*-value)	Resistance (*P*-value)
N95 FFRs		
N95-A[a]	0.0635	0.1233
N95-B[a]	0.5761	**0.0035**[b]
N95-C[a]	0.8067	0.7572
Surgical N95 FFRs		
SN95-D[a]	0.7688	**0.0170**[b]
SN95-E[c]	0.2189	0.2448
SN95-F[a]	0.9409	**0.0014**[b]
P100 FFRs		
P100-G[a]	0.2185	0.7446
P100-H[a]	0.3046	0.4970
P100-I[c]	**0.0438**[a]	0.2580

[a]For each FFR model with the exceptions of SN95-E and P100-I, one-way ANOVAs compare observed filter aerosol penetration or filter airflow resistance values for six test treatments [five different decontamination treatments and one as-received (control) group].
[b]Values in bold font are *P*-value <0.05. Probability (*P*-value) of observing the given *F*-statistic or larger by chance.
[c]The one-way ANOVAs compare observed filter aerosol penetration and filter airflow resistance values for five test treatments [four different decontamination treatments and one as-received (control) group]. Respirator component materials melted for these FFRs during microwave oven irradiation and subsequently samples were not evaluated for initial filter aerosol penetration and initial filter airflow resistance.

Filter airflow resistance

For each 'FFR model/decontamination treatment' combination, the average initial filter airflow resistances are summarized in Table 3. The six FFRs in which melting occurred could not be tested for filter airflow resistance. For the remaining 129 post-decontamination samples tested, average initial filter airflow resistance measurements were $\leq$17.0 mm H_2O. Previous studies using the same test method on 21 models of NIOSH-approved N95 FFRs observed filter airflow resistance levels between 7 and 30 mmH_2O (Viscusi *et al.*, 2009). For filter airflow resistance, three of the nine ANOVA tests, including N95-B ($P = 0.0035$), SN95-D ($P = 0.0170$), and SN95-F ($P = 0.0014$), showed significantly different means (see Table 4). Although statistically significant, the levels of differences in filter airflow resistance between treatments are not practically meaningful as small changes in filter airflow resistance are unlikely to be noticed by the user (Vojtko *et al.*, 2008).

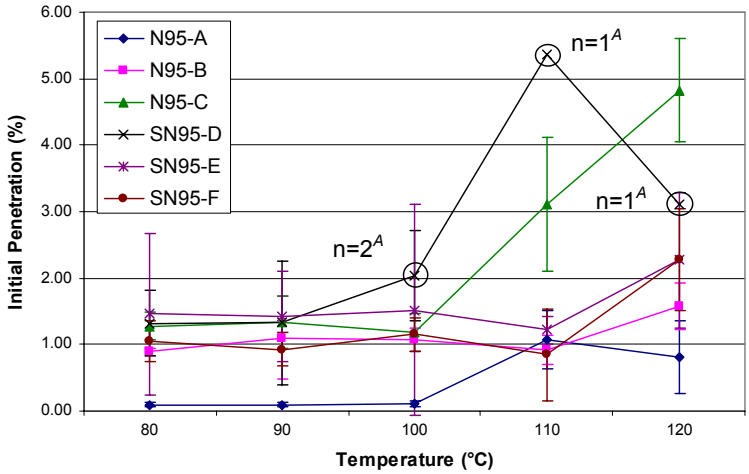


Fig. 1. N95 FFR average initial sodium chloride filter aerosol penetration versus temperature. Each data point represents the average initial penetration of three samples ($n = 3$), unless otherwise noted. 'A' indicates five SN95-D FFRs melted, one at 100°C, two at 110°C, and two at 120°C and could not be penetration or airflow resistance tested.

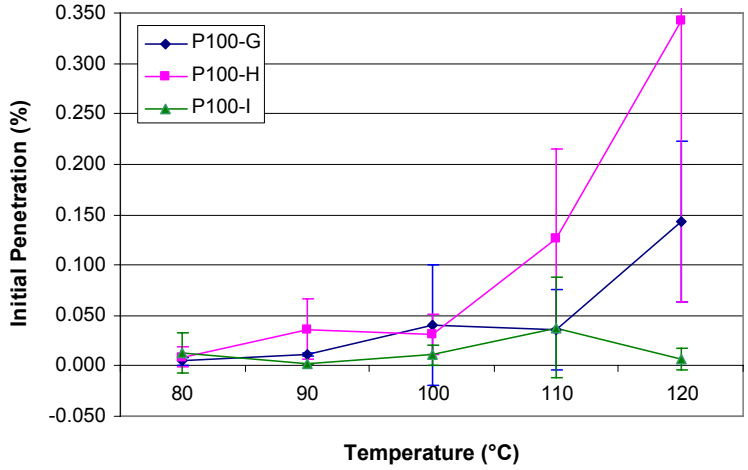


Fig. 2. P100 FFR average initial sodium chloride filter aerosol penetration versus temperature. Each data point represents the average initial penetration of three samples ($n = 3$).

Dry oven experiments

The degree to which temperature affects initial filter aerosol penetration and component melting was observed to be model specific (Figs 1 and 2). The average initial penetration ($n = 3$) for each N95 model is shown in Fig. 1. Only three tested N95 FFR samples had filter aerosol penetrations >5% (therefore failed to maintain their expected filtration efficiency level of ≥95%). These three failing samples were one SN95-D (5.37% at 110°C) and two N95-C (5.18 and 5.37%, both at 120°C). Five of the SN95-D samples could not be analyzed following treatments of 100°C (one sample), 110°C (two samples), and 120°C (two samples) because their inner moisture barrier melted into the filtration media rendering those samples unsuitable for testing. For the three P100 FFR models, average initial filter aerosol penetration values for P100-G and P100-H exceeded 0.03% beginning at 100°C for P100-G and beginning at 90°C for P100-H (Fig. 2). P100-I averaged an initial filter aerosol penetration value <0.03% for all evaluated temperature increments with the exception of one 110°C temperature experiment. This unexpectedly high average result was due to a single test ($\%P = 0.096$).

Hydrophobicity testing

All nine FFR models demonstrated differences in their number of media layers and the hydrophobicity of their filter media (Table 5). Common to all three models of surgical N95 respirator was the fact that their outer layer was hydrophobic. This is not surprising since surgical N95 respirators cleared by the US FDA undergo fluid resistance testing

Table 5. FFR media layer hydrophobicity

FFR model	Total layers	Outer layer	Middle layers	Inner layer
N95 FFRs				
N95-A	4	—	Second, —	+
			Third, —	
N95-B	2	+/—	No middle layer	—/+
N95-C	5	Plastic webbing	Second, —	—
			Third, —	
			Fourth, plastic webbing	
Surgical N95 respirators				
SN95-D	5	—	Second, —	—
			Third, —	
			Fourth, —	
SN95-E	5	—	Second, —	—
			Third, —	
			Fourth, —	
SN95-F	4	—	Second, —	+
			Third, —	
P100 FFRs				
P100-G	5	—	Second, —	+
			Third, —	
			Fourth, —	
P100-H	12	Plastic webbing	Second, —	—
			Third, —	
			Fourth, —	
			Fifth, —	
			Sixth, plastic webbing	
			Seventh, —	
			Eighth, —	
			Ninth, —	
			10th, —	
			11th, plastic webbing	
P100-I	6	+	Second, —	+
			Third, —	
			Fourth, —	
			Fifth, —	

—, both sides of layer are hydrophobic; +, both sides of layer are hydrophilic; +/—, outer side of layer is hydrophilic and inner side of layer is hydrophobic; —/+, outer side of layer is hydrophobic and inner side of layer is hydrophilic; plastic webbing, not tested.

and are used as barriers against disease transmission by airborne respiratory fluids, including blood, and other small infectious droplets (Bailar *et al.*, 2006). The N95 FFRs and P100 FFRs varied by having either hydrophobic or hydrophilic outer and inner layers. All middle layers, with the exception of those that were plastic webbing, were hydrophobic on both sides.

Chlorine off-gassing experiments

Initial concentrations of chlorine gas (2–12 p.p.m.) were measured on FFRs wet with bleach immediately following submersion for 30 min (Fig. 3). FFRs that were treated using bleach and allowed to air-dry overnight (as described in Table 1) had initial concentrations of ~0.05 p.p.m. followed by no detectable off-gassing (0 p.p.m.) after the initial data point. FFRs which were submerged in bleach, immediately rinsed (entirely under a stream of deionized water for ~1 min) and then allowed to air-dry overnight had concentrations similar to FFRs which were not rinsed, indicating that the water rinse had no effect. When FFRs were rehydrated by rinsing with deionized water following overnight air-drying, low-level chlorine off-gassing concentrations were measured at ~0.1 p.p.m. (Fig. 4).

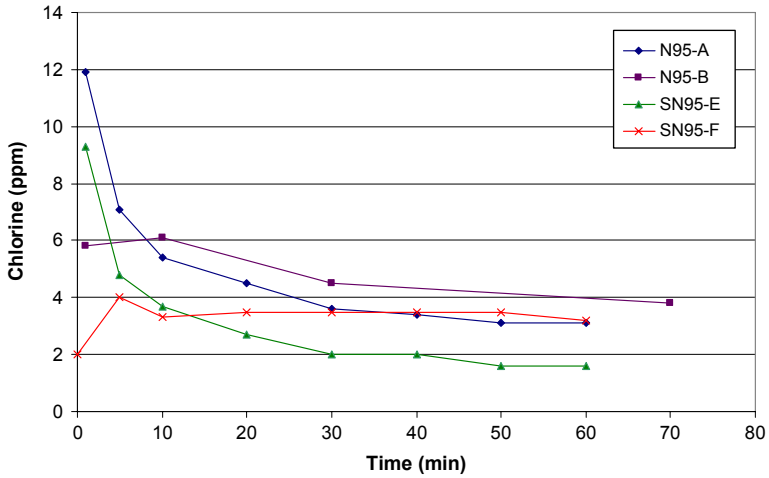


Fig. 3. Chlorine off-gassing of FFRs after 30 min submersion in bleach (tested wet).

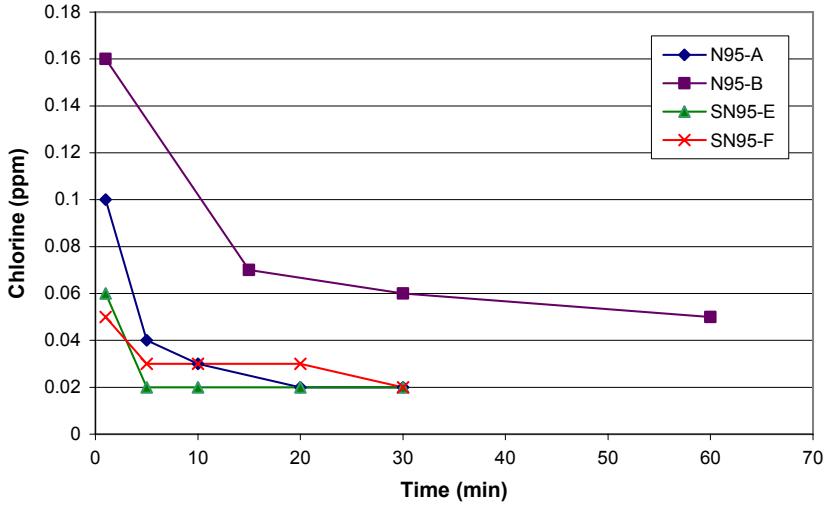


Fig. 4. Chlorine off-gassing of FFRs submerged in bleach (dried overnight then rinsed with deionized water and tested wet).

DISCUSSION

The goal of this study was to evaluate five decontamination methods using nine FFR models from three FFR types (three N95 models, three surgical N95 respirator models, and three P100 models) to determine which methods should be considered for future research studies. The five decontamination methods were selected based on previous research from the NPPTL laboratory (Viscusi *et al.*, 2007). Criteria for assessing methods of decontaminating disposable N95 FFRs have been suggested by the National Academies (Bailar *et al.*, 2006); the decontamination method must remove the viral threat, be harmless to the user, and not compromise the integrity of the various elements of the respirator. This manuscript utilizes and expands upon the second and third criteria. For purposes of discussion, a successful FFR decontamination method is considered to be a physical or chemical treatment which does not degrade laboratory performance (filter aerosol penetration and filter airflow resistance) beyond expected performance levels, is able to be performed on enough FFRs in a short period of time to be practical in the event of a pandemic-induced shortage, and should not pose any additional health risk to the user. In this study, assessment of potential health risks (e.g. possible dermal contact with residuals and/or inhalation of off-gassing residuals) was done using the observational analysis data, off-gassing test results, and general knowledge of the physical/chemical characteristics of the decontamination method. Chemical off-gassing is of particular concern because of the close proximity of the FFR to the wearer's face and breathing zone. A limited assessment of the throughput capability was also done

using general knowledge of the various decontamination methods. Additional studies on dry heat laboratory oven exposure and FFR media layer hydrophobicity were conducted to collect data on various aspects of FFR resilience and construction in order to further optimize decontamination strategies and assess the practicality for FFR decontamination during a shortage. In the following sections, the results of laboratory performance testing and observational analysis, additional testing, and assessment of throughput and health concerns will be discussed for each of the five decontamination methods evaluated in order to provide recommendations on which decontamination methods should be considered in future research studies.

Bleach

Bleach is available as an aqueous solutions containing 5–15% sodium hypochlorite (active ingredient) which is a highly active oxidizing agent known to be effective against a broad spectrum of bacteria and viruses (Rutala and Weber, 1997; McDonnell and Russell, 1999). Bleach decontamination did not affect the FFRs' filter aerosol penetration and filter airflow resistance. The metallic nosebands of all models that had them were slightly tarnished following decontamination and the inner nose cushion on the SN95-E FFRs was discolored. Throughput capability of a bleach method similar to the one used in this study is likely to be high; the main limiting factors are the size of the vessel containing the bleach and FFRs, adequate space to dry the FFRs, and sufficient time for air-drying.

All FFR models had a scent of bleach following overnight air-drying. Residual bleach remaining on FFRs is of concern given its known health effects. Hypochlorite powder, solutions, and vapor can be irritating and corrosive to the eyes, skin, and respiratory tract. For example, Nixon *et al.* (1975) reported that a 5.25% sodium hypochlorite solution caused severe irritation to human skin over a 4-h exposure. Other studies also reported skin irritation for long-term exposure down to a 1% solution (Eun *et al.*, 1984; Habetes *et al.*, 1986; Hostynek *et al.*, 1990). Low concentrations of bleach have been shown to trigger respiratory events in asthmatics and sensitized individuals (Medina-Ramon, 2005; Mirabelli *et al.*, 2007). The chlorine off-gassing measurements showed that overnight air-drying significantly reduced off-gassing; however, when the FFR was rehydrated with deionized water, an increase in off-gassing was measured. This observation may be significant when viewed in light of the moisture in the exhaled breath of an individual; it gives rise to the possibility of an individual being exposed to low levels of chlorine ($<$0.2 p.p.m.) from a bleach-decontaminated FFR. Comparing Table 5 and data shown in Fig. 4, a relationship between hydrophobicity of outer and inner respirator surface layers to off-gassing concentration could not be established.

Considering the potential health risks, the bleach method evaluated in this study is not recommended for further study without modification. Possible modifications worth further investigation would include reduced initial bleach concentration, chemical methods for neutralizing residuals, additional rinse steps, and more aggressive air-drying procedures.

Ethylene oxide

EtO is used in a wide range of work settings as a sterilant or fumigant, including healthcare, diagnosis, and treatment facilities; medical products manufacturing; and libraries and museums (NIOSH, 1981). EtO decontamination did not affect the filter aerosol penetration, filter airflow resistance, or physical appearance of the FFRs in this study. The EtO process used in this study has a 5-h total processing cycle (1-h EtO exposure followed by 4 h of aeration) and has a 4.8 ft^3 (0.14 m^3) chamber volume (3M, 2007). The 5-h total processing time may be a limiting factor in the timely processing of a large volume of FFRs. Residual EtO remaining on FFRs following EtO vapor-phase decontamination is not believed to be a concern because the sterilization process includes a final aeration cycle of 4 h to remove residual EtO gas.

Vaporized hydrogen peroxide

VHP has been shown to be sporicidal at temperatures ranging from 4 to 80°C, with sterilant concentrations ranging from 0.5 to $<$10 mg l^{-1} (Joslyn, 1991). VHP decontamination for a single warm cycle did not significantly affect FFR filter aerosol penetration or filter airflow resistance. The only visible physical effect on the FFRs was a slight tarnishing of the metallic nosebands. The VHP process used in this study has a short cycle time (55 min) and a usable processing volume of 3.5 ft^3 (0.1 m^3) (Advanced Sterilization Products, 2007). Although the 55-min cycle time is short compared to the lengthy EtO total process time, the throughput capability of VHP processing is limited by the fact that cellulose-based products (e.g. cotton, which may be present in some head straps or some FFR layers) absorb hydrogen peroxide and can cause the STERRAD® cycle to abort due to low hydrogen peroxide vapor concentration. Significant levels of residual hydrogen peroxide vapors off-gassing from FFR materials following the STERRAD® process are unlikely and not of concern because the vapors decompose readily into water vapor and oxygen, both of which are environmentally benign (Advanced Sterilization Products, 2007).

Microwave oven irradiation

Biological decontamination of FFRs using a domestic microwave oven is an attractive idea since it

has the advantages of convenience and short treatment times. The decontamination method used here treats the microwave oven as a source of dry heat, similar to other studies. Elhafi *et al.* (2004) demonstrated that four avian viruses (infectious bronchitis virus, avian pneumovirus, Newcastle disease virus, and avian influenza virus) were inactivated on dried cotton swab samples using a domestic microwave oven for as little as 20 s. Rosaspina *et al.* (1994) demonstrated destruction of *Mycobacterium bovis* dried onto scalpel blades after 4 min of microwave exposure.

Of the nine FFR models that underwent microwave oven irradiation, filter aerosol penetration and filter airflow resistance were not affected for seven models. Material components melted on the two remaining models. Correlation could not be established for filter aerosol penetration results between dry oven-treated and microwave oven-irradiated samples. In microwave oven irradiation tests, all three SN95-D samples had penetration values $<5\%$ and did not melt; however, some SN95-D samples partially melted at 100, 110, and 120°C during dry oven treatment (Fig. 1). All SN95-E samples and all P100-I samples partially melted in the microwave oven, but no melting was observed for these two models, even at 120°C following dry oven treatment (Table 3, Figs 1 and 2).

The throughput capability of a method similar to the one in this study was limited by microwaving one FFR at a time; however, the 2-min treatment time per FFR was relatively short. Although it is likely that processing more than one FFR at a time is feasible (limited only by the internal volume of the oven), maximizing throughput was beyond the scope of this investigation. No known health risks to the user were identified. The data presented here suggest that the dry microwave oven irradiation method requires improvement before it could be recommended for decontamination and subsequent reuse. Possible modifications worth further investigation would include microwave irradiation of wet FFRs, shorter exposure times, and lower power settings.

Ultraviolet germicidal irradiation

UVGI has been demonstrated to be effective for the disinfection of drinking water and wastewater (Sykes, 1965; Angehrn, 1984; Lazarova *et al.*, 1999; Craik *et al.*, 2001; Lazarova and Savoye, 2004; Wu *et al.*, 2005) and for hospital air disinfection as a method for controlling airborne infectious disease (Macher *et al.*, 1992; Nardell, 1993; CDC, 1994; Gorsuch *et al.*, 1998; Miller and Macher, 2000). This study found that UVGI treatment did not affect the filter aerosol penetration, filter airflow resistance, or physical appearance of the FFRs. Throughput capability of a method similar to the one in this study is benefited by a relatively short irradiation time (30 min); however, it is limited by the available working surface area of a biosafety cabinet equipped with a UV-C source or other area being irradiated by a UVGI source. No known health risks to the user were identified.

Study limitations

These findings are exploratory and the data presented in this study are applicable only to the FFRs and decontamination methods tested; other FFRs may be more easily degraded while others may be less affected and slight modifications to the decontamination methods could result in different findings. Future studies are still needed to evaluate whether the decontamination processes evaluated in this study will inactivate infectious microorganisms (or appropriate surrogates), if FFR decontamination influences respirator fit, and the effect of multiple decontamination treatments on FFR performance. Future studies should also investigate the depths that infectious organisms (or appropriate surrogates) penetrate into each FFR layer, assess the relative cost of various decontamination strategies, and determine how effective various decontamination methods are at reducing the number of viable virus in all layers of the FFRs. Recent work in the NPPTL laboratory toward developing a system for studying the virucidal capability of decontamination methods for FFRs appears promising (Fisher *et al.*, 2009).

CONCLUSIONS

The effects of the various decontamination methods on the laboratory performance (filter aerosol penetration and filter airflow resistance) and physical appearance of FFRs were found to be model specific. The respirators tested have differences in their design, materials of construction, and hydrophobicity of their layers (including the filter media layers). Microwave oven irradiation melted all six samples from two FFR models. The remainder of the FFR samples that were evaluated exhibited average initial filter airflow resistances ≤ 17.0 mmH_2O and average initial sodium chloride filter aerosol penetration values $\leq 1.86\%$ for N95 FFRs and $\leq 0.012\%$ for P100 FFRs. Although there were statistically significant differences found between control respirators and those that have undergone decontamination for both filter aerosol penetration and filter airflow resistance, the practical significance is minimal as the range of numerical differences is quite small. The scent of bleach remained noticeable on all FFR models following overnight drying and low levels of chlorine were found to off-gas from bleach-decontaminated FFRs when rehydrated with deionized water, thus giving rise to the possibility of low-level exposure to a subsequent wearer.

In light of these results, the microwave oven irradiation and bleach decontamination methods investigated in this study were determined to be the least desirable among the five methods tested for consideration in future studies. UVGI, EtO, and VHP were found to be the most promising decontamination methods; however, concerns remain about the throughput capabilities for EtO and VHP. Further research is needed before any specific decontamination methods can be recommended.

FUNDING

National Institute for Occupational Safety and Health (CAN #921Z6PT).

Acknowledgements—The authors would like to express their sincere gratitude to Charles Hughes and Donald Tumminelli (SPSmedical Supply Corp., Rush, NY, USA), Douglas P. Landsittel (Duquesne University, NIOSH/NPPTL), Samy Rengasamy (NIOSH/NPPTL), Evanly Vo (NIOSH/NPPTL), Debra Novak (NIOSH/NPPTL), Ziqing Zhuang (NIOSH/NPPTL), and Edward Fisher (EG&G Technical Services, Inc.) for their contributions and consultation. The authors would also like to extend thanks to Les Boord (NIOSH/NPPTL), Roland Berry Ann (NIOSH/NPPTL), Maryann D'Alessandro (NIOSH/NPPTL), Lisa Delaney (NIOSH/OD), Ray Roberge (NIOSH/NPPTL), Jay Parker (NIOSH/NPPTL), and Brian Heimbuch (Applied Research Associates) for their reviews, comments, and suggestions.

Disclaimer—The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the NIOSH. Mention of company names or products does not constitute endorsement by NIOSH.

REFERENCES

3M. (2007) 3M Steri-Vac ethylene oxide sterilization systems and accessories. St Paul, MN: 3M Inc. Available at http://www.3M.com/medicalspecialties. Accessed 24 March 2009.

Advanced Sterilization Products. (2007) STERRAD® 100S. Available at http://www.sterrad.com/Products_&_Services/STERRAD/STERRAD_100S/Literature/100S_compare_eto.pdf. Accessed 24 March 2009.

Angehrn M. (1984) Ultraviolet disinfection of water. Aqua; 2: 109–15.

Bailar JC, Brosseau LM, Cohen HJ *et al*. (2006) Reusability of facemasks during an influenza pandemic. Washington, DC: Institute of Medicine, National Academies Press.

CDC. (1994) Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care facilities. MMWR Recomm Rep; 43: 1–132.

CDC. (2007) Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings 2007. Atlanta, GA: J.D. Siegel and the Healthcare Infection Control Practices Advisory Committee, Centers for Disease Control and Prevention. Available at http://www.cdc.gov/ncidod/dhqp/gl_isolation.html. Accessed 24 March 2009.

Craik SA, Weldon D, Finch GR *et al*. (2001) Inactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. Water Res; 35: 1387–98.

Elhafi G, Naylor CJ, Savage CE *et al*. (2004) Microwave or autoclave treatments destroy the infectivity of infectious bronchitis virus and avian pneumovirus but allow detection by reverse transcriptase–polymerase chain reaction. Avian Pathol; 33: 303–6.

Eun HC, Lee AY, Lee YS. (1984) Sodium hypochlorite dermatitis. Contact Dermatitis; 11: 45.

Fisher E, Rengasamy S, Viscusi DJ *et al*. (2009) Development of a test system to apply virus containing particles to air permeable materials for the evaluation of decontamination procedures for filtering facepiece respirators. J Appl Environ Microbiol; 75: 1500–7.

Gorsuch EL, Grinshpun SA, Willeke K *et al*. (1998) Method for evaluating germicidal ultraviolet inactivation of biocontaminated surfaces. Int J Occup Saf Ergon; 4: 287–97.

Habetes JMW, Geursen-Reitsma AM, Stolz E *et al*. (1986) Sensitization to sodium hypochlorite causing contact dermatitis. Contact Dermatitis; 15: 140–2.

Hostynek JJ, Wilhelm KP, Cua AB *et al*. (1990) Irritation factors of sodium hypochlorite solutions in human skin. Contact Dermatitis; 23: 316–24.

Joslyn LJ. (1991) Gaseous chemical sterilization. In: Block SS, editor. Disinfection, sterilization and preservation. 4th edn. Philadelphia, PA: Lea and Febiger; pp. 344–5.

Lazarova V, Savoye P. (2004) Technical and sanitary aspects of wastewater disinfection by UV irradiation for landscape irrigation. Water Sci Technol; 50: 203–9.

Lazarova V, Savoye P, Janex ML *et al*. (1991) Advanced wastewater disinfection technologies: state of the art and perspective. Water Sci Technol; 40: 203–13.

Macher JM, Alevantis LE, Chang YL *et al*. (1992) Effect of ultraviolet germicidal lamps on airborne microorganisms in an outpatient waiting room. Appl Occup Environ Hyg; 7: 505–13.

McDonnell D, Russell D. (1999) Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev; 12: 147–79.

Medina-Ramon M. (2005) Asthma, chronic bronchitis, and exposure to irritant agents in occupational domestic cleaning: a nested case-control study. Occup Environ Med; 62: 598–606.

Miller SL, Macher JM. (2000) Evaluation of a methodology for quantifying the effect of room air ultraviolet germicidal irradiation on airborne bacteria. J Aerosol Sci Technol; 33: 274–5.

Mirabelli MC, Zock J, Plana E *et al*. (2007) Occupational risk factors for asthma among nurses and related healthcare professionals in an international study. Occup Environ Med; 64: 474–9.

Nardell EA. (1993) Fans, filters, or rays? Pros and cons of the current environmental tuberculosis control technologies. Infect Cont Hosp Epidemiol; 14: 681–5.

NIOSH. (1981) Current intelligence bulletin 35-ethylene oxide. National Institute for Occupational Safety and Health. Available at http://www.cdc.gov/niosh/81130_35.html. Accessed 24 March 2009.

NIOSH. (2005) Determination of inhalation resistance (Procedure RCT-APR-STP-0007). Pittsburgh, PA: National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory. Available at http://www.cdc.gov/niosh/npptl/stps/respirator_testing.htm#STP_APR. Accessed 24 March 2009.

NIOSH. (2007) Determination of particulate filter efficiency level for N95 series filters against solid particulates for non-powered, air-purifying respirators (procedure TEB-APR-STP-0059). Pittsburgh, PA: National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory. Available at http://www.cdc.gov/niosh/npptl/stps/respirator_testing.htm#STP_APR. Accessed 24 March 2009.

Nixon GA, Tyson CA, Wertz WC. (1975) Interspecies comparisons of skin irritancy. Toxicol Appl Pharmacol; 31: 481–90.

OSHA. (2007) Pandemic influenza preparedness and response guidance for healthcare workers and healthcare employers. Washington, DC: Occupational Safety and Health Administration Pub no. OSHA 3328-05.

Rosaspina S, Salvatorelli G, Anzanel D. (1994) The bactericidal effect of microwaves on *Mycobacterium bovis* dried on scalpel blades. J Hosp Infect; 26: 45–50.

Rutala WA, Weber DJ. (1997) Uses of inorganic hypochlorite (bleach) in health-care facilities. Clin Microbiol Rev; 10: 597–610.

Sykes G. (1965) Disinfection and sterilization. 2nd edn. London: E & EN Spon.

Viscusi DJ, Bergman MS, Sinkule EJ *et al*. (2009) Evaluation of the filtration performance of 21 N95 filtering facepiece respirators after prolonged storage. Am J Infect Control; 37: 381–6.

Viscusi DJ, King WP, Shaffer RE. (2007) Effect of decontamination on the filtration efficiency of two filtering facepiece respirator models. J Int Soc Respir Prot; 24: 93–107.

Vojtko MR, Roberge MR, Vojtko RJ *et al*. (2008) Effect of breathing resistance of a surgical mask worn over a N95 filtering facepiece respirator. J Int Soc Respir Prot; 25: 1–8.

Wu Y, Clevenger T, Deng B. (2005) Impacts of goethite particles on UV disinfection of drinking water. Appl Environ Microbiol; 71: 4140–3.

16

RESEARCH ARTICLE

Human Coronavirus 229E Remains Infectious on Common Touch Surface Materials

Sarah L. Warnes, Zoë R. Little, C. William Keevil

Centre for Biological Sciences, University of Southampton, Southampton, United Kingdom

ABSTRACT **The evolution of new and reemerging historic virulent strains of respiratory viruses from animal reservoirs is a significant threat to human health. Inefficient human-to-human transmission of zoonotic strains may initially limit the spread of transmission, but an infection may be contracted by touching contaminated surfaces. Enveloped viruses are often susceptible to environmental stresses, but the human coronaviruses responsible for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) have recently caused increasing concern of contact transmission during outbreaks. We report here that pathogenic human coronavirus 229E remained infectious in a human lung cell culture model following at least 5 days of persistence on a range of common nonbiocidal surface materials, including polytetrafluoroethylene (Teflon; PTFE), polyvinyl chloride (PVC), ceramic tiles, glass, silicone rubber, and stainless steel. We have shown previously that noroviruses are destroyed on copper alloy surfaces. In this new study, human coronavirus 229E was rapidly inactivated on a range of copper alloys (within a few minutes for simulated fingertip contamination) and Cu/Zn brasses were very effective at lower copper concentration. Exposure to copper destroyed the viral genomes and irreversibly affected virus morphology, including disintegration of envelope and dispersal of surface spikes. Cu(I) and Cu(II) moieties were responsible for the inactivation, which was enhanced by reactive oxygen species generation on alloy surfaces, resulting in even faster inactivation than was seen with nonenveloped viruses on copper. Consequently, copper alloy surfaces could be employed in communal areas and at any mass gatherings to help reduce transmission of respiratory viruses from contaminated surfaces and protect the public health.**

IMPORTANCE **Respiratory viruses are responsible for more deaths globally than any other infectious agent. Animal coronaviruses that "host jump" to humans result in severe infections with high mortality, such as severe acute respiratory syndrome (SARS) and, more recently, Middle East respiratory syndrome (MERS). We show here that a closely related human coronavirus, 229E, which causes upper respiratory tract infection in healthy individuals and serious disease in patients with comorbidities, remained infectious on surface materials common to public and domestic areas for several days. The low infectious dose means that this is a significant infection risk to anyone touching a contaminated surface. However, rapid inactivation, irreversible destruction of viral RNA, and massive structural damage were observed in coronavirus exposed to copper and copper alloy surfaces. Incorporation of copper alloy surfaces in conjunction with effective cleaning regimens and good clinical practice could help to control transmission of respiratory coronaviruses, including MERS and SARS.**

Received 1 October 2015 **Accepted** 13 October 2015 **Published** 10 November 2015

Citation Warnes SL, Little ZR, Keevil CW. 2015. Human coronavirus 229E remains infectious on common touch surface materials. mBio 6(6):e01697-15. doi:10.1128/mBio.01697-15.

Editor Rita R. Colwell, University of Maryland

Address correspondence to C. W. Keevil, cwk@soton.ac.uk.

This article is a direct contribution from a Fellow of the American Academy of Microbiology.

Treatment of infectious disease is currently facing a crisis. Widespread antibiotic resistance has reduced therapeutic options against bacterial pathogens. However, there is also a significant threat from reemerging, newly evolving, and zoonotic viral pathogens. In addition, new technologies are also able to identify previously unknown pathogenic viruses. The majority of these are RNA viruses transmitted through the mucosal or respiratory route and manifesting as respiratory disease (1). Respiratory viruses can cause a wide range of lung disorders ranging from mild upper respiratory tract infections to more-severe life-threatening pathologies, including bronchiolitis, fever, pneumonia, and acute respiratory distress syndrome. The World Health Organization (WHO) estimated that there are 450 million cases of pneumonia per year resulting in 4 million deaths, and approximately 200 million of these are cases of viral community-acquired pneumonia (reviewed in reference 2). Common viruses include respiratory syncytial virus (RSV), rhinoviruses, influenza virus, parainfluenzavirus, and coronaviruses. Coinfections with two or more pathogens and comorbidities often affect disease severity and prognosis and complicate initial diagnosis (3).

Many coronavirus species are important animal pathogens and are often host species specific. In humans, several species, e.g., human coronavirus 229E (HuCoV-229E) and NL63 (*Alphacoronavirus*) and HKU1 and OC43 (*Betacoronavirus*), are a common cause of upper respiratory tract infection. There is an ever-present risk of pathogens emerging from animal reservoirs that have at-

tained the ability to infect humans. The risk can be increased when individuals have continuous and close contact with animals; also, climate changes can change the distribution of insect vectors and hosts (4, 5).

In 2003, a highly pathogenic coronavirus believed to have originated in bats and palm civet cats transferred to humans in Guangdong Province, China, resulting in cases of severe acute respiratory syndrome (SARS). Over 8,000 people were infected in 37 different countries, but mostly in Southeast Asia, with 10% mortality. Inefficient human-to-human transmission, severe restrictions on air travel, closure of many wild-animal markets, and quarantine procedures have successfully contained the outbreak so far. However, zoonotic transmission of a coronavirus from reservoirs in bats and possibly camels gave rise to severe respiratory infection in individuals in the Arabian Peninsula in 2012. The resulting Middle East respiratory syndrome (MERS), which affects the lower respiratory tract, is clinically similar to SARS but pathologically different. A ubiquitous host cell receptor often leads to extrapulmonary disease, often in the kidneys, and viral progeny are released through apical and basolateral respiratory cell surfaces, contributing to the high (up to 40%) mortality rate (reviewed in references 6 and 7). Late uncontrolled inflammation leads to severe pathologies which are not dependent on viral load, and human-to-human spread does occur (reviewed in references 3 and 4). This, combined with a low infectious dose, suggests that transmission of very few virus particles via person-to-person or contact with contaminated surfaces may be an infection risk. Although camels and associated food products have been found to contain the virus, a recent study of individuals constantly in contact with infected herds suggested that zoonotic transmission is rare (8) but that the risk may be highest from juvenile animals. The risk of transmission is increased, however, in clinical facilities (9) and possibly in other crowded public areas, including care homes and areas of mass gatherings, such as the Hajj Muslim pilgrimage to Mecca. In a recent outbreak in South Korea, MERS has so far (July 2015) killed 36 people and infected 186 patients in hospital-associated cases associated with the first imported case arising from travel to the Middle East (10, 11).

Surface contamination has recently been found to be more significant than originally thought in the spread of many diseases (12). Symptoms of respiratory disease often result in continuous recontamination of surfaces which are then touched, and infectious virus particles may be transferred to facial mucosa. In addition, ineffective cleaning agents may leave residual particles that can initiate infection (13). The use of biocidal surfaces may help to reduce the incidence of infections spread by touching contaminated surfaces. Copper alloys have demonstrated excellent antibacterial and antifungal activity against a range of pathogens in laboratory studies (14–19). Copper ion release has been found to be essential to maintaining efficacy, but the mechanism of action is variable (20, 21). A reduction in microbial bioburden and acquisition of nosocomial infection has now been observed in clinical trials of incorporation of copper alloy surfaces in health care facilities (22–25).

Previous studies have shown that murine norovirus (MNV) and human norovirus, highly infectious nonenveloped viruses that are resistant to environmental stress and impervious to many cleaning agents, are destroyed on copper and copper alloy surfaces (26–28). HuCoV-229E is associated with a wide range of respiratory disease from mild colds to severe pneumonia in immunocompromised people and has been implicated as an autoimmune trigger in multiple sclerosis (29, 30). Infection with this virus occurs in a high proportion of the population in approximately 3-year cycles, incurring considerable hidden costs in lost work hours, and in this study was also used as a surrogate for the more virulent coronaviruses responsible for SARS and MERS (rather than using animal viruses or coronaviruses that primarily infect the gastrointestinal tract). In addition, a recent study also observed that HuCoV-229E shares important characteristics with MERS-coronavirus and also has an ancestral link with bats (31). In this study, the ability of HuCoV-229E to retain infectivity on a range of common surface materials was investigated to understand the risk of disease dissemination. The potential use of biocidal surfaces to provide constant antiviral activity against continual surface recontamination could help to limit the spread of respiratory viruses; accordingly, the efficacy of a range of copper alloys to inactivate HuCoV-229E was also determined.

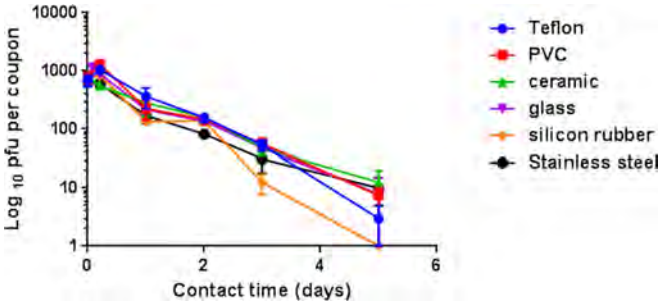


FIG 1 Persistence of infectious human coronavirus on common surface materials. Approximately 10^3 PFU HuCoV-229E (20 μl infected-cell lysate) was applied to 1-cm^2 coupons of test surface materials and incubated at ambient conditions (21°C; relative humidity, 30% to 40%). Virus was removed and assayed for infectivity at various time points as described in the text. Although the initial inoculum concentration was quite low, the virus retained infectivity for 5 days on all surfaces, except silicon rubber. Therefore, natural contamination of common surface material with very few coronavirus particles could represent a considerable risk of infection spread if touched and transferred to facial mucosa. Error bars represent ± SEM, and data are from the results of multiple experiments.

RESULTS

Coronavirus persists in an infectious state on common surface materials for several days. An inoculum of 10^3 plaque forming units (PFU) persisted on polyfluorotetraethylene (Teflon; PTFE), polyvinyl chloride (PVC), ceramic tiles, glass, and stainless steel for at least 5 days (and 3 days for silicon rubber) at 21°C and a relative humidity of 30% to 40% (Fig. 1).

Rapid inactivation of human coronavirus occurs on brass and copper nickel surfaces at room temperature (21°C). Brasses containing at least 70% copper were very effective at inactivating HuCoV-229E (Fig. 2A), and the rate of inactivation was directly proportional to the percentage of copper. Approximately 10^3 PFU in a simulated wet-droplet contamination (20 μl per cm^2) was inactivated in less than 60 min. Analysis of the early contact time points revealed a lag in inactivation of approximately 10 min followed by very rapid loss of infectivity (Fig. 2B). As observed previously for norovirus, zinc demonstrated a slight antiviral effect compared to that seen with stainless steel (neither metal contains copper).

Copper nickels were also effective at inactivating HuCoV-229E

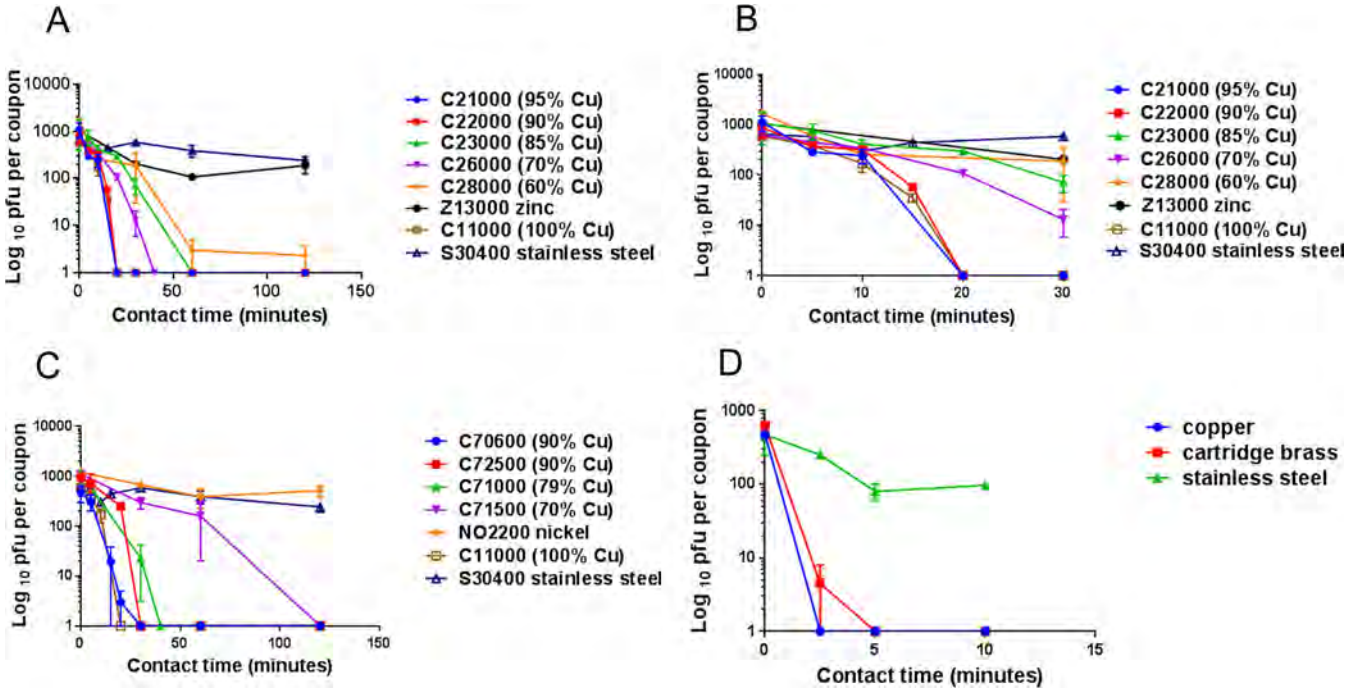


FIG 2 Rapid inactivation of human coronavirus occurs on brass and copper nickel surfaces. Approximately 10^3 PFU HuCoV-229E (20 μl infected-cell lysate) was applied to 1-cm^2 coupons of a range of brasses (A and B [early time points only]), copper nickels (C), and control metal surfaces that did not contain copper (stainless steel, zinc, and nickel). Virus was removed at various time points and assayed for infectivity as described in the text. Coronavirus was inactivated in ≤40 min on brasses and 120 min on copper nickels containing less than 70% copper. Analysis of the initial 30 min of contact between virus and brasses (Fig. 2B) reveals an initial lag followed by rapid inactivation. Stainless steel and nickel did not demonstrate any antiviral activity, although mild antiviral activity was observed on zinc (this was significant only at 60 min [$P = 0.046$]). (D) The same inoculum was applied as 1 μl/cm^2, was dried immediately to simulate fingertip touch contamination, and was found to have inactivated the virus approximately 8 times faster. Error bars represent ± SEM, and data are from the results of multiple experiments.

but required higher (90%) copper content to produce a degree of inactivation equivalent to that seen with brasses containing 70% copper (Fig. 2C). The inactivation time was reduced further in the rapidly drying fingertip contamination model by approximately 8-fold to 5 min for C26000 cartridge brass (Fig. 2D).

Using the same data for simulated droplet contamination, a comparison between brasses and copper nickels containing the same percentage of copper, 90% or 70%, is demonstrated in Fig. 3. At the higher copper content level, there was little difference in efficacy between C22000 and C70600 (Fig. 3A). However, copper nickel C72500 was less effective than C70600 although it contains the same percentage of copper. The superior antiviral properties of C70600 have been observed previously for norovirus and may involve the cuprous oxide layer visible as a removable layer (27). However, at a lower percentage of copper, the cartridge brass was far superior to copper nickel C71500, inactivating virus in approximately one-third the time (Fig. 3B).

Copper ion release and generation of reactive oxygen species (ROS) are involved in inactivation of HuCoV-229E on copper and copper alloy surfaces. HuCoV-229E was inoculated onto copper and cartridge brass surfaces (100% and 70% copper, respectively; Table 1) in the presence of ethylenediaminetetraacetic acid (EDTA) and bathocuproine disulfonate (BCS), chelators of Cu(II) and Cu(I), respectively. Both chelators initially protected the virus from inactivation for up to 2 h (although BCS was still protective after 2 h of contact with brass) (Fig. 4A and C). This suggests that both ionic species of copper are required directly and/or indirectly for virus inactivation and that Cu(I) may be more significant in the longer term.

Inoculation of coronavirus in the presence of D-mannitol and Tiron (4,5-dihydroxy-1,3-benzene disulfonic acid) to quench hydroxyl radicals and superoxide anions, respectively, was done to determine if these moieties were involved in the coronavirus inactivation mechanism (Fig. 4B and D). Tiron protected the virus for the first hour of contact, suggesting that superoxide generation is important. However, D-mannitol was minimally protective on copper but protected the virus for the duration of the test on brass. Increasing the concentration of D-mannitol did not prolong survival of infectivity on copper (not shown). This suggests that rapid inactivation of coronavirus on copper surfaces is primarily due to copper ion release and that the effect of reactive oxygen species is minimal. However, as the percentage of copper in the alloy decreased, ROS generation played a more significant role.

EDTA, BCS, D-mannitol, and Tiron did not significantly affect the virus on stainless steel control surfaces or in suspension (not shown).

Inactivation of coronavirus on copper and copper alloy surfaces results in fragmentation of the viral genome, ensuring that inactivation is irreversible. Coronavirus was exposed to metal surfaces and recovered, and the positive-stranded viral RNA genome was extracted and purified. A one-step reverse transcriptase real-time quantitative PCR (RTqPCR) was performed to detect a 139-bp region of ORF1 within nonstructural protein 4 (nsp4). Virus that had been exposed to copper and brass surfaces demon-

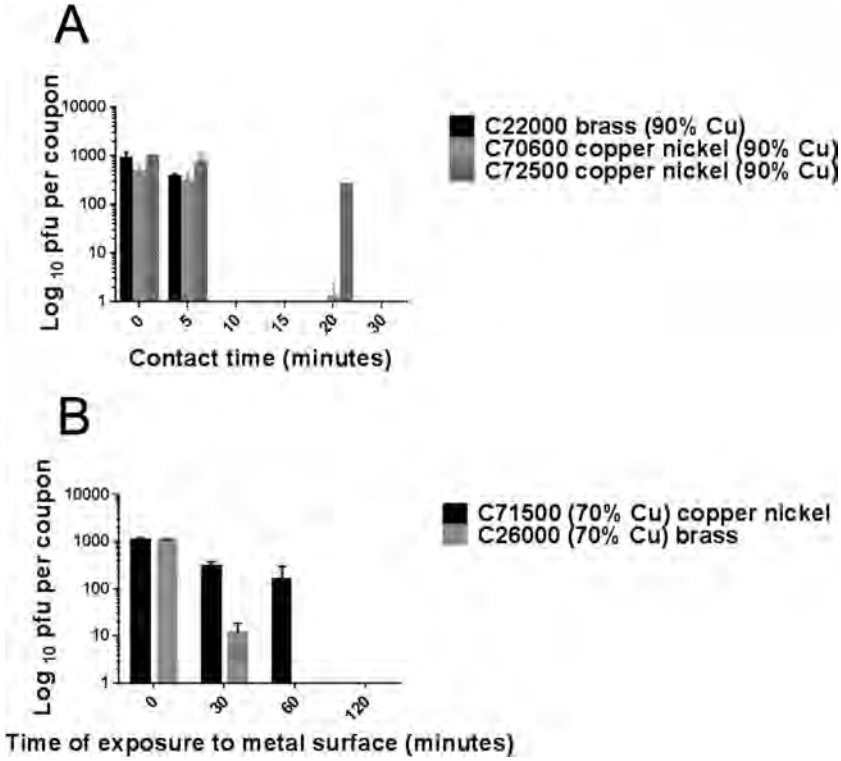


FIG 3 Comparison between brasses and copper nickels (containing the same percentage of copper) used to inactivate human coronavirus to determine if zinc content enhances the antiviral effect. Approximately 10^3 PFU was inoculated onto alloys containing 90% copper for 0, 5, and 20 min (A) or 70% copper for 0, 30, and 60 min (B) and was then removed and assessed for infectivity as described in the text. Alloys containing 90% copper were very effective at inactivating human coronavirus (A), but variations in efficacy did not appear to be related only to the presence of zinc. The presence of copper nickel C70600 resulted in increased efficacy compared to that of copper nickel C72500; that result may be linked to surface oxide layer or copper ion release from this alloy. However, at a lower percentage of copper (B), synergy with zinc or Cu(I) release may be important because contact with cartridge brass resulted in virus inactivation that was at least 3 times faster than that seen with copper nickel C71500.

strated reduced copy numbers of this fragment with increasing contact times (Fig. 5A). Comparison of the entire viral genome by agarose gel electrophoresis confirmed that nonspecific fragmentation occurred on copper and brass, with fragments becoming smaller with increasing contact time (Fig. 5B).

Exposure to copper surfaces results in morphological changes to human coronavirus particles visible in transmission electron microscopy (TEM). There was a significant difference in appearance between purified HuCoV-229E exposed to stainless steel and that exposed to copper surfaces (Fig. 6). On stainless steel, uniform virions were visible following a 10-min exposure (Fig. 6A), but on copper, clumps of damaged virus particles (Fig. 6B) as well as a few intact particles could be seen. The extent of damage increased upon further exposure to copper (Fig. 6C).

DISCUSSION

A combination of genetic reassortment in viruses with segmented genomes and point mutations, particularly evident in viruses that cause disease in the respiratory tract such as influenza virus and coronaviruses, results in constantly changing antigenicity and host immune response evasion. This can also affect the attachment to the host cell receptor and the "host jump" from animals to human that can occur if the mutation results in an increased ability of the virus to bind to human cells. If this is accompanied by a decrease in binding to the original host, then human-to-human transmission can occur, presenting a substantial threat of rapid spread of a novel virus throughout the community (reviewed in reference 32).

Viruses causing respiratory infections are spread by droplets expelled by coughs and sneezes, which can also contaminate the environment 2 m and 6 m away, respectively (33), and a single droplet may easily contain an infectious dose (34). Enveloped respiratory viruses, although more susceptible to environmental stress than nonenveloped viruses, have been shown to persist on surfaces and contaminate more than 50% of surfaces in household and day care centers (35). Animal coronaviruses, including transmissible gastroenteritis virus (TGEV) and mouse hepatitis virus (MHV), have been shown to retain infectivity for long periods on hard surfaces (36) and for several hours on health care gowns, gloves, and masks (37), but human coronavirus 229E (HuCoV-229E) did not persist for above a few hours on surfaces (38). In contrast, we have observed that a relatively low titer of infectious human coronavirus 229E persisted on 5 surface materials, common to communal and domestic environments, for at least 5 days. Our virus preparation contained a high proportion of lung cell debris to mimic natural contamination in respiratory secretions, which may have protected the virus from desiccation, and a human lung cell line was used for the assay, which may have been more sensitive. The relatively low virus concentration used suggests that higher viral concentrations which can occur in sputum may persist for longer periods. During coronavirus infection, the

TABLE 1 Composition of metal alloys used for the study

Metal(s)	UNS ID[a]	Common name	Main constituent (%)						
			Copper	Zinc	Nickel	Tin	Iron	Chromium	Manganese
Copper nickels	C70600	Copper nickel 10	89–90		10		<1		
	C72500	Cupronickel with Sn	89–90		9		<2		
	C71000	Cupronickel	79		20				1
	C71500	Copper nickel 30	70		30				
Brasses	C21000	Gilding brass	95	5					
	C22000	Commercial "bronze" (does not contain Sn)	89–90	10					
	C23000	Red brass	85	15					
	C26000	Cartridge brass	70	30					
	C28000	Muntz metal	60	40					
Phosphor bronze	C51000	"5% A" (contains <0.26% phosphorus)	95			5			
Nickel "silver"	C75200	"65/18"	65	17	18				
Copper	C11000	Copper	100						
Nickel	NO2200	Nickel			100				
Zinc	Z13000	Zinc		100					
Stainless steel	S30400	"18/8"			8		74	18	

[a] UNS ID, Unified Numbering System identifier.

viral load is highest later in the infection and large numbers of infectious virus which may also contaminate the surrounding environment can be shed as symptoms subside over long periods (4). There is scant information on minimum infectious doses, but for many respiratory viruses, the minimum infectious dose is believed to be low, i.e., just a few virus particles. Coronavirus persistence on surfaces represents a considerable infection risk if contaminated surfaces are touched and infectious virus transferred to the mouth, nasal mucosa, or conjunctiva. Nicas and Best (39) observed that individuals in office environments touched their face an average of 15 times an hour, giving ample opportunities for infection spread. The use of antiviral surfaces in health care and community facilities could help to reduce infection spread in this way. HuCoV-229E was rapidly inactivated on copper surfaces, with the inactivation rate being roughly proportional to the percentage of copper in the alloy. Alloys containing >90% copper inactivated 10^3 PFU coronavirus in <30 min, and a surface oxidation layer or increased copper ion release on C70600 increased efficacy, which has been observed for this alloy before (27). Brasses were more efficacious than copper nickels at a lower percentage of copper.

Previous studies by our laboratory have shown release of copper ionic species to be essential to the efficacy of copper surfaces in killing bacteria and inactivating norovirus (26). Using chelators, we have determined that Cu(I) and Cu(II) are also essential for inactivation of coronaviruses. On brass (70% copper), BCS, the chelator for Cu(I), was still protective at 2 h of contact, suggesting that inactivation may have been due to Cu(II) immediately and to Cu(I) in the long term. Copper ions have been shown to directly inhibit proteases by reacting with surface cysteine and to inflict damage to the viral genome in HIV and herpes simplex virus (40, 41).

The mechanism of bacterial death on copper surfaces is complex, involving not only direct action of copper ion on multiple targets but also the generation of destructive oxygen radicals, resulting in "metabolic suicide" (20). This was not observed for norovirus destruction on copper, presumably because of the lack of respiratory machinery (26). However, it appears that superoxide and hydroxyl radical generation may be important in the inactivation of coronaviruses on copper alloys but that inactivation on 100% copper surfaces is primarily due to the direct effect of copper ions. Following application of a wet droplet to a copper surface, the predominant ionic species to dissolute from the metal surface is Cu(II), but reduction to Cu(I) and the Fenton reaction with oxidative intermediates from cell debris, molecular oxygen, or viral envelope could produce the highly toxic hydroxyl radical. ROS are generated in the natural course of coronavirus infection (42) and contribute to pathogenesis and apoptosis. Fujimori et al. (43) observed inactivation of influenza A H1N1 pandemic 2009 strain by Cu(I) iodide nanoparticles which involved hydroxyl radicals and resulted in degradation of hemagglutinin and neuraminidase viral proteins. They surmised that, although there was no exogenous hydrogen peroxide to fuel the Fenton reaction (equation 3), Cu(I) reacted with molecular oxygen to generate superoxide (equation 1) and, subsequently, hydrogen peroxide (equation 2) (which could also produce hydroxyl radicals via the Haber Weiss reaction) as follows:

$$2Cu^{+} + 2O_2(aq) \rightarrow 2Cu^{2+} + 2O_2^{-} \quad (1)$$

$$2O_2^{-} + 2H^{+} \rightarrow H_2O_2 + O_2 \quad (2)$$

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + OH^{\cdot} \quad (3)$$

In our results, the Cu(I) chelator BCS protected coronavirus on brass surfaces, suggesting that Cu^{+} migrating from the metal is important in toxicity and supporting the Fenton reaction generation of hydroxyl radicals that was observed. Perhaps the reason brasses were more effective at inactivating coronavirus than copper nickels was the increased Cu(I) release and subsequent ROS

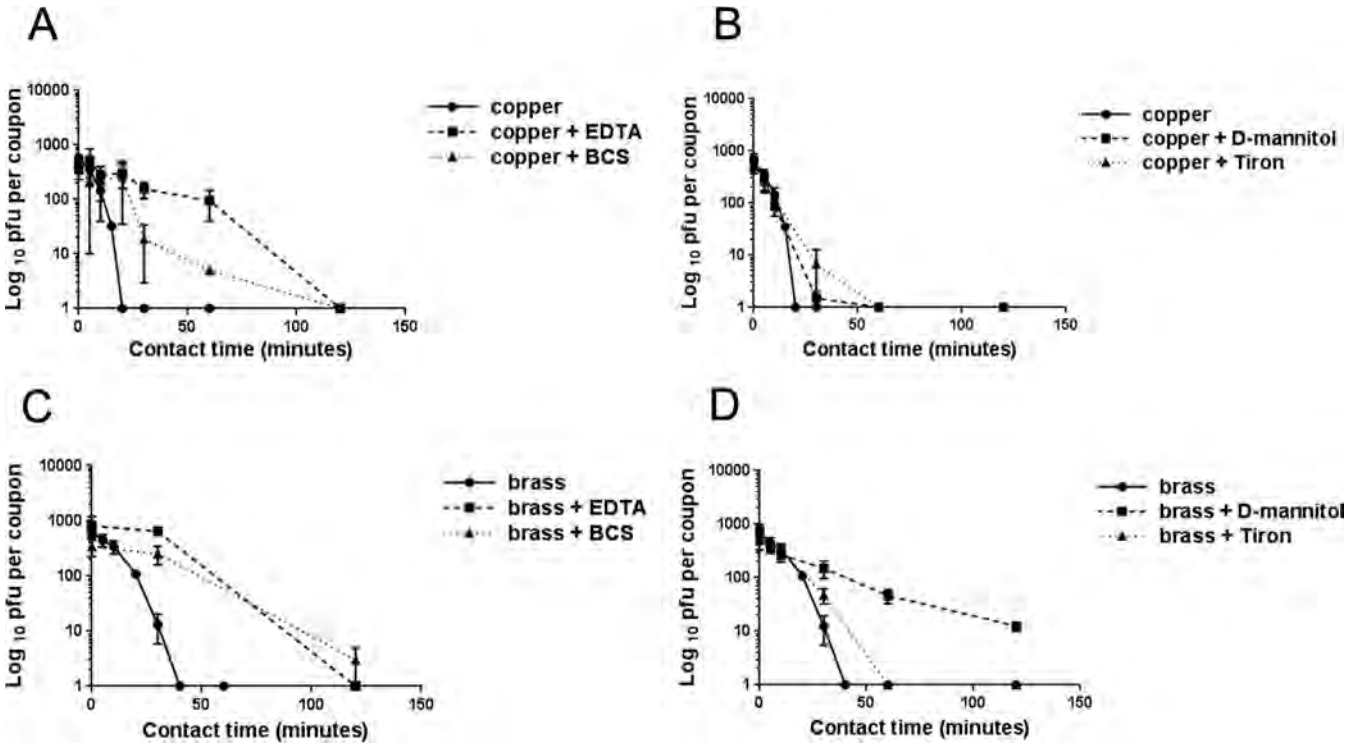


FIG 4 Inactivation of coronavirus on copper and cartridge brass surfaces in the presence of chelators EDTA and BCS (A and C) and quenchers D-mannitol and Tiron (B and D) to remove Cu(II) or Cu(I) ionic species and hydroxyl radical or superoxide, respectively. Both chelators protected coronavirus from inactivation on copper and brass surfaces, suggesting that release of Cu(I) and Cu(II) is required for antiviral activity. Tiron was protective for the first hour of contact on copper and brass surfaces, indicating that superoxide is directly or indirectly involved in the inactivation mechanism. However, D-mannitol gave minimum protection on copper but prolonged protection on brass surfaces. Increasing the concentration of D-mannitol did not affect the results (not shown). This suggests that copper ions are the main moieties responsible for inactivation of coronavirus on 100% copper surfaces but that generation of hydroxyl radicals becomes more significant as the concentration of copper in the alloy is reduced. EDTA, BCS, D-mannitol, and Tiron did not significantly affect the infectivity of HuCoV-229E on stainless steel controls or in suspensions (not shown). Error bars represent ± SEM, and data are from the results of multiple experiments.

generation rather than the zinc content, which had only mild antiviral activity. Presumably, as in bacteria, a multitarget attack on enveloped viruses by copper ions and ROS may result in nonenzymatic peroxidation of the envelope (44) and damage to membrane proteins and the nucleoproteins.

We have observed previously (27) that exposure to copper surfaces resulted in significant morphological changes to nonenveloped norovirus, where possible disassociation of the capsid subunits exposed the viral genome to copper inactivation. In this study, we observed rapid damage, including clumping, breakage, membrane damage, and loss of surface spikes, to the coronavirus particles following exposure to copper, and some particles appeared smaller and seemed to have lost rigidity, folding up on themselves. These changes were not observed with virus recovered from stainless steel surfaces.

Analysis of coronavirus genomic RNA from viruses exposed to copper and copper alloys revealed a nonspecific fragmentation of the entire genome that can also be observed at the gene level by the reduction in copy number of a small fragment of nsp4 proteins, and the extent of damage increased with contact time. We have observed that the reduction in the capsid integrity of norovirus allows access of copper ions to the genome inactivating the virus. For coronavirus, the envelope and nucleoprotein are likewise compromised, and the process occurs more rapidly than with nonenveloped norovirus, which has a resistant capsid, to allow copper ion and/or ROS to destroy the genome. Interestingly, there was a 10-min delay in inactivation of simulated wet-droplet contamination which may reflect the time taken to breach the envelope and disrupt the nucleoprotein which allows access of copper ions to the coronavirus genome. Further studies may determine if the use of synergistic cleaning agents to weaken the envelope could reduce this delay. Sagripanti et al. (45) also reported increased sensitivity to solutions of copper ions of enveloped viruses compared to nonenveloped phages.

There are concerns about the pandemic potential of MERS, especially if the efficiency of interhuman transmission increases (46). The majority of cases have been in the Middle East, and concerns have been expressed because >2.5 million pilgrims attend the Hajj in Mecca, Saudi Arabia, aggregating from >180 countries. Analysis of data since June 2012 resulted in estimates that the risks of transmission are low (47, 48), but members of the Health Protection Agency (HPA) UK Novel Coronavirus Investigation team (49) have observed person-to-person transmission within a family cluster in the United Kingdom contracted from a family member who had visited Saudi Arabia. They also observed that the spectrum of symptoms of MERS, including mild and asymptomatic disease, is wider than initially realized and that spread of the virus may therefore already be greater than expected. MERS has so far killed 36 people and infected 186 patients in hospital-associated cases in South Korea associated with the first

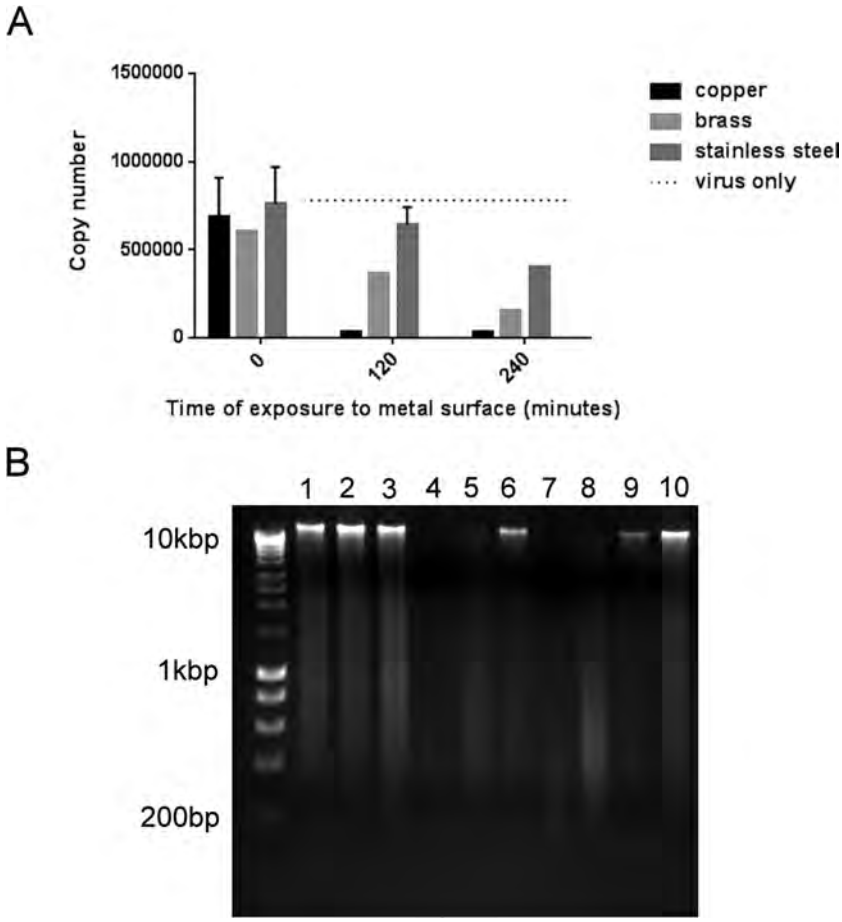


FIG 5 Destruction of human coronavirus viral genome on copper and copper alloy surfaces. (A) Analysis of a small fragment (136-bp region of the nsp4 gene) of the coronavirus genome revealed a reduction in copy number from virus exposed to copper and cartridge brass surfaces in reverse transcriptase real-time PCR. There was some reduction on stainless steel but none in viral suspension (lightest gray bars), suggesting that this was due to sample drying. (B) Analysis of the entire viral genome is represented in electrophoretic separation of viral RNA extracted from virus exposed to copper (lanes 1, 4, and 7), cartridge brass (lanes 2, 5, and 8), and stainless steel (lanes 3, 6, and 9) for 0 min (lanes 1 to 3), 120 min (lanes 4 to 6), and 240 min (lanes 7 to 9). The genomic RNA from virus exposed to copper and brass degraded with increased contact time. This did not occur on stainless steel; the genomic RNA remained as fragments too large to pass through the gel. However, the total amount of intact RNA was reduced at 4 h, possibly due to drying damage as seen in panel A. Lane 10 represents untreated virus, and the unmarked lane is a Bioline marker (Hyperladder I). The same procedure was used with mock-infected cells, revealing the same pattern of RNA breakdown following application to copper surfaces (not shown).

imported case arising from travel to the Middle East (10, 11). The current increase in the incidence of MERS has been described as a "subcritical epidemic," but statistics have concentrated on severe cases only. It remains to be seen if the number of cases continues to escalate, and the evolution of SARS and MERS is a timely reminder of the constant threat of other coronaviruses making the jump from a large reservoir in wild and domestic animals to the human population. Several Hajj pilgrims returning to Austria had contracted serious respiratory disease caused by influenza A and B virus and not MERS (50), emphasizing that there are multiple risks of contacting infectious diseases in any highly populated areas.

The results from this study have shown that a relatively low concentration of enveloped respiratory viruses may retain infectivity on common hard surfaces for longer than previously thought and may present a real risk of infection to anyone who contacts a contaminated surface. However, human coronavirus 229E, an important pathogenic virus but also a surrogate for MERS coronavirus, which is structurally very similar, was rapidly inactivated on copper alloys. Inactivation results from a combination of direct copper ion attack and reactive oxygen species generation. The latter is particularly important as the copper content decreases, ensuring that rapid inactivation still occurs in alloys with lower percentages of copper. Therefore, incorporation of copper alloys in communal areas could help to reduce infection spread from touching surfaces contaminated with coronaviruses. This is especially important in infectious disease where the infectious dose is low, surface contamination is high, and effective therapies are limited. The mechanism of action of copper is complex and may be enhanced by radical formation but is ultimately nonspecific, ensuring continuous kill and inactivation of a wide range of pathogenic microorganisms with completely different morphologies. Concerns about the biocide resistance, possible concomitant drug resistance, and horizontal gene transfer that have been observed with other biocides (51) can be allayed because of the destruction of viral nucleic acid observed following exposure to copper surfaces. It is not feasible to cover every surface in copper, and many materials in the built environment, including stain-

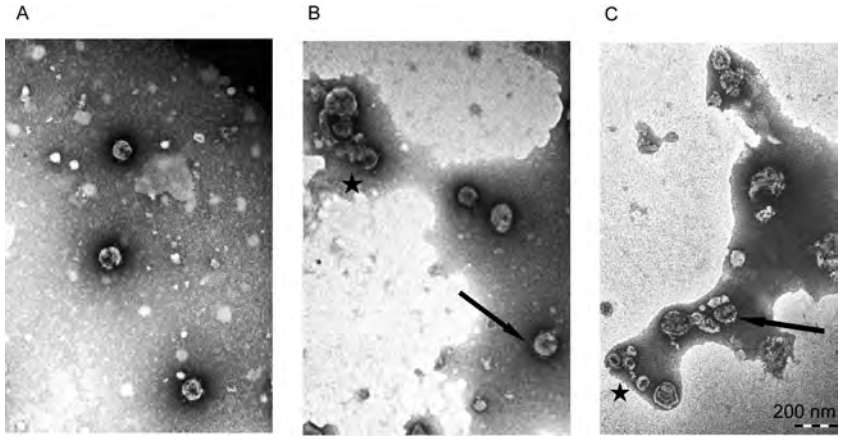


FIG 6 Exposure to copper surfaces results in morphological changes to human coronavirus. Purified HUCoV-229E was applied to metal surfaces and then removed, and a negatively stained preparation was observed using transmission electron microscopy. (A) Intact virions were visible following exposure to stainless steel for 10 min. (B) However, following exposure to copper for 10 min, many virus particles appeared to be disintegrating (indicated by a star), although some intact virions were still present (arrow). (C) After a 30-min exposure to copper, further damage had occurred and virions appeared shrunken (indicated by a star), with damage to surface spikes (arrow).

less steel, will continue to be used because of resilience, anticorrosion, and other beneficial attributes. Incorporation of even a few copper surfaces may have an impact in effectively reducing transmission of infectious material from a surface to an individual, provided that stringent, regular, and effective cleaning regimens are employed for all surfaces. The use of copper does not serve as an excuse to relax cleaning regimens. However, the choice of cleaning reagents is critical for copper alloys because it is essential to maintain copper ion release for efficacy, so avoidance of chelators is necessary.

There is now a large body of evidence from laboratory studies and small clinical trials to suggest that incorporation of copper surfaces could play a significant role in reducing infection transmission from contaminated surfaces. The time is nigh to investigate this further on a larger scale, but fears of the installation costs appear to be hampering the progress. Given the huge costs, human and monetary, associated with the treatment and care of patients with hospital-acquired infections, preliminary studies have suggested that the initial costs could be recouped within a few months (52). New technologies in copper coatings are being developed which may allow large-scale community areas, such as transport facilities, to be rendered antimicrobial at reduced costs. A note of caution: for these to be effective, there must be actual contact between copper and the contaminating pathogenic microorganisms, because any interference from matrix components could result in false economy.

MATERIALS AND METHODS

Viral strains and cell lines. Human coronavirus 229E (HuCoV-229E) and a fetal fibroblast cell line, MRC-5, were supplied by Public Health England (PHE), United Kingdom. Cells were maintained in minimal essential medium (MEM) supplemented with GlutaMax-1, nonessential amino acids, and 5% fetal calf serum and incubated at 37°C and 5% CO_2. Cells were passaged twice a week using trypsin (0.25%)-EDTA and were not used beyond passage 30 (P30) (which occurred before the onset of senescence, but susceptibility to infection diminished greatly from P30). Viral stocks were prepared by infecting cells at multiplicity of infection of 0.01 for 4 to 7 days until a significant cytopathic effect (CPE) was observed. Infected cells were subjected to 3 freeze/thaw cycles, and infected-cell lysate was stored at −80°C.

Preparation of sample surfaces. Metal coupons (10 by 10 by 0.5 mm) were degreased in acetone, stored in absolute ethanol, and flamed prior to use as described previously (19). Metal samples were supplied by the Copper Development Association and are described in Table 1. Coupons of nonmetal surfaces (PTFE, polyvinyl chloride [PVC], ceramic tiles, glass, and silicone rubber) of the same size were sterilized by autoclaving at 121°C and 1.06×10^5 pascals (1.06 bar) for 15 min. Stainless steel controls for comparison were also autoclaved for method consistency for these experiments.

Infectivity assay for HuCoV-229E exposed to surfaces. Infected cell lysate preparations of HuCoV-229E were spread over coupons of the test surface and incubated at room temperature. The virus was removed from the coupons at various times and assayed for infectious virus by a plaque assay which was a modification of the murine norovirus 1 (MNV-1) assay described previously (26). Briefly, coupons inoculated with virus and incubated for various times at room temperature were added to a tube containing 5 ml growth medium and 2-mm-diameter glass beads and subjected to vortex mixing for 15 s. Dilutions were prepared in growth medium, and 1-ml aliquots were plated onto confluent monolayers of MRC-5 cells that had been prepared 24 h earlier in 6-well trays. The inoculum was removed after 90 min and replaced with agarose overlays, and plates were incubated at 37°C and 5% CO_2 for 4 to 7 days until CPE was evident. The monolayers were stained with vital stain and neutral red, and plaques in the monolayer were enumerated. Triplicate samples were processed for each time point.

The effect of copper chelators and reactive oxygen species quenchers on infectivity of human coronavirus exposed to copper and copper alloy surfaces. Incorporation of chelators ethylenediaminetetraacetic acid (EDTA) (20 mM) and bathocuproine disulfonic acid (BCS) (20 mM) to chelate Cu(II) and Cu(I), respectively, at the time of inoculation of virus onto the metal surfaces was investigated using a plaque assay. In addition, 20 mM D-mannitol and 20 mM Tiron (4,5-dihydroxy-1,3-benzene disulfonic acid) were used to quench hydroxyl radicals and superoxide, respectively. Stainless steel was used as a control surface and to determine if quenchers and chelators affect viral replication.

Purification of viral RNA and analysis of integrity by agarose gel electrophoresis. The total RNA of untreated virus or virus exposed to metal surfaces (5 coupons per test, with virus removed from coupons by pipetting up and down in a small volume [100 μl]) was extracted using a Qiagen QIAamp viral RNA minikit according to the manufacturer's instructions and the carrier RNA provided to prevent degradation.

Purified RNA fragments were separated on a nondenaturing 1% aga-

rose gel using a GelRed nucleic acid prestaining kit (Biotium, United Kingdom) according to the manufacturer's instructions. The staining intensity is reduced because GelRed binds to single-stranded RNA (ssRNA) approximately half as much as to double-stranded nucleic acid. DNA ladders were supplied by Bioline. Gels were observed and photographed using GeneSnap software and a Syngene UV light box.

Detection and quantification of a 139-bp region of the coronavirus nonstructural gene encoding nsp4 in virus exposed to copper and brass surfaces. To determine if exposure to copper affected the viral genome at the gene level, a 139-bp region of the gene encoding nsp4 (within polyprotein 1ab replicase) was investigated using a One-Step real-time quantitative PCR (RTqPCR) diagnostic kit (supplied by PrimerDesign, United Kingdom). The kit is based on sequences from HuCoV-229E (GenBank accession number NC_002645; anchor nucleotide position 8205). Amplification was performed on a BioRad iQ5 cycler, and standard curves were prepared from known copy number standards to determine copy numbers in test samples. PCR products were analyzed by gel electrophoresis as described above.

Detection of morphological changes to HuCoV-229E using transmission electron microscopy (TEM). HuCoV-229E was purified from crude infected-cell lysate. Polyethylene glycol (PEG) precipitation (Bio-Vision PEG virus precipitation kit) was followed by sucrose density (25% to 55%) centrifugation at 96,000 × *g* for 16 h at 4°C. The virus band was resuspended in water and the virus pelleted at 77,000 × *g* for 1 h at 4°C. The supernatant was discarded, the tubes were allowed to drain, and the final pellet was resuspended in ice-cold nuclease-free deionized distilled water after incubation on ice for 30 min. The preparation was applied to copper and stainless steel as described in the Figure 6 legend and was removed by gentle pipetting. Samples were fixed, applied to TEM grids, washed with water, and stained with 5% ammonium molybdate for 10 s.

Statistical analysis. Data are expressed as means ± standard errors of the mean (SEM) and are from the results of multiple independent experiments. Statistical analyses and representational graphic depictions were performed using GraphPad Prism 6.

ACKNOWLEDGMENTS

This research was supported by the Copper Development Association, New York, and the International Copper Association, New York.

We thank Anton Page, Patricia Goggin, Lizzie Angus, and everyone at the Biomedical Imaging Unit, University of Southampton, Southampton, United Kingdom, for their help with the use of the transmission electron microscope.

REFERENCES

1. **Zambon M.** 2014. Influenza and other emerging respiratory viruses. Medicine **42:**45–51 http://dx.doi.org/10.1016/j.mpmed.2013.10.017.
2. **Ruuskanen O, Lahti E, Jennings LC, Murdoch DR.** 2011. Viral pneumonia. Lancet **377:**1264–1275. http://dx.doi.org/10.1016/S0140-6736(10)61459-6.
3. **Van den Brand JM, Smits SL, Haagmans BL.** 2015. Pathogenesis of Middle East respiratory syndrome coronavirus. J Pathol **235:**175–184. http://dx.doi.org/10.1002/path.4458.
4. **Gralinski LE, Baric RS.** 2015. Molecular pathology of emerging coronavirus infections. J Pathol **235:**185–195. http://dx.doi.org/10.1002/path.4454.
5. **Coleman CM, Frieman MB.** 2014. Coronaviruses: important emerging human pathogens. J Virol **88:**5209–5212. http://dx.doi.org/10.1128/JVI.03488-13.
6. **Chan JFW, Lau SKP, To KKW, Cheng VCC, Woo PCY, Yuen K.** 2015. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev **28:**465–522. http://dx.doi.org/10.1128/CMR.00102-14.
7. **Cong Y, Ren X.** 2014. Coronavirus entry and release in polarized epithelial cells: a review. Rev Med Virol **24:**308–315. http://dx.doi.org/10.1002/rmv.1792.
8. **Hemida MG, Al-Naeem A, Perera RAPM, Chin AWH, Poon LLM, Peiris M.** 2015. Lack of Middle East respiratory syndrome coronavirus transmission from infected camels. Emerg Infect Dis **21:**699–701. http://dx.doi.org/10.3201/eid2104.141949.
9. **Oboho IK, Tomczyk SM, Al-Asmari AM, Banjar AA, Al-Mugti H, Aloraini MS, Alkhaldi KZ, Almohammadi EL, Alraddadi BM, Gerber SI, Swerdlow DL, Watson JT, Madani TA.** 2015. 2014 MERS-CoV outbreak in Jeddah—a link to health care facilities. N Engl J Med **372:**846–854. http://dx.doi.org/10.1056/NEJMoa1408636.
10. **Butler D.** 2015. South Korean MERS outbreak spotlights lack of research. Nature **522:**139–140. http://dx.doi.org/10.1038/522139a.
11. **Ki M.** 2015. 2015 MERS outbreak in Korea: hospital-to-hospital transmission. Epidemiol Health **37:**e2015033. http://dx.doi.org/10.4178/epih/e2015033.
12. **Otter JA, Yezli S, Salkeld JAG, French GL.** 2013. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. Am J Infect Control **41:**S6–S11. http://dx.doi.org/10.1016/j.ajic.2012.12.004.
13. **Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH, Jr.** 1982. Survival of influenza viruses on environmental surfaces. J Infect Dis **146:**47–51. http://dx.doi.org/10.1093/infdis/146.1.47.
14. **Wilks SA, Michels H, Keevil CW.** 2005. The survival of *Escherichia coli* O157 on a range of metal surfaces. Int J Food Microbiol **105:**445–454. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.04.021.
15. **Noyce JO, Michels H, Keevil CW.** 2006. Potential use of copper surfaces to reduce survival of epidemic meticillin-resistant *Staphylococcus aureus* in the healthcare environment. J Hosp Infect **63:**289–297. http://dx.doi.org/10.1016/j.jhin.2005.12.008.
16. **Noyce JO, Michels H, Keevil CW.** 2006. Use of copper cast alloys to control *Escherichia coli* O157 cross-contamination during food processing. Appl Environ Microbiol **72:**4239–4244. http://dx.doi.org/10.1128/AEM.02532-05.
17. **Weaver L, Michels HT, Keevil CW.** 2008. Survival of *Clostridium difficile* on copper and steel: futuristic options for hospital hygiene. J Hosp Infect **68:**145–151. http://dx.doi.org/10.1016/j.jhin.2007.11.011.
18. **Weaver L, Michels HT, Keevil CW.** 2010. Potential for preventing spread of fungi in air-conditioning systems constructed using copper instead of aluminium. Lett Appl Microbiol **50:**18–23. http://dx.doi.org/10.1111/j.1472-765X.2009.02753.x.
19. **Warnes SL, Green SM, Michels HT, Keevil CW.** 2010. Biocidal efficacy of copper alloys against pathogenic enterococci involves degradation of genomic and plasmid DNAs. Appl Environ Microbiol **76:**5390–5401. http://dx.doi.org/10.1128/AEM.03050-09.
20. **Warnes SL, Keevil CW.** 2011. Mechanism of copper surface toxicity in vancomycin-resistant enterococci following wet or dry surface contact. Appl Environ Microbiol **77:**6049–6059. http://dx.doi.org/10.1128/AEM.00597-11.
21. **Warnes SL, Caves V, Keevil CW.** 2012. Mechanism of copper surface toxicity in *Escherichia coli* O157:H7 and *Salmonella* involves immediate membrane depolarization followed by slower rate of DNA destruction which differs from that observed for Gram-positive bacteria. Environ Microbiol **14:**1730–1743. http://dx.doi.org/10.1111/j.1462-2920.2011.02677.x.
22. **Casey AL, Adams D, Karpanen TJ, Lambert PA, Cookson BD, Nightingale P, Miruszenko L, Shillam R, Christian P, Elliott TSJ.** 2010. Role of copper in reducing hospital environment contamination. J Hosp Infect **74:**72–77. http://dx.doi.org/10.1016/j.jhin.2009.08.018.
23. **Karpanen TJ, Casey AL, Lambert PA, Cookson BD, Nightingale P, Miruszenko L, Elliott TSJ.** 2012. The antimicrobial efficacy of copper alloy furnishing in the clinical environment: a crossover study. Infect Control Hosp Epidemiol **33:**3–9. http://dx.doi.org/10.1086/663644.
24. **Schmidt MG, Attaway HH, Sharpe PA, John J, Jr, Sepkowitz KA, Morgan A, Fairey SE, Singh S, Steed LL, Cantey JR, Freeman KD, Michels HT, Salgado CD.** 2012. Sustained reduction of microbial burden on common hospital surfaces through introduction of copper. J Clin Microbiol **50:**2217–2223. http://dx.doi.org/10.1128/JCM.01032-12.
25. **Salgado CD, Sepkowitz KA, John JF, Cantey JR, Attaway HH, Freeman KD, Sharpe PA, Michels HT, Schmidt MG.** 2013. Copper surfaces reduce the rate of healthcare-acquired infections in the intensive care unit. Infect Control Hosp Epidemiol **34:**479–486. http://dx.doi.org/10.1086/670207.
26. **Warnes SL, Keevil CW.** 2013. Inactivation of norovirus on dry copper alloy surfaces. PLoS One **8:**e75017. http://dx.doi.org/10.1371/journal.pone.0075017.
27. **Warnes SL, Summersgill EN, Keevil CW.** 2015. Inactivation of murine