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;

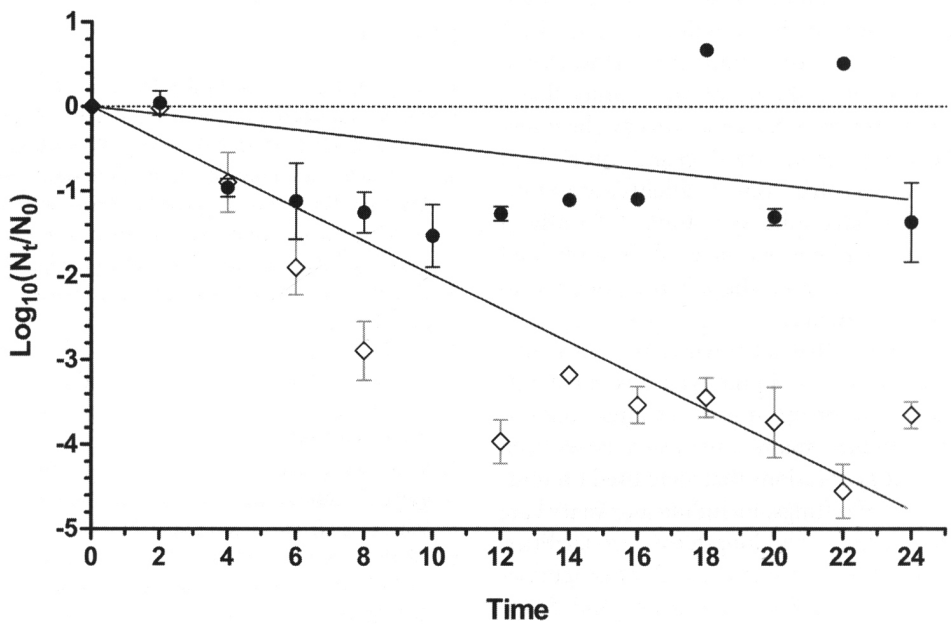

FIGURE 1. Survival of bacteriophage Φ6 over 24 hours at 22°C at 40% and 60% relative humidity (6 replicates per point). Circles, 40%; diamonds, 60%. Regression lines: solid lines, 40%; dashed line, 60%. Bars, 95% confidence interval.

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$P < .0001$). 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.

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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-