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Assessment of influenza virus exposure and recovery from contaminated surgical masks and N95 respirators

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Abstract

Healthcare workers (HCWs) are at significantly higher risk of exposure to influenza virus during seasonal epidemics and global pandemics. During the 2009 influenza pandemic, some healthcare organizations recommended that HCWs wear respiratory protection such as filtering facepiece respirators, while others indicated that facemasks such as surgical masks (SMs) were sufficient. To assess the level of exposure a HCW may possibly encounter, the aim of this study was to (1.) evaluate if SMs and N95 respirators can serve as “personal bioaerosol samplers” for influenza virus and (2.) determine if SMs and N95 respirators contaminated by influenza laden aerosols can serve as a source of infectious virus for indirect contact transmission. This effort is part of a National Institute for Occupational Safety and Health 5-year multidisciplinary study to determine the routes of influenza transmission in healthcare settings. A coughing simulator was programmed to cough aerosol particles containing influenza virus over a wide concentration range into an aerosol exposure simulation chamber virus/L of exam room air), and a breathing simulator was used to collect virus on either a SM or N95 respirator. Extraction buffers containing nonionic and anionic detergents as well as various protein additives were used to recover influenza virus from the masks and respirators. The inclusion of 0.1% SDS resulted in maximal influenza RNA recovery (41.3%) but with a complete loss of infectivity whereas inclusion of 0.1% bovine serum albumin resulted in reduced RNA recovery (6.8%) but maximal retention of virus infectivity (17.9%). Our results show that a HCW’s potential exposure to airborne influenza virus can be assessed in part through analysis of their SMs and N95 respirators, which can effectively serve as personal bioaerosol samplers.

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Keywords

Influenza A; Aerosol transmission; Healthcare worker; PPE contamination; Virus extraction; Quantitative PCR

1. Introduction

Genetic and environmental factors are constantly influencing the transmissibility and infectivity of influenza viruses. As a result, millions of people worldwide are at risk of developing an acute viral infection, and seasonal epidemics as well as global pandemics continue to cause significant morbidity and mortality. The CDC estimates that 9.2–35.6 million influenza illnesses and 12,000–15,000 deaths in the United States have occurred annually since 2010 (CDC, 2017. While vaccination is considered one of the first lines of defense against influenza virus, vaccines may not be immediately available during an outbreak of a novel influenza virus. A better understanding of influenza exposure and transmission is needed to determine the best interventions to avoid the spread of this virus.

Current literature shows that transmission occurs through direct and indirect contact with infectious respiratory secretions (Brankston et al., 2007; Killingley and Nguyen-Van-Tam, 2013; Tellier, 2009; Weber and Stilianakis, 2008) and growing experimental evidence indicates that influenza viruses are transmitted through airborne respiratory particles (Bischoff et al., 2013; Blachere et al., 2009; Lednicky and Loeb, 2013; Leung et al., 2016; Lindsley et al., 2010a; Thompson et al., 2013; Tseng et al., 2010; Yang et al., 2011). Engineering and administrative controls are important in mitigating the spread of infectious diseases. However, transmission-based precautions such as hand washing and the use of personal protective equipment (PPE) including gloves, gowns and masks, also play a major role in protecting healthcare workers and preventing healthcare-associated infections. Although respiratory PPE greatly limits exposure to airborne particles, recommendations for PPE usage vary and depend on the application. To reduce exposure to seasonal influenza, the Centers for Disease Control and Prevention (CDC) recommends that HCWs wear SMs during routine patient care and respiratory protection such as N95 respirators while performing aerosol-generating procedures (CDC, 2009). Surgical masks offer limited protection against infectious bioaerosols, yet effectively protect healthcare workers from contact with large particles and are frequently worn to prevent contamination of sterile environments. In comparison, filtering facepiece respirators such as N95 respirators are designed to filter infectious airborne contaminants but healthcare workers often find them to be less comfortable than facemasks, and they must be fit-tested to ensure effective protection. Several laboratory studies have shown that N95 respirators are nearly completely effective at blocking infectious influenza bioaerosols but SMs are not (Bischoff et al., 2013; Harnish et al., 2013; Janssen et al., 2013; Makison Booth et al., 2013; Noti et al., 2012).

Studies investigating the incidence of influenza among HCWs suggest that healthcare employees are at high risk for exposure particularly during an influenza pandemic (Kuster et al., 2011; OSHA, 2015; Peterson et al., 2016; Santos et al., 2010; Wise et al., 2011). Given the elevated demand for HCWs during a pandemic, in 2011 NIOSH initiated a 5-year

multidisciplinary study entitled “Why Healthcare Staff Catch the Flu” (WHSCF) to improve our understanding of how influenza is transmitted including the potential for both aerosol and contact transmission routes. To monitor exposure to influenza aerosols within healthcare settings, studies have used stationary or personal aerosol samplers (Bischoff et al., 2013; Blachere et al., 2009; Lindsley et al., 2010a). Stationary aerosol samplers are usually placed in a patient room and the aerosol is collected on a filter over a period of time. Unfortunately with stationary aerosol samplers, sample collection often occurs away from the patient and is not indicative of direct exposure to the healthcare worker. Personal aerosol samplers have been placed on healthcare workers while conducting patient care activities and analyzed for influenza virus. The use of personal aerosol samplers permits the collection of bioaerosols that are more representative of a healthcare employee’s exposure when in close contact with a patient. Nonetheless, personal aerosol samplers can be cumbersome to wear, are expensive to purchase, and are often available for a limited number of study participants. As part of the WHSCF study, PPE from HCWs and aerosol samples from the Johns Hopkins Student Health Facility and the Adult Emergency Department were collected and analyzed for influenza to determine the relationship between levels of airborne influenza virus and PPE contamination. In particular, SMs and N95 respirators were assessed to determine whether these PPE can serve as “personal bioaerosol samplers” to evaluate potential airborne exposure to influenza virus and also determine if contaminated PPE could serve as a source for infectious virus. Surgical masks and respirators are ubiquitous in a healthcare setting during influenza season and may serve as a tool to assess HCW exposure during specific patient encounters and care activities which may increase exposure potential, such as aerosol generating procedures. However, collection and subsequent detection of influenza from PPE can be difficult. Experimental challenges such as the effect of storage conditions on virus infectivity and nucleic acid stability, low virus recovery efficiency from porous PPE materials, and potentially low virus concentrations of virus expected on respirator and masks used in the field are a few of the concerns this study aimed to address. To establish whether contaminated PPE could be used to assess levels of airborne influenza exposure within a healthcare setting, laboratory studies were performed utilizing a previously described aerosol exposure simulation chamber, with coughing and breathing simulators (Lindsley et al., 2013; Noti et al., 2013; Noti et al., 2012). Aerosol samples along with SMs and N95 respirators placed on the breathing simulator, were analyzed to determine the lowest concentration of influenza virus that could be detected both in the air and on respiratory PPE.

2. Materials and methods

2.1. Cell and virus stock

Madin-Darby canine kidney (MDCK) cells (ATCC CCL-34) and influenza A(H1N1) strain A/WS/33 (ATCC VR-825) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Complete growth medium for MDCK cells consisted of Eagle’s Minimum Essential Medium (EMEM) (ATCC) containing 10% fetal bovine serum (Hyclone Laboratories Inc, Logan, UT), 200 units/ml penicillin G, 200 μg/ml streptomycin (Invitrogen, Carlsbad, CA). MDCK cells were incubated at 35 °C in a humidified 5% CO_2 incubator until approximately 80% confluent. Propagation of influenza A(H1N1) [1.0×10^7

$TCID_{50}$] and dilution in Viral Transport Media (VTM) consisting of Hank's Balanced Salt Solution (1X HBSS; ThermoFisher Scientific) supplemented with 0.1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA), 100 units/ml penicillin G and 100 units/ml streptomycin (ThermoFisher Scientific), was performed as previously described (Blachere et al., 2011).

2.2. RNA Isolation/cDNA transcription/Quantitative PCR

Viral RNA was isolated using the MagMAX™−96 viral RNA Isolation Kit (ThermoFisher Scientific, Waltham, MA) as described previously (Blachere et al., 2011). The entire volume of eluted RNA (32 μl) was transcribed into 40 μl cDNA using the High Capacity RNA to cDNA Transcription Kit (ThermoFisher Scientific) in accordance with the manufacturer's instructions. Quantitative PCR (qPCR) of the influenza matrix (M1) gene expression was performed as described previously (Blachere et al., 2011).

2.3. Virus infectivity following storage

To assess the effects of temperature and length of storage on influenza A(H1N1) infectivity, viral suspensions with a tissue culture infectious dose $(TCID)_{50}$ of 10^6 (high concentration), $TCID_{50}$ 10^4 (medium concentration) and $TCID_{50}$ 10^2 (lowest concentration), were prepared by directly inoculating virus into VTM and storing at either 4 °C, −20 °C or −80 °C for 1, 2, 4, 6, 14 or 18 days. Following storage, viral infectivity (as measured by plaque forming units per milliliter (pfu/mL) of virus solution) was determined by viral plaque assay (Blachere et al., 2011).

2.4. Aerosol exposure simulation chamber

To simulate exposure of a healthcare worker to airborne infectious influenza, a SM or N95 respirator was sealed to the breathing manikin's face and a coughing simulator was programmed to cough influenza virus. All aerosol studies were conducted within a 3.2m×3.2m×2.3m high environmental chamber that was set up to simulate a patient examination room (Lindsley et al., 2013). A schematic diagram of the aerosol exposure simulation chamber can be found in publications by Noti et al. (Noti et al., 2013; Noti et al., 2012) The room included a HEPA filtration system to remove airborne particles before/after testing, an ultraviolet germicidal irradiation system to disinfect the room between experiments, NIOSH BC 251 two-stage cyclone samplers (Lindsley et al., 2006), and coughing and breathing simulators to mimic a coughing patient and breathing healthcare worker. The coughing simulator was programmed to cough a size range of 0.1–30 μm aerosol particles containing influenza virus (0.1 μm is the approximate size on a single influenza virion) over a wide range of concentrations (Lindsley et al. 2013). Influenza A(H1N1) was aerosolized with an Aeroneb 2.5–4-μm volume median diameter micropump nebulizer (Aerogen, Galway, Ireland), as described previously (Noti et al., 2013), and loaded into the cough simulator remotely for a total of 5 coughs at approximately 2-minute intervals as described (Noti et al., 2012). The coughing simulator uses a metal bellows driven by a computer-controlled linear motor (Model STA2506; Copley Controls, Canton, MA) to mimic the flow and aerosol pattern of a human cough. The volume of the coughs was either 2.1 l (peak flow of 8.45 L/s and mean flow of 2.64 L/s) or 4.2 l (peak flow 16.9 L/s and mean flow 5.28 L/s). The digital breathing simulator (Warwick Technologies LTD, Warwick,

UK) was equipped with a standard medium-sized head form (Sheffield model 189,003; ISI, Lawrenceville, GA). The breathing waveform was sinusoidal with a flow rate of 32 L/min (ISO standard for an adult 1.88 m tall with a mass of 85 kg engaged in moderate work) (ISO and ISO, 2007). The coughing and breathing simulators were synchronized so that each cough was initiated at the start of inhalation. A SM (Kimberly Clark 47,625; Irving, TX) or N95 respirator (3 MM1860; 3 M, St. Paul, MN) was sealed with silicone adhesive over the mouth of the breathing simulator to obtain a best-fit scenario and exposed to viral-laden aerosols over the course of one hour. The fit factor of each mask or respirator was measured using a standard respirator fit-testing device (Model 8038 PortaCount Pro Plus; TSI, Shoreview, MN). The mouths of the coughing and breathing simulators and the NIOSH sampler air inlets were positioned 152 cm above the floor (approximate mouth height of a patient sitting on a medical examination table) and 183 cm apart facing directly towards each other. NIOSH samplers collected air samples from positions 10 cm to the left, right and through the breathing manikin's mouth.

2.5. Elution of virus from PPE

Both electrostatic (charged differences between PPE and virus) and hydrophobic interactions (exclusion of water molecules between PPE and virus) account for much of the adsorption of bacteriophage viruses including MS2 and phi X174 to microporous filters (Bean et al., 1982; Coulliette et al., 2013; Greatorex et al., 2011) and various PPE (Sakaguchi et al., 2010). Since proteins are charged molecules, VTM was supplemented with various proteinaceous additives to establish if they could effectively neutralize charge and enhance recovery of influenza virus from SMs and N95 respirators. Additionally, VTM was supplemented with various nonionic and anionic surfactants, which have been shown to minimize hydrophobic interactions and enhance viral recovery from membrane filters (Lytle and Routson, 1995). After a one hour exposure of respiratory PPE to influenza aerosols inside the aerosol exposure simulation chamber, four 25-mm coupons were punched from the central portion of each PPE and virus was eluted from the coupons for approximately 12 h at 4 °C in 8 ml of VTM containing [0.1%] of either brain heart infusion, malt extract, peptone, nutrient broth (Becton Dickenson, Franklin Lakes, NJ), tryptone (Sigma-Aldrich, St. Louis, MO), BSA (Sigma-Aldrich), the nonionic detergents sarcosine, Nonidet P-40, Triton X-100 and Tween-20 (Sigma-Aldrich, St. Louis, MO), or the anionic detergent sodium dodecyl sulfate (SDS) (Fisher Scientific, Hampton, NH). Efficiency of viral recovery was assessed by qPCR analysis of M1 gene copies.

2.6. PEG precipitation

Recovery of influenza virus from contaminated PPE can require large elution volumes that may consequently, lower the detection limit threshold and/or not be amenable with downstream sample analysis. To concentrate virus from large sample volumes, the PEG (polyethylene glycol) Virus Precipitation Kit (BioVision, Mountain View, CA) was used according to the manufacturer's instructions. Briefly, a 1:4 ratio of 5X PEG Solution to VTM inoculated with a concentration range of influenza A(H1N1) was used. To maximize viral recovery, PEG-laden samples were stored overnight at 4 °C. On the following day, samples were centrifuged at 3,200×g for 30 min at 4 °C. The resulting viral pellet was re-

suspended in 500 μl of Lysis Bind Concentrate (ThermoFisher Scientific) and analyzed by M1 qPCR.

3. Results

3.1. Effects of sample storage on influenza infectivity

To determine how long influenza virus extracted onsite from facemasks into VTM could be stored, VTM was directly inoculated with virus at low, medium and high $TCID_{50}$ concentrations, and stored at either 4 °C, −20 °C or −80 °C over a period of 18 days. Using qPCR analysis, we found no statistical significance in M1 gene copies regardless of either temperature or length of storage (Fig. 1A–C). At a $TCID_{50}$ concentration of 10^6, 10^4 or 10^2, the average M1 copy numbers per one mL sample were 4.8×10^7 (SE $=1.7\times10^7$), 4.4×10^5 (SE $= 2.4\times10^5$) and 5.6×10^3 (SE $=2.9\times10^3$), respectively. Similarly, temperature and length of storage did not significantly alter viral infectivity (Fig. 2A–C). At a $TCID_{50}$ concentration of 1×10^6, 1×10^4 or 1×10^2, the average PFU's per one mL sample were 9.6×10^5 (SE $= 1.2\times10^5$), 8.7×10^3 (SE $=1.5\times10^3$) and 8.5×10^1 (SE $= 1.1\times10^1$), respectively.

3.2. Effects of protein and detergent supplementation on the recovery of influenza from exposed PPE

To optimize extraction of A(H1N1) from exposed PPE, VTM was supplemented with various proteins and detergents. Viral recovery, assessed by M1 qPCR analysis, was not improved with the addition of any protein supplement but instead was significantly decreased, particularly with the addition of peptone, tryptone, or nutrient broth (Fig. 3). In contrast, 2–3 fold greater M1 gene copies was detectable with the addition of Tween 20, Nonidet P-40, or Triton X-100, and 5-fold greater with the addition of SDS to VTM (Fig. 4). However, all detergents in part or completely lysed MDCK cells used in the viral plaque assay (data not shown). Attempts to extract influenza viral RNA by directly lysing the virus while bound to the coupons were also not successful. Specifically, the woven fibers of SMs and N95 s were found to interfere with the magnetic binding properties during viral RNA extraction (data not shown).

3.3. Recovery of influenza from large sample volumes using PEG precipitation

To enhance influenza A(H1N1) recovery from large sample volumes, PEG precipitation was employed. Quantitative PCR results in Fig. 5 indicates that despite the concentration of virus, PEG precipitation did not significantly enhance viral recovery from large sample volumes. At high concentrations, more influenza virus was recovered from large sample volumes without the addition of PEG (Fig. 5, samples E–I). Albeit minor, PEG precipitation of viral samples E–I resulted in a 1.1 to 2.0-fold reduction in total M1 gene copies.

3.4. Aerosol exposure of facemasks and analysis of influenza

To simulate the occupational exposure of a HCW to airborne flu particles expelled by a sick patient, a coughing simulator was programmed to cough influenza aerosols inside an exposure chamber. A SM or N95 respirator was sealed to a breathing manikin's face to assess the potential exposure to influenza virus. Analysis of aerosol samples from the exposure chamber studies (N = 19) showed that 10.2% ±10.2 SD of the virus was collected

in the >4 μm aerosol fraction, 59.2% +/− 14.4 SD was in the 1–4 μm fraction, and 30.6% +/−10.3 SD was in the <1.0 μm fraction (Fig. 6). The total virus coughed was adjusted for each experiment to cover a range from 7.0 to 2.7×10^3 influenza virus/L chamber air (Table 1). Over the course of the 19 experiments, the mean percentage of infectious virus collected by the NIOSH samplers was 68% +/−22.5 SD (Table 1). The total amount of virus predicted to be collected on 25 mm diameter coupons punched out from the centers of each SM or N95 respirator affixed to the breathing manikin was determined based on the total surface area of the PPE, the total time of exposure to influenza aerosols, the collection rate of the NIOSH sampler positioned in the manikin's mouth (3.5 L/min), and the breathing rate of the manikin (32 L/min). To maintain infectivity of the virus deposited on the SM or N95 respirator, VTM was used for extraction. The total virus (infectious and non-infectious) extracted per facemask (SM or N95) was 18.9%. The extraction efficiency from SMs was lower (9.9% +/−8.2 SD) than from N95 s (20.6% +/−14.8 SD). The mean percentage of infectious virus extracted from the facemasks was 17.9% +/−21.1 SD. The mean extraction efficiency from facemask coupons in the four chamber studies with the lowest levels of aerosolized virus (7–26 influenza virus/L chamber air, studies 1–4) was 26.1% +/−23.3 SD, similar to the mean extraction efficiency of 16.8% +/−16.4 SD in the four chamber studies with highest levels of aerosolized virus (619–2,714 influenza virus/L chamber air). These aerosol exposure chamber studies demonstrate that a HCWs potential exposure to influenza can in part be revealed by monitoring for virus on their facemask.

4. Discussion

HCWs are at high risk for exposure to influenza during a pandemic, and as such, preparedness is the key to prevention and control of the transmission of influenza. As part of a multi-year study examining the possible routes of influenza transmission in a healthcare setting, our research was aimed to determine whether SMs and N95 respirators could serve as personal bioaerosol samplers for HCWs exposed to influenza during the course of treating patients and explore laboratory methods to recover infectious virus from contaminated PPE. Our experimental setting designed to mimic a HCWs potential exposure to airborne influenza virus showed that efficient extraction and retention of infectivity of influenza-contaminated SMs and N95 respirators could be obtained to provide insight into exposure risks for these HCWs in real-world settings such as hospitals and patient examination rooms. These results also support previous studies that suggest that virus trapped on the outside of SMs and N95 respirators may pose an indirect contact transmission risk as the HCW doffs these PPE after seeing a patient or continues to wear their PPE for an extended period of time.

Our exposure studies on the aerosol transmission of influenza highlight the importance of proper donning and doffing of respiratory PPE, however, limitations and future considerations exist. Influenza A viruses display a pleomorphic morphology that is, in part, related to viral and host factors (Badham and Rossman, 2016). Such factors can affect the amino acid composition and overall net charge on viral surface proteins. Because experimentation was exclusively performed with influenza strain A/WS/33, which has a spherical structure, further studies should examine the binding capacity of the filamentous form of influenza A viruses as well as clinical influenza A viruses, which typically contain

mixed populations of spherical and filamentous virions. Similarly, other influenza A virus subtypes and influenza B viruses should be examined. Conversely, in the event of a flu pandemic, potential shortages of disposable SMs and N95 respirators could mandate their reuse. While disinfection with ultraviolet germicidal irradiation (UVGI) has been shown to decontaminate disposable N95 respirators, efficacy is dependent upon the UVGI dosage, respiratory PPE model and the contaminating microorganism (Lindsley et al., 2015). Future research assessing UVGI disinfection of influenza contaminated disposable SMs and N95 respirators would prove invaluable in protecting HCWs during pandemic and seasonal influenza.

The survival of influenza virus on porous and non-porous surfaces has been investigated by a number of researchers. Buckland showed that influenza deposited and dried on glass inactivated relatively quickly (Buckland and Tyrrell, 1962). Bean et al. (1982) assessed the recovery of influenza from stainless steel, plastic, and cloth by measuring the $TCID_{50}$ of the virus over 48 h and showed that the virus could survive for 24 to 48 h on stainless steel and plastic however, survival was essentially gone after 8 to 12 h when virus was recovered from porous material such as paper or cotton. Greatorex et al. (2011) showed that the $\log_{10}$ reduction in recoverable influenza virus (determined by PCR) inoculated onto porous and non-porous materials varied considerably from 0.06 to 3 after 24 h but that infectious influenza (determined by fluorescent plaque assay) could still be recovered from cloth fabric within 4 h of deposition and within 9 h from stainless steel. Casanova et al. (2009) evaluated various combinations and concentrations of beef extract protein and Tween 80 as eluents to extract MS2 virus from cotton gowns and found that recoveries (determined by plaque assay) varied over a broad range from approximately 8% to 63%. Coulliette et al. (2013) evaluated the survival of influenza virus on N95 respirators (determined by cytopathic effects on infected cells) and concluded that the virus remained infectious up to 6 days with only approximately a 1 $\log_{10}$ reduction after 6 days, in stark contrast to (Bean et al. (1982) and (Sakaguchi et al. (2010) who observed ~3 $\log_{10}$ reduction in infectivity within 8 to 24 h.

Conflicting data regarding virus recovery and survival on porous surfaces led us to evaluate and optimize for extraction and survival of influenza viruses from PPE material. The inclusion of 0.1% SDS in the extraction buffer was most effective for viral RNA recovery (41.3% recovered) but was not conducive for cell-based viability studies. The addition of various proteins did not improve the extraction efficiency beyond that of BSA. After inoculation with influenza virus, overnight storage of coupons at −20°C or −80°C vs 4°C did not significantly alter the extraction efficiency or infectivity of the virus, while storage up to 6 days at the lower temperatures led to considerable decline in infectivity only (data not shown). Therefore, when processing HCWs respiratory PPE to evaluate their potential exposure to influenza aerosols, it would be prudent to perform two extractions, one that included SDS for maximal extraction of both infectious and non-infectious virus and one without SDS to assess infectivity, and to process within 24 h of obtaining the respiratory PPE.

Aerosol collection using the NIOSH samplers showed that 68% of the virus remained infectious. The relative humidity in the exposure simulation chamber was maintained at 20–22% for maximum stability of the aerosolized virus as was previously determined (Noti et

al., 2013). The concentration of influenza virus coughed into the simulated chamber ranged from a high of 2714 virus/L of air to as low as 7 virus/L of air. The amount of influenza virus detected in health-care facilities by Lindsley et al. (Lindsley et al., 2010a) was 12 viruses/L of air and by Yang et al. (Yang et al., 2011) was 16 viruses/L of air, while Tseng et al. (Tseng et al., 2010) reported that the lowest level was 168 viruses/L of air in a pediatric clinic. In a professional examination room setting, the number of aerosolized virus that a HCW could potentially inhale would be dependent on the number of viral particles shed by infected individuals in close proximity and the air flow within the room. Using VPA, Lindsley et al. (Lindsley et al., 2010b) detected only 142 PFU's of influenza in 6 coughs which was potentially much lower than would be seen in a severe pandemic, because patients were young and otherwise healthy outpatients who typically didn't present at the clinic until after peak viral shedding. However, Alford et al. (Alford et al., 1966) determined that the dose required for infection by the aerosol route was approximately 0.7–3.5 PFUs, sufficient to cause seroconversion in 50% of their subjects tested. Milton et al. (Milton et al., 2013) reported that naturally infected patients shed 33 copies/minute in aerosol particles ≥5 μm and 187 viral copies/minute in particles <5 μm. Tellier et al. (Tellier, 2009) calculated that 150–169 viruses were equivalent to 1 $TCID_{50}$, and Alford et al (Alford et al., 1966) reported an influenza virus HID_{50} of 0.6–3 $TCID_{50}$, equivalent to 90–1950 viruses. Teunis et al. (Teunis et al., 2010) developed an influenza A dose response model for infectivity and pathogenicity and concluded that the probabilities of infection by either aerosol or droplet transmission are approximately equal, and that the probability of infection is significant (P_{inf} =0.2–0.4) at low doses (10^{1-2} $TCID_{50}$ infectious units). They discussed that most of the freshly shed virus are potentially infectious, but environmental conditions may rapidly render the virus non-infectious. If the reported detectable concentration of virus throughout a healthcare facility is 12–16 viruses/L air then a HCW breathing 32 l of air/min could potentially inhale 23,040–30,720 viruses every hour.

A healthcare worker's exposure to virus can also vary greatly due to distance from a coughing patient, whether the patient is wearing a facemask for source control (CDC, 2009), the roles of gravitational settling, ventilation, and virus inactivation at a relative humidity ranging from 10 to 90%. Based on the assumption that virus was uniformly distributed within particles, Yang et al. (Yang and Marr, 2011) calculated that ten minutes after a cough, settling can remove over 80% of airborne influenza, and raising the relative humidity increases the removal efficiency only slightly. We applied a similar model in an earlier report (Noti et al., 2013) by coughing influenza virus from a cough simulator for 5 min and then assessed how much virus remained airborne and infectious in our exposure simulation chamber 1 h and 5 h later. We predicted that the amount of virus in the largest particle fraction (> 4 μm) collected during the fifth hour would be reduced to 6% of that seen during the first hour; the second fraction (1–4 μm) would be reduced to 30%; and the smallest particle fraction (< 1 μm) would be reduced to 58%. Our actual results showed that during the fifth hour the amount of virus fell to 13%, 28%, and 50% in the >4 μm, 1–4 μm and <1 μm fraction, respectively, of that detected during the first hour due to gravitational settling (ventilation was not a factor as it was turned off and relative humidity was constant at 20%), which compared very well to the model predictions. Although most of the >4 μm particles were removed from the exam room after 4 to 5 h, a further decline in infectivity associated

with the larger viral particles to nearly zero was shown when the relative humidity was raised to 45% and the potential for infection from influenza carried on the smaller particles was also further reduced (Noti et al., 2013). However, the longer retention time in the air of these smaller aerosol particles emphasizes the exposure risk they still pose long after an exam room is vacant.

Lastly, Bishchoff et al. (Bischoff et al., 2013) showed that 19% of hospitalized patients were super emitters who released up to 20,400 viruses in 20 min, significantly more than the majority that released <1300 viruses. They also showed that all their patients shed within the lower HID_{50} of 0.6, yet 8% (5/61) of patients shed more than the higher HID_{50} of 3. Lindsley et al. (Lindsley et al., 2010b) showed that 45% of the virus collected from patient's coughs using the NIOSH aerosol sampler came from just 4 of 38 subjects with influenza. These findings demonstrate that a HCWs risk of exposure is related not just to the number of patients they treat, but also the concentration of infectious airborne particles that they shed, which likely depends upon the severity of the patient's infection and other factors such as the genetic makeup of the virus and environmental constraints (Schrauwen and Fouchier, 2014).

5. Conclusions

Our experimental setting designed to mimic a HCWs potential exposure to influenza virus demonstrated efficient viral extraction and retention of infectivity on contaminated SMs and N95 respirators. Moreover, influenza-contaminated SMs and N95 respirators provide insight into the aerosol exposure risks that HCWs may encounter in real-world settings such as hospitals and patient examination rooms. These results also support previous studies that suggest that virus trapped on the outside of facemasks and respirators may pose an indirect contact transmission risk as the HCW doffs these PPE after seeing a patient or continues to wear their PPE for an extended period of time, and testing of contaminated PPE can help advise emergency preparedness plans to decontaminate and re-use respiratory PPE in a pandemic (Fisher et al., 2014; Fisher and Shaffer, 2014).

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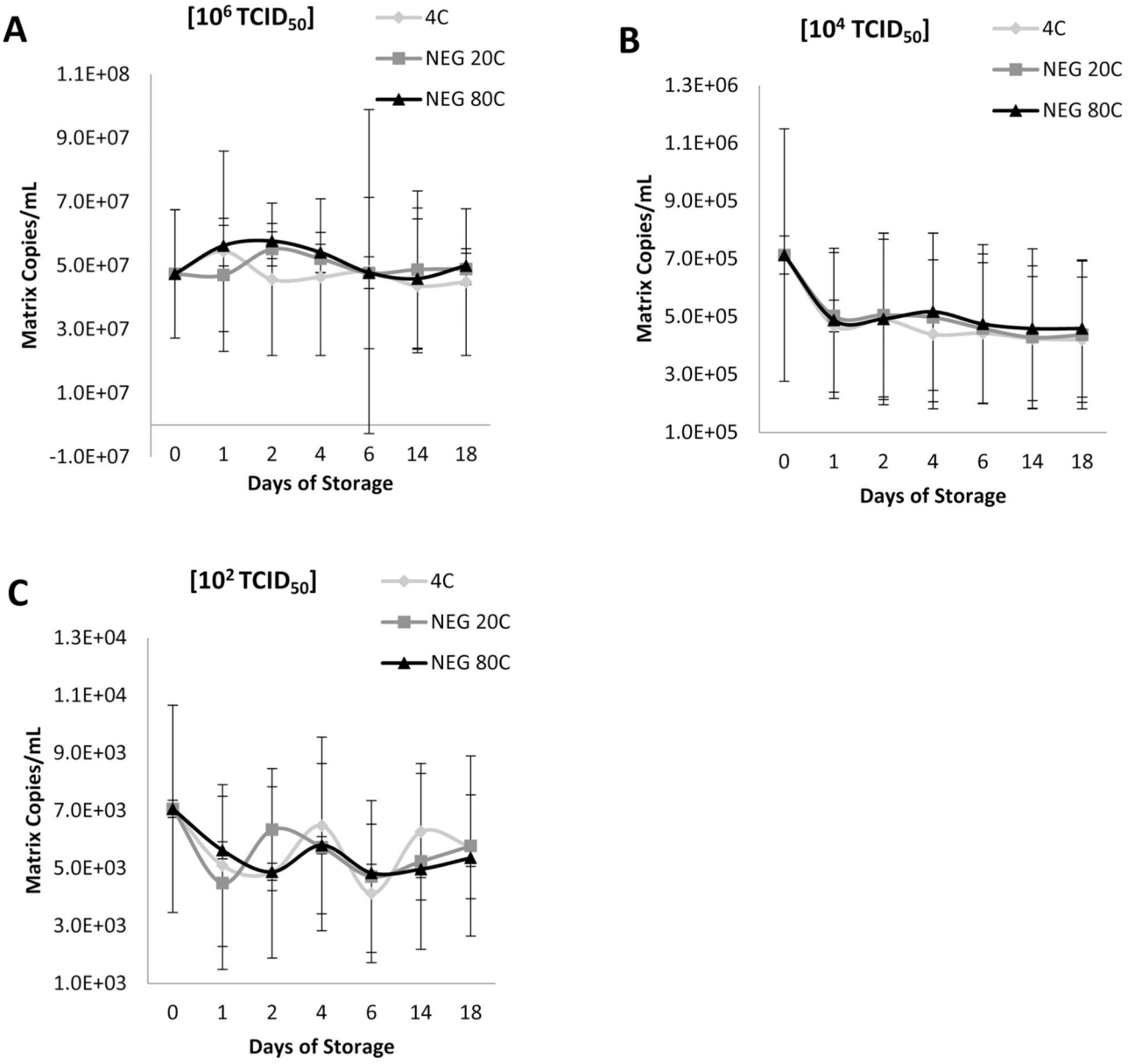

Fig. 1.

A, B, C. Effects of temperature and length of storage, as measured by M1 gene copies, on the stability of influenza A(H1N1) at a $TCID_{50}$ concentration of (A) 7×10^3 virus/ml of VTM (B) 7×10^4 virus/ml VTM, and (C) 5×10^6 virus/ml VTM, stored for 0–18 days at 4°C, −20°C, or −80°C. Data shown represent the arithmetic mean (n =3) with error bars showing the standard error.

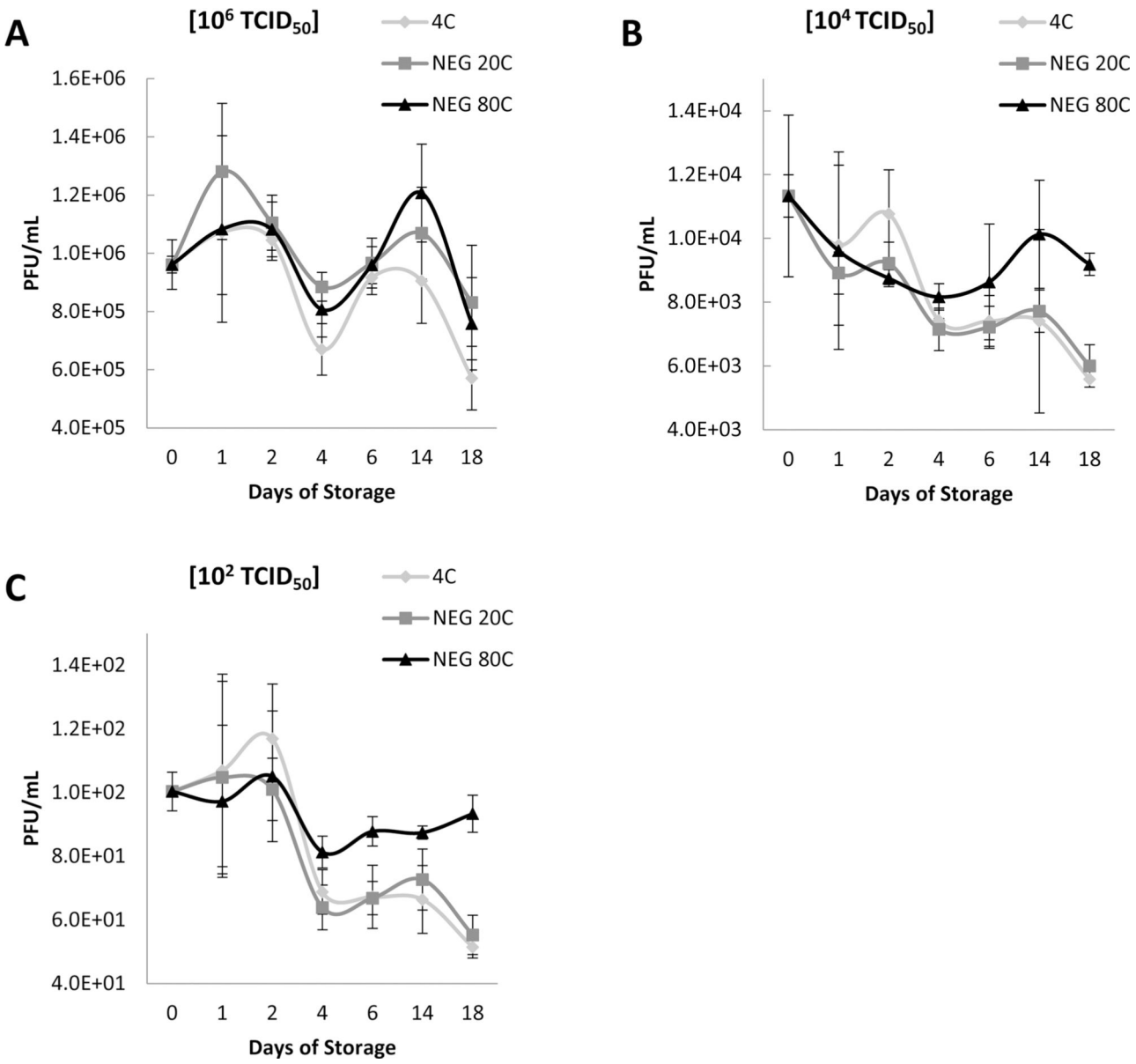

Fig. 2.

A, B, C. Effects of temperature and length of storage, as measured by viral plaque assay (VPA), on the infectivity of influenza A(H1N1) at a $TCID_{50}$ concentration of (A) 7×10^3 virus/ml of VTM (B) 7×10^4 virus/ml VTM, and (C) 5×10^6 virus/ml VTM, stored for 0–18 days at 4 °C, −20 °C, or −80 °C. Data shown represent the arithmetic mean (n = 3) with error bars showing the standard error.

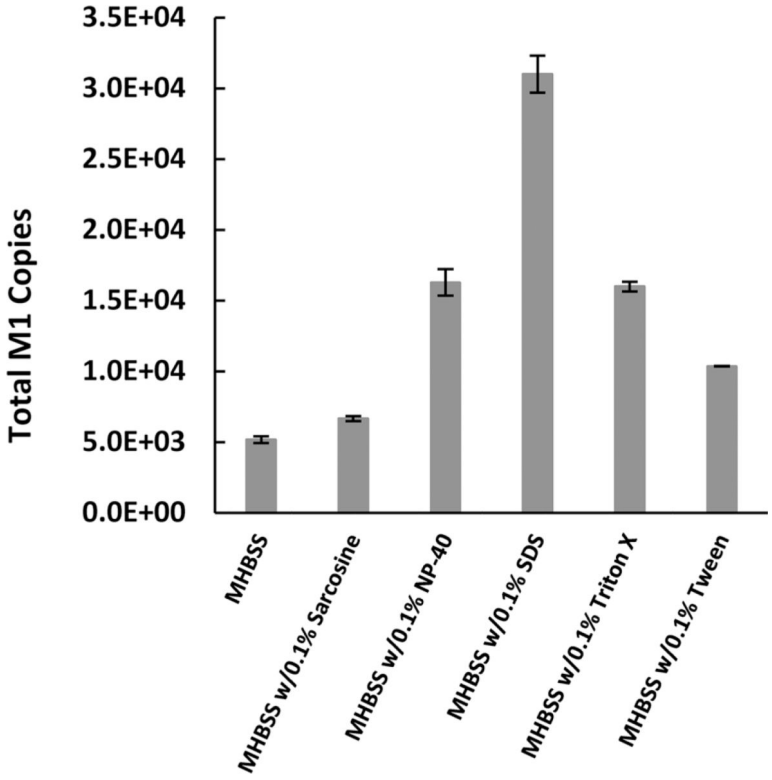

Fig. 3.

Effects of protein supplements on influenza A(H1N1) recovery from N95 respirator coupons. Influenza virus was inoculated onto 25 mm diameter coupons punched out from an N95 respirator. The coupons were stored overnight at 4°C in 2 ml VTM supplemented with either brain heart infusion, malt extract, peptone, tryptone, nutrient broth, or BSA to a final concentration of 0.1%. The eluents were analyzed by qPCR for M1 gene copies. Data shown represent the arithmetic mean (n =2) with error bars showing the standard error.

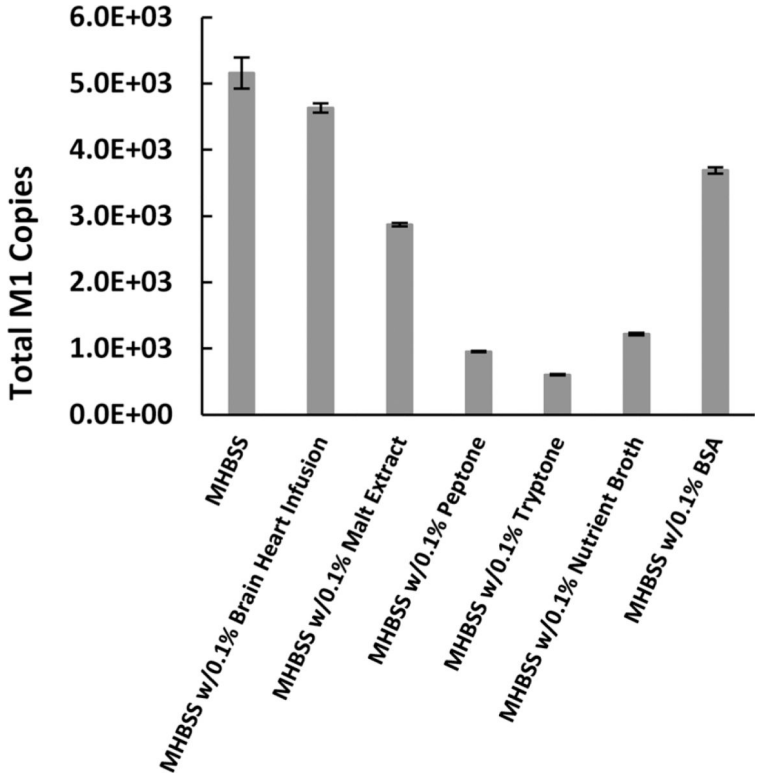

Fig. 4.

Effects of detergents on influenza A(H1N1) recovery from N95 respirator coupons. Influenza virus was inoculated onto 25 mm diameter coupons punched out from an N95 respirator.The coupons were stored overnight at 4°C in 2 ml VTM supplemented with either the nonionic detergents sarcosine, NP40, Tween 80 or Triton X-100, or the anionic detergent SDS, to a final concentration of 0.1%. The eluents were analyzed by qPCR for M1 gene copies. Data shown represent the arithmetic mean (n =2) with error bars showing the standard error.

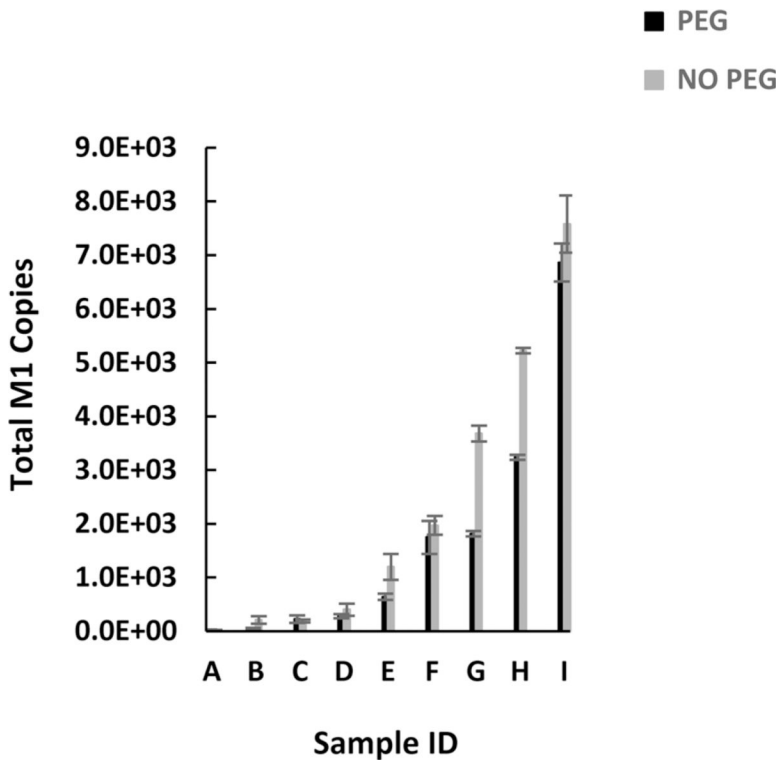

Fig. 5.

Recovery of influenza A(H1N1) by PEG precipitation. Influenza virus was inoculated into 10 ml VTM at a wide concentration range equivalent to (A) 3.2×10^2, (B) 1.6×10^3, (C) 3.2×10^3, (D) 6.3×10^3, (E) 1.6×10^4, (F) 3.2×10^4, (G) 4.8×10^4, (H) 9.5×10^4, and (I) 1.6×10^5 M1 gene copies. The prepared 10 ml samples were either processed directly or PEG-precipitated and then processed. All samples were analyzed by qPCR for M1 gene copies. Data shown represent the arithmetic mean (n =4) with error bars showing the standard error.

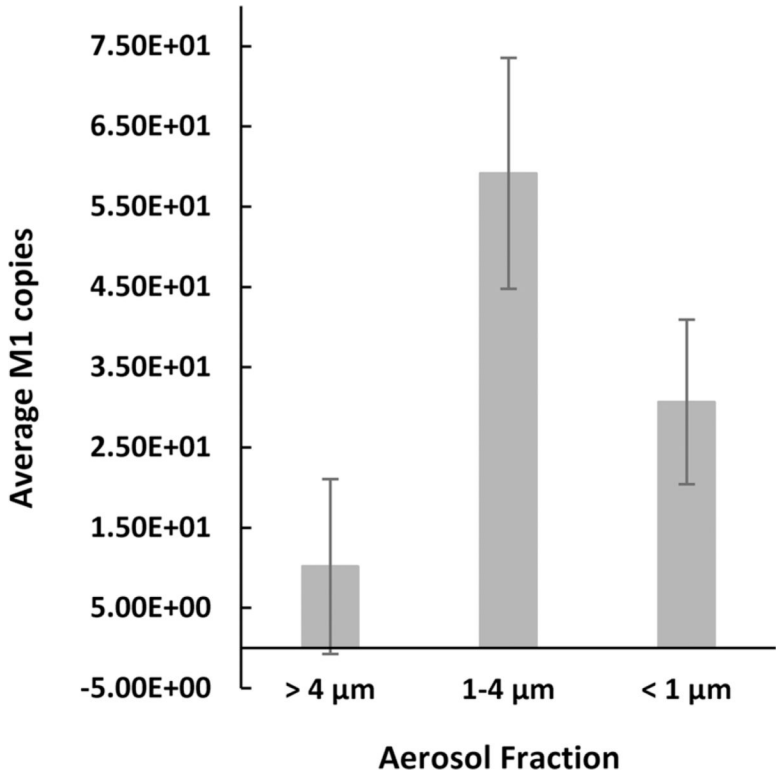

Fig. 6.

Aerodynamic particle size distribution of influenza A(H1N1) in a cough exposure simulation chamber. Data shown represent the arithmetic mean (n = 19) of detectable M1 gene copies with error bars showing the standard error.

Table 1

Environmental chamber studies examining influenza A(H1N1) extraction efficiency and infectivity from exposed surgical a masks and N95 respirators.

Chamber Study Number	Total Virus per L of Chamber Air	% Infectious Virus in NIOSH Sampler	% Total Virus Extracted from SM or N95	% Total Virus Extracted from SM	% Total Virus Extracted from N95	% Infectious Virus Extracted from SM or N95
1	7	100	49.8		49.8	4
2	14	ND	42.3		42.3	ND
3	14	48	4.5		4.5	0
4	26	100	7.6	7.6		43
5	32	ND	12.7		12.7	ND
6	47	ND	20.9		20.9	ND
7	76	50	22.1		22.1	0
8	81	57	11.6		11.6	0
9	133	ND	19	19		ND
10	171	ND	24.2		24.2	0
11	205	53	3.1	3.1		9.2
12	248	82	35.2		35.2	18
13	405	35	12.5		12.5	71
14	524	51	9.2		9.2	26
15	619	64	3.1		3.1	ND
16	762	ND	45		45	ND
17	2152	84	10.3		10.3	15
18	2619	100	16.4		16.4	33
19	2714	60	9.3		9.3	14
	Mean	68	18.88	9.9	20.57	17.94
	SD	22.46	14.34	8.20	14.84	21.05
	SE	6.23	3.30	4.73	8.57	5.84

% Extraction Efficiency from surgical masks (SM) and N95 respirators (N95) for chamber studies 1,2,3,4 (Lowest virus concentration in chamber): Mean 26.05 (SD: 23.33; SE: 9.04).

% Extraction Efficiency from SM and N95 for studies 15, 16, 17, 18, 19 (Highest virus concentration in chamber): Mean 16.82 (SD: 16.44; SE: 7.35). ND = Not Determined.