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Measurement of Particle Sizes Associated with Airborne Viruses

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3. List of terms and abbreviations

AIV: avian influenza virus

SIV: swine influenza virus

aMPV: avian pneumovirus

MS2: bacteriophage MS-2

TGEV: transmissible gastroenteritis virus

HAdV-1: human adenovirus type 1

ACI: Andersen cascade impactor

DMA: differential mobility analyzer

SMPS: scanning mobility particle sizer

MOUDI: micro-orifice uniform deposit impactor

HVAC filters: heating ventilation, and air conditioning filters

AH: absolute humidity

RH: relative humidity

PCR: polymerase chain reaction

UVGI: ultra-violet germicidal irradiation

4. Abstract

Health care personnel, swine and poultry farm workers, airline and public transportation workers, and others may be infected by a variety of viruses that can be transmitted through air. Conventional wisdom suggests that most transmission of infectious viruses occurs by droplet transmission although more recent research indicates that at least some viruses can be transmitted by the airborne route. This project assessed the sizes of particles with which viruses are associated in occupational environments. Methods were developed to quantify the amount of virus associated with airborne particles of different sizes and the viability of these viruses in the environment. The airborne particles were separated into different size ranges. We compared the eight-stage non-viable Andersen cascade impactor (ACI) and a micro-orifice uniform deposit impactor (MOUDI) and found that although both impactors were capable of size-selectively sampling viral aerosols, the ACI achieved higher relative recoveries than the MOUDI. The second step was to analyze the particles divided into each size range for the amount of virus present within them. The quantification of live viruses (avian influenza virus, swine influenza virus, avian metapneumovirus, human adenovirus type 1, and transmissible gastroenteritis virus) was accomplished by inoculation of appropriate cell culture systems. The bacteriophage MS-2 was quantified using its *Escherichia coli* host. Molecular methods such as polymerase chain reaction (PCR) and RT-PCR were also used as a semi-quantitative method to detect both live and inactivated viruses. We found that virus survival in air depended on both virus type and particle size. In general, virus survival was higher at large particle size (300-450 nm) than at 100-200 nm. Most of the published studies have used airborne viruses generated from artificial nebulizer suspensions. We conducted a study by aerosolizing MS-2 suspended in human saliva and found that actual human saliva was much less protective than cell culture media or artificial saliva. In another study, three different models of respirators were evaluated. Results suggest that the current NIOSH (photometer based) certification method is appropriate to prescreen respirators for infection control applications. An environmental chamber,which simulated a typical indoor environment, was then used to study viral aerosol survivability, transmission, and sampling. Aerosols of MS-2, human adenovirus type 1, and avian influenza virus were sampled size-selectively using a non-viable Andersen impactor. MS2 and AIV showed higher survival at lower temperature. Absolute humidity (AH) was found to be a better predictor of virus survival than RH, and the interaction between AH and temperature was not significant. In another study, susceptibility of aerosolized virus to ultra-violet germicidal irradiation (UVGI) was examined; all three viruses were significantly inactivated by UVGI. Due to damaged nucleic acid, most of the virus inactivated by UVGI could not be detected by either culture-based titration or by molecular methods.In summary, the results of this study has improved our understanding of virus behavior in air and will influence the procedures and technology used to prevent virus transmission in health care facilities, animal facilities, public venues, and other workplaces.

5. Section 1:

a. Significant findings

We found that stable experimental aerosols of human adenovirus (HAdV), bacteriophage MS-2, avian influenza virus (AIV), swine influenza virus (SIV), avian metapneumovirus (aMPV), and transmissible gastroenteritis virus (TGEV) can be generated without loss of infectivity as a result of nebulization. This indicates that viral aerosols can be generated experimentally with minimal loss of virus viability due to the aerosolization process. Cascade impactors were able to sample airborne virus particles larger than about 0.5 μm in diameter. The type of impactor did not make a significant difference in virus recovery across all six viruses tested. However, the use of deionized, filtered water and MEM led to higher recovery on average than MEM with glycerol. Recovery of live virus also varied substantially as a function of virus type. TGEV and MS-2 showed the highest recovery; on the order of 30% averaged across all other test factors. The AIV, aMPV, and HAdV had significantly lower recovery (approximately 2-3%). SIV had the lowest recovery of all (less than 1%) although there was no statistically significant difference the recovery of SIV and AIV. The results demonstrate that MS-2, despite being used widely as a general virus surrogate for aerosol testing, is a poor surrogate for influenza viruses. Recovery of live virus with impactors was affected by relative humidity (RH). MS-2 recovery was the highest at 50% RH while TGEV, AIV, and HAdV had the lowest recovery at 50% RHs. Averaged across all particle diameters, recovery of HAdV and AIV was the highest at 85% RH.

Classification of virus-containing particles followed by sampling with gelatin filters can effectively measure virus concentrations in particles with diameters of 100 to 500 nm. For MS-2, approximately 30% of airborne virus was recovered live, independent of particle size. This is comparable to impactor sampling for MS-2 in larger particles. For TGEV, AIV, and SIV, recovery of live virus depended strongly on particle diameter; the recovery was much lower at 100-200 nm than at 300 nm and above. Recovery of live virus was generally greater for TGEV and SIV than for MS-2 and AIV at particle sizes of 300 nm and larger.

Field tests using impactors in two swine barns that had laboratory proven SIV infection indicated that virus is substantially more difficult to recover in real workplace environments than in laboratory experiments. In the first barn, all samples were negative for SIV by both rRT-PCR and virus isolation. In the second barn, results from virus isolation were negative but rRT-PCR was positive for SIV RNA from 5 of 7 impactor stages for a 30-minute sample. This corresponded to particles of 0.65-2.1 and 3.3-9.0μm in diameter. rRT-PCR was also positive for 2 of 7 impactor stages for 15-minute samples, corresponding to particle sizes of 0.65-2.1 μm in diameter. The detection of viral RNA and not infectious virus in field samples indicates that either infectious virus was not present in these environments or that impactor collection plates may not have been effective at keeping sampled viruses live from the time they were collected to the time they were analyzed in the laboratory.

b. Translation of findings

Although we found that impactors can sample viruses successfully in laboratory settings for analysis using molecular techniques, they were not as effective at sampling live influenza viruses in these tests. Furthermore, impactors have not been very successful at providing samples of infectious influenza viruses in field sampling we have performed, although they effectively

sample viral RNA. Laboratory tests using size separation by electrical mobility classification followed by filtration on gelatin filters at low velocity provided much higher recovery of live influenza virus than impactors did in equivalent tests. This comparison suggests that a high-volume, size-differentiating sampler for virus-containing particles that collects the size-separated particles on filters at low velocity may be our best option for measuring concentrations of infectious airborne viruses in workplace atmospheres as a function of particle size. A challenge for this approach is keeping the sampler small enough to be portable for practicing industrial hygienists.

Further research is warranted to understand how to sample airborne viruses effectively. Over the last decade, emerging viruses such as severe acute respiratory syndrome (SARS), H5N1 avian influenza virus, novel 2009 H1N1 influenza virus, and 2012 H3N2 variant influenza virus have posed real or potential risks to workers. Among those at risk from viral exposures are healthcare workers, clinical laboratory workers, mortuary workers, poultry and swine workers, veterinary workers, transportation workers, business travellers, educators, and members of the armed services. Most of the emerging viruses are thought to be able to be transmitted through air among people or between animals and people. To know how far infectious virus-containing particles may travel through air, to understand where they deposit in human respiratory systems, and to identify the most effective methods tor limiting exposure to the particles, we must first be able to measure the sizes of particles with which live viruses are associated. In this project, our research team developed the knowledge and experience to answer the critical research question that few, if any, other groups are capable of addressing: How do you accurately measure the sizes of particles with which infectious viruses are associated in workplace atmospheres?

c. Outcomes/Impact

Intermediate outcome: Subsequent to the conclusion of this project, the techniques developed in this study have been used to assess concentrations of influenza viruses in swine and poultry production operations. The Andersen cascade impactor (ACI) in particular, has been effective at measuring size-differentiated concentrations of influenza virus RNA in swine and poultry facilities. Measurements using the ACI have shown that RNA of the emerging, highly-pathogenic H5N2 avian influenza virus, that is currently affecting turkeys and chickens in the Midwest area, is present in particles larger than about 3 μm in diameter. The collection of live virus from airborne samples has been less common. These results demonstrate that the virus aerosol sampling techniques developed in this research can be applied to outbreak investigations to evaluate the risk that workers face from airborne transmission of potentially-infectious viruses.

Potential outcomes: The techniques developed in this research could be used to measure size-by-size concentrations of virus in mass transit vehicles. Influenza and rhinoviruses, which are relevant in exposures to mass transit workers and commuting workers, could be measured in buses and light rail vehicles during high-occupancy periods because virus concentrations are likely to be high due to the confinement of riders. In addition, the same techniques could be used to measure size-by-size concentrations of virus in healthcare facilities. Influenza-, parainfluenza- and rhinoviruses could be measured in healthcare facility waiting rooms to evaluate the exposure of healthcare workers to airborne viral agents. The size-differentiated measurements made in various workplaces could be used to choose optimal control measures to manage worker exposures to viral agents.

6. Section 2:

Viral aerosol transmission has become an important focus of research because aerosolized viruses can travel long distances and remain infective for a long time. In spite of a large number of studies on viral aerosols a number of questions remain due possibly to the great variety of viruses, the uncertainty of their measurement, and the differences in test methods. Two major studies were designed to answer the question of particle sizes with which viruses are associated. Study 1 was designed to: develop and validate a method to determine virus concentrations in air as a function of particle size, to use the method to measure the particle sizes with which airborne viruses are associated in occupational settings, and to begin to assess the effectiveness of control measures for viral aerosols as a function of particle size. Study 2 was designed to size-selectively study six different viruses under various conditions of temperature and humidity. In addition, the effect of ultraviolet germicidal irradiation (UVGI) and HVAC filters (heating, ventilation, and air conditioning) was also studied on the removal/inactivation of viruses. All tests for this study were conducted in an environmental chamber with relatively low viral aerosol concentration to simulate a common indoor situation.

Study 1: The potential involvement of virus aerosols in the transmission of human respiratory diseases has led to increased public concern. Although laboratory generated virus aerosols have been widely studied in terms of infectivity and survivability, how they are related to particle size, especially in the submicron size range, is not understood. We aerosolized four viruses (MS2 bacteriophage, transmissible gastroenteritis virus, swine influenza virus, and avian influenza virus), size classified (100–450 nm) them using a differential mobility analyzer (DMA), and collected particles onto gelatin filters. Uranine dye was also nebulized with the virus, serving as a particle tracer. Fig. 1 shows a schematic of experimental setup used.

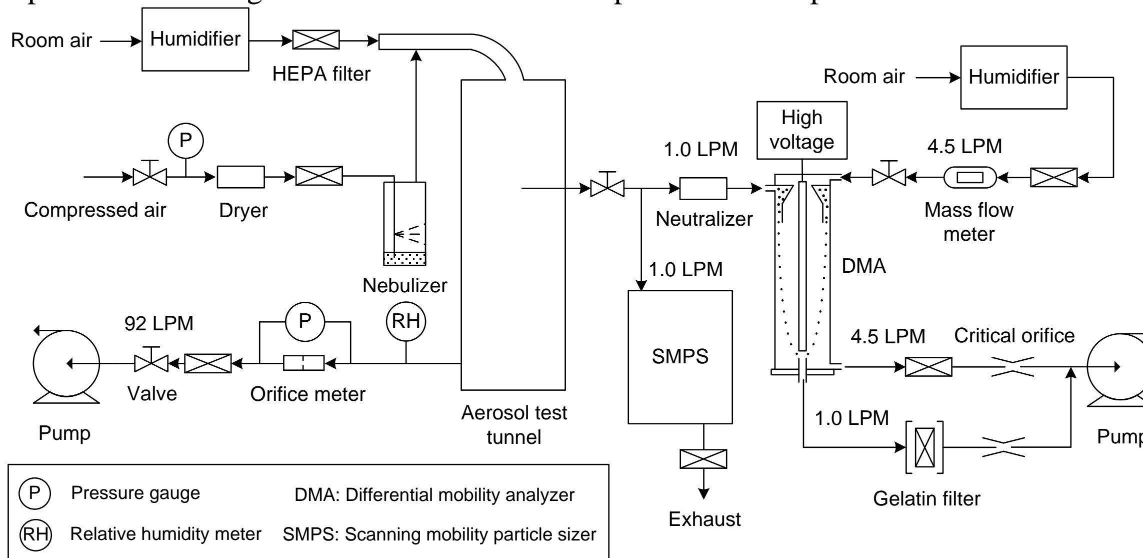

Figure 1. Schematic diagram of the experimental setup for the measurement of virus aerosols.

Virus infectivity assay and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) were then used to quantify the amount of infectious virus and total virus present in the samples, respectively. The virus distribution was found to be better represented by the particle

volume distribution rather than the particle number distribution. The capacity for a particle to carry virus increased with the particle size and the relationship could be described by a power law. Virus survivability was dependent on virus type and particle size. Survivability of the three animal viruses at large particle size (300–450 nm) was significantly higher than at particle size close to the size of the virion (100–200 nm), which could be due to the shielding effect. The data suggest that particle size plays an important role in infectivity and survivability of airborne viruses and may therefore have an impact on the airborne transmission of viral illness and disease. Our data do not support the use of MS2 bacteriophage as a general surrogate for animal and human viruses.

Laboratory studies of virus aerosols have been criticized for generating airborne viruses from artificial nebulizer suspensions (e.g., cell culture media), which do not mimic the natural release of viruses (e.g., from human saliva). Therefore, we designed a study to determine the effect of human saliva on the survival of airborne virus and to compare it with those of artificial saliva and cell culture medium (i.e., 3% tryptic soy broth). A stock of MS2 bacteriophage was diluted in one of the three nebulizer suspensions, aerosolized, size selected (100 to 450 nm) using a DMA, and collected onto gelatin filters. Uranine was used as a particle tracer. The resulting particle size distribution was measured using a scanning mobility particle sizer. The amounts of infectious virus, total virus, and fluorescence in the collected samples were determined by infectivity assays, qRT-PCR, and spectrofluorometry, respectively. For all nebulizer suspensions tested, the virus content generally followed a particle volume distribution rather than a number distribution. The survival of airborne MS2 was independent of particle size but was strongly affected by the type of nebulizer suspension. Human saliva was found to be much less protective than cell culture medium and artificial saliva. These results indicate the need for caution when extrapolating laboratory results, which often use artificial nebulizer suspensions. To better assess the risk of airborne transmission of viral diseases in real-life situations, the use of natural suspensions such as saliva or respiratory mucus is recommended.

In the next study, particle number penetration (one form of physical penetration) and infectivity penetration of human adenovirus and swine influenza virus aerosols through respirators were measured to better characterize the effectiveness of filtering face piece respirators against airborne viruses. Particle number penetration was found to range from 2% to 5%. However, aerosol loading and large sample-to-sample variation made it difficult to quantify the difference in particle number penetration caused by the different virus aerosols. Infectivity penetration of human adenovirus was much lower than particle number penetration, indicating that the latter provides a conservative estimate for respirator performance against airborne viruses.

In another experiment, infectivity, viral RNA, photometric, fluorescence (particle volume), and particle number penetration of MS2 bacteriophage through three different models of respirators were compared to better understand the correlation between infectivity and physical penetration. Although infectivity and viral RNA penetration were better represented by particle volume penetration than particle number penetration, they were several-fold lower than photometric penetration, which was partially due to the difference in virus survival between upstream and downstream aerosol samples. Results suggest that the current NIOSH (photometer-based) certification method may be used to prescreen respirators for infection control applications.

Study 2: Particle size is a significant factor for viral aerosol transmission, since it affects the amount of virus carried by each particle, the route of travel, and the deposition in the human respiratory system. Viral aerosol survivability, transmission, and sampling were studied size-selectively in an environmental chamber, which simulated a typical indoor environment featuring a complex flow field and low viral aerosol concentration. Bacteriophage MS2, human adenovirus type 1 (HAdV-1), avian influenza virus (AIV), swine influenza virus (SIV), transmissible gastroenteritis virus (TGEV, a coronavirus of pigs), and avian metapnumovirus (aMPV) were tested as surrogates for common human viruses. Live virus titer, total virus concentration, and fluorescence intensity were measured to calculate virus relative recovery and survival. Because of the low viral aerosol concentration in the chamber, long-term sampling had to be conducted for virus quantification.

In this study, the Andersen cascade impactor (ACI) was used to sample viral aerosols size-selectively. Before studying viral aerosols, the performance of the ACI was first tested for its long-term sampling capability. The tests estimated the inactivation of collected viruses in the ACI, and determined the suitable sampling duration and other parameters for the rest of the viral aerosol tests. In the second task, viral aerosols were tested under different temperatures and humidities. The effects of RH and absolute humidity (AH) were also compared. Viral aerosols were then tested with two UVGI lamps. The inactivation effect of UVGI was assessed and compared for the test viruses. Viral aerosols were also tested with two chemical-free HVAC filters of different minimum efficiency reporting values (MERV) using a test duct installed in an environmental chamber (see appendix for a description of the environmental chamber). All six viruses were sampled for one and six hours at 25 ˚C and 50% RH. The six-hour samples did not show much higher live virus titers than the one-hour samples, suggesting significant inactivation in the impactor. The six-hour tests did collect much higher total virus concentrations that resulted from PCR analysis. Impactor plate overloading which caused a decrease in sampling efficiency was observed in the six-hour tests.

Due to higher survivability and lower uncertainty, MS2, HAdV-1, and AIV were further tested at different temperatures and relative humidities. All three viruses had lower inactivation rates at 25˚C than at 30˚C, but the effect was not significant for HAdV-1. Absolute humidity (AH) was found to be a better predictor of survival than relative humidity (RH). Using AH also removed the significant interaction between temperature and humidity, which exists when RH is used. MS2 and HAdV-1 had the lowest inactivation rates at low AH, and AIV had the lowest inactivation rate at high AH for the AH range from 8.8 to 15.2 g/m^3, which is common in most indoor environments. Future tests are recommended at more extreme humidity levels.

In the HVAC filter tests, the overall filtration efficiencies for fluorescein and for total virus were similar, but the filtration efficiency for total virus was significantly higher than that for fluorescein at about 1 µm particle diameter. This result suggests that using fluorescein may not accurately predict the filtration behavior of viruses for the small particles. For the chemical-free filters tested, no significant inactivation of MS2 was found in the filtration process. More than 95% of the aerosol mass collected was smaller than 4.7 µm, with the mass median diameter of about 1.5 µm. The particle size distribution was affected by suspension medium but not virus. If the nucleic acid was not damaged during a test, the physical loss of virus was better predicted by total virus (PCR) rather than fluorescein, and their difference could be larger for larger viruses. In the nebulizer fluid, no significant virus inactivation was found after the one-hour tests with 20 psi (138 kPa) compressed air, or after the six-hour tests with 10 psi (69 kPa) compressed

air. The evaporation effect was more obvious for longer test duration or greater compressed air pressure. The nebulization rate of fluorescein was higher than that of virus.

Description of the environmental chamber used in Study 2.

In the lab environment, viral aerosol tests can be conducted in a rotating drum, a wind tunnel, or an environmental chamber. We chose to use an environmental chamber as a half-scale model of an indoor environment, such as an office, a hospital ward, or a barn (Fig. 2 and 3). The inside of the environmental chamber is 1.95 m (width) by 1.95 m (depth) by 1.45 m (height). Its interior was painted white for better illumination.

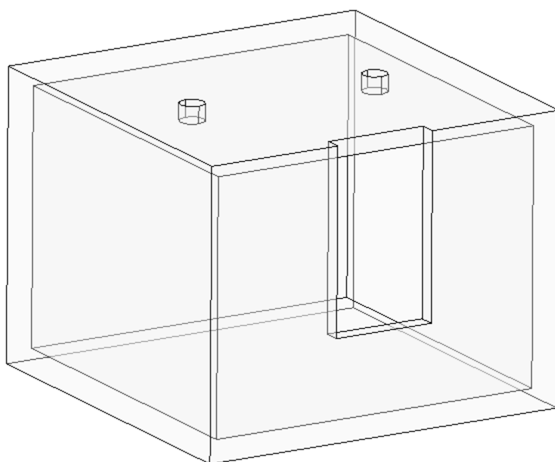

Fig. 2. Schematic of empty environmental chambershown here without access door, supply air diffuser, return air grille, lamps, and other interior components.

A door made of acrylic glass is located on the front wall of the chamber and is 1.09 m (height) by 0.57 m (width) (Fig. 3). During testing, the door was sealed with rubber gaskets and fastened by three clamps. A pressure tap was placed on the door to monitor the negative pressure in the chamber during a test to prevent the escape of viral aerosol to the surrounding laboratory space. The negative pressure was measured with a Magnehelic differential pressure gauge (Dwyer Instruments, Michigan City, IN) with a range of 0-0.5 inch water (0-124 Pa) and a resolution of 0.01 inch water (2.5 Pa). In order to safely manipulate the instruments in the chamber during a test, two glove ports with Hypalon gloves were placed on the door. An access window between the two gloves was used to retrieve the nebulizer and impactors from the chamber after each test when the chamber interior had not been disinfected. The operation of the small window strictly followed the requirement for a biosafety level-2 (BSL-2) cabinet in that the inward velocity of at least 75 ft/min (0.38 m/s) must be maintained when the window was open. A test indicated that the minimum velocity was reached when the negative pressure in the chamber was above 0.16 inch water (40 Pa) before the window was opened. For the actual operations with the fan running at its maximum speed, the negative pressure was about 0.36 inch water (90 Pa).

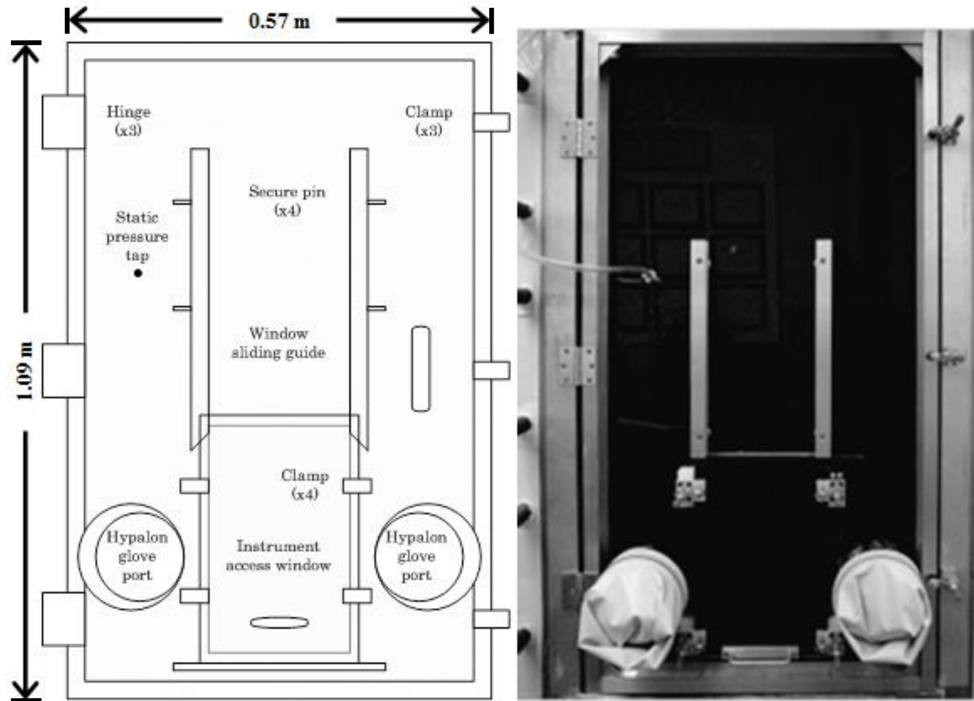

Fig. 3. Schematic drawing (left) and photo (right) of the chamber door with two glove ports, an access window, and a static pressure tap.

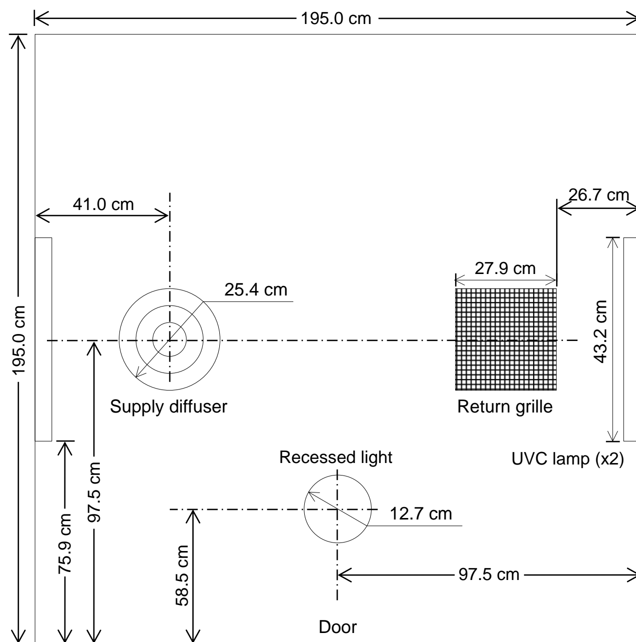

Fig. 4. Top view of the chamber, showing the main dimensions and positions of round supply diffuser, square return grille, recessed light, and two UVGI lamps.

The chamber ceiling was built with foam boards covered in plastic sheeting which provided thermal insulation (Fig. 4). A round supply diffuser (10 in or 25.4 cm diameter) and a square

return grille (11 in or 27.9 cm side length) were placed on the centerline of the ceiling. A recessed light (5 in or 12.7 cm diffuser diameter) was installed to illuminate the chamber interior. The light fixture was thermally insulated, and its diffuser was vapor proof. Two linear fluorescent UVGI lamps using G25T8 tubes (General Electric) with 1 in (2.5 cm) diameter and approximately 17 in (43.2 cm) length were installed for viral aerosol inactivation tests and for chamber disinfection after each test as recommended by several studies (Hocking 2000; Booth 2005; Sze To 2009). The switches for the recessed light and the two UVGI lamps were placed outside the chamber.

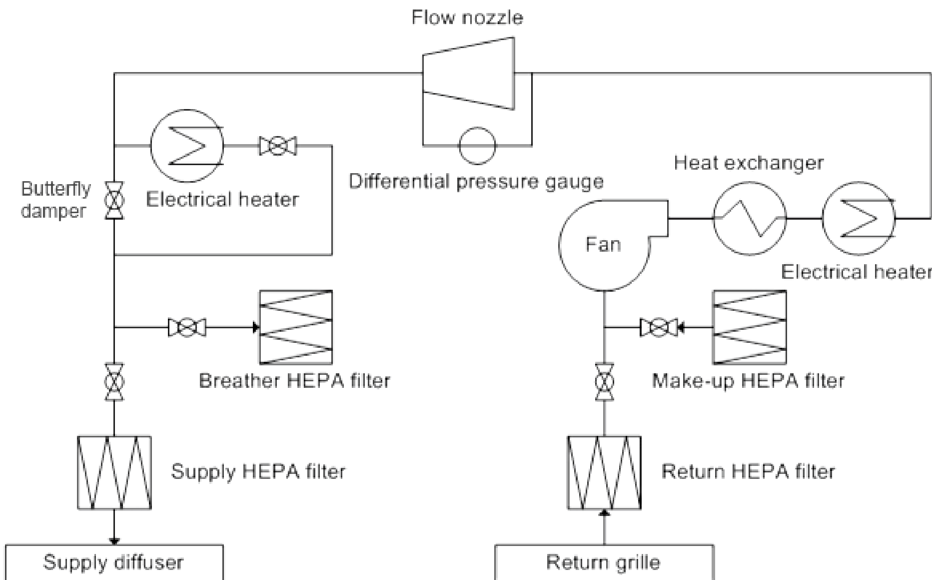

Fig. 5. The chamber air handling unit (AHU) for air circulation and conditioning.

In the air handling unit (AHU), PVC pipe and insulated flexible ducts of 6 in (15.2 cm) inside diameter (ID) were used to connect a variable speed fan, four HEPA filters, and other air conditioning equipment (Fig. 5). The fan could provide a maximum volumetric flow rate of about 150 ft^3/min (4250 l/min) to the chamber. The instantaneous chamber air flow rate was measured with a nozzle flow meter, which was calibrated using a duct traversing method shortly before the viral aerosol tests (ASHRAE 2009). The air flow rate was calculated from the pressure drop in the nozzle, which was measured with a Magnehelic differential pressure gauge (Dwyer Instruments, Michigan City, IN) with a range of 0-5 inch water (0-1244 Pa) and a resolution of 0.1 inch water (24.9 Pa). Four HEPA filters in the AHU were placed on top of the chamber, and used to capture the viral aerosol generated in the chamber. Two of the HEPA filters were for supply and return air. A pre-filter was placed immediately upstream of the return air HEPA filter to collect large particles. The other two HEPA filters were used for make-up and breather air respectively. During the aerosol tests, these two HEPA filters were sealed otherwise the by-pass flow would cause an unknown offset in the measurement of the chamber air flow rate by the nozzle flow meter. A certification test was conducted for the four HEPA filters before the viral aerosol tests, and no leakage was detected.

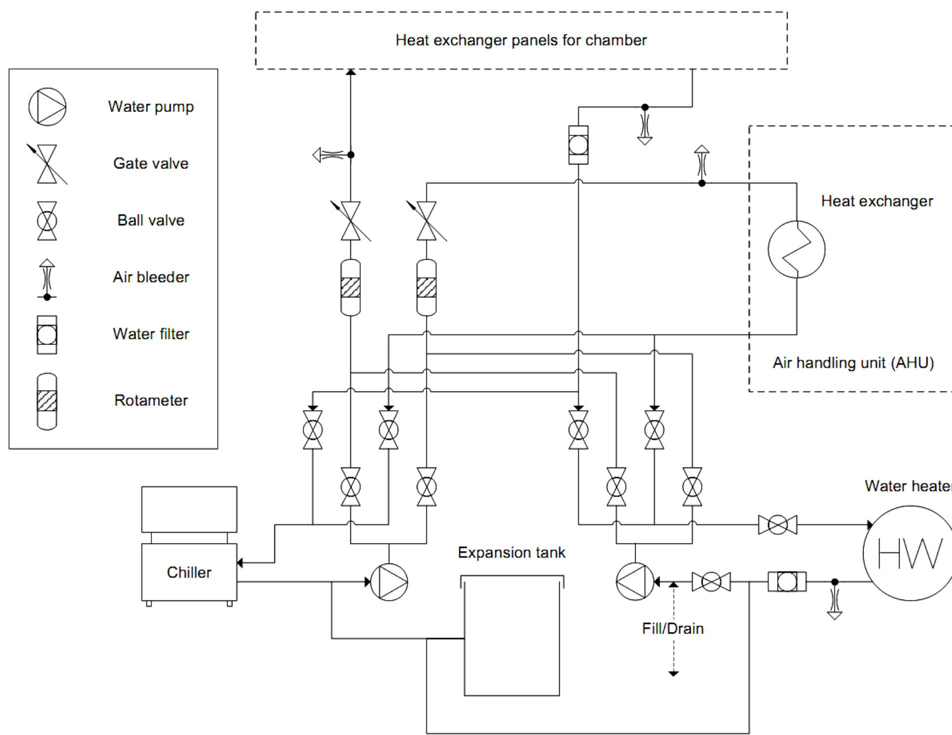

Fig. 6. The piping and equipment for glycol solution for heat exchangers.

To change the temperature of the supply air, heat exchanger coils were installed in the AHU (Fig. 6). The chamber walls and floor were constructed using plate heat exchangers attached to aluminum plates that formed all the interior surfaces of the chamber except the ceiling. The working fluid for all the heat exchangers was a 50% ethylene glycol solution in water. A water heater and a water chiller were used to adjust the temperature of the working fluid. Two water pumps circulated the working fluid through the heater and chiller separately. Eight ball valves on the control panel were used to change the passages for hot and cold flows. Before the viral aerosol tests began, the working fluid was replaced, and new filters were installed. The heat exchanger coil in the AHU could operate in combination with the downstream electrical heater (Fig. 5) to dry the air when needed. The electrical heater also provided more precise temperature control than using the heat exchanger coil alone.

Type T thermocouples (TFE-T-20, Omega Engineering, Stamford, CT) were installed on the interior surfaces of the walls, ceiling, floor, the UVGI lamps, and at various locations in the AHU to monitor temperature. A hygrometer (HX94C, Omega Engineering, Stamford, CT) was installed downstream of the return air HEPA filter to measure both temperature and relative humidity. Before the viral aerosol tests began, the thermocouples were calibrated using an ice bath. The hygrometer accuracy was checked using saturated salt solutions in a temperature controlled container, since it was purchased new with valid factory calibration. The temperature and humidity measured by the hygrometer were taken as the average values in the chamber environment, and were referred to when the test condition was being monitored. The electrical signals from the thermocouples and the hygrometer were picked up by a multimeter (2700, Keithley Instruments, Cleveland, OH) through a multimeter card (7706, Keithley Instruments,

Cleveland, OH). The signals were first processed by the multimeter, and then transferred to a computer data logging program (Excelinx-1A, Version C04, Keithley Instruments, Cleveland, OH).

The Collison nebulizer: In this study, viral aerosols were generated from virus suspensions using a 6-jet modified MRE Collison nebulizer (BGI, Waltham, MA) (Fig. 7). The Collison nebulizer generates aerosol from liquid using compressed air. During operation, only a small portion of the fluid jets coming out of the nozzles is carried out by the compressed air as aerosol; the rest flows back to the jar.

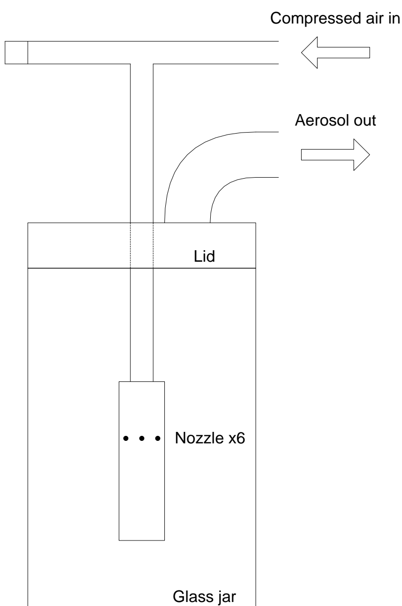

Fig. 7. The 6-jet Collison nebulizer used in this study.

The Andersen impactor: In a previous study, the eight-stage non-viable Andersen cascade impactor (ACI) (Thermo Scientific, Franklin, MA) (Fig. 8) and a micro-orifice uniform deposit impactor (MOUDI) (MSP Co., Shoreview, MN) were compared. Both impactors were capable of size-selectively sampling viral aerosols, but the ACI achieved higher relative recoveries than the MOUDI. Therefore, the ACI was further tested in the current study to investigate viral aerosol survivability and transmission. The ACI collected size-selective samples on eight 80 mm aluminum plates. At its standard sampling flow rate of 1 ft^3/min (28.3 l/min), the size range spanned from 0.4 to 10 µm, covering that of droplet nuclei and simulating particle deposition in the human respiratory system (Table 1).

Table 1. Size ranges of the Andersen impactor stages at the standard sampling flow rate.

Impactor stage	0	1	2	3	4	5	6	7
Lower limit (µm)	9.0	5.8	4.7	3.3	2.1	1.1	0.7	0.4
Upper limit (µm)	10.0	9.0	5.8	4.7	3.3	2.1	1.1	0.7

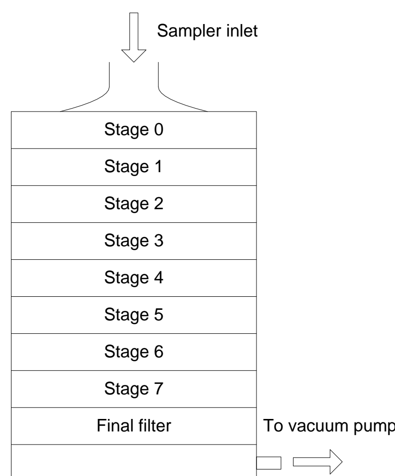

Fig. 8. The eight-stage non-viable Andersen cascade impactor (ACI) used in this study.

In an ACI stage, aerosol is first accelerated in nozzles upstream of the collection plate. As the air flow direction is abruptly changed due to the presence of the plate, particles which are too large to follow the streamlines impact on the plate and are retained on it. With a number of impactor stages arranged in series, size-selective sampling is achieved. In the ACI, each following impactor stage in the downstream direction has smaller nozzles, gradually increasing the momentum of particles so that smaller particles can be collected. The sampling efficiencies of impactors for viral aerosols were generally satisfactory in previous studies.

Other equipment and test setup: Most of the test equipment was placed inside the chamber for the viral aerosol tests (Fig. 9 and 10). The nebulizer and up to two ACIs were secured on a cart close to the chamber door for easy access. The compressed air for the nebulizer was dried, filtered, and regulated outside the chamber. Flexible Tygon tubing was attached to the nebulizer outlet for controlling the aerosol injection position and direction. For aerosol sampling, a 0.75 HP (0.55 kW) vacuum pump (NT10, Oerlikon Leybold Vacuum, Cologne, Germany) was used to draw air through up to two ACIs in this study. One mass flow meter (4100, TSI, Inc., Shoreview, MN) was used to monitor the sampling flow rate through each ACI. All of the mass flow meters were calibrated regularly during the study with a bubble meter (Sensidyne Gilian Gilibrator). Flexible Tygon tubing was attached to the inlet of each ACI to control the sampling position and direction. Neutralizers were not attached to either the nebulizer or the impactors, since their effect on virus inactivation was not known, and might not remain constant over time. One ultrasonic humidifier (Vicks V-5100NS) and one warm mist humidifier (Duracraft DWM250) were placed on the chamber floor to adjust humidity. The vacuum pump, the mass flow meters, and the humidifiers could be turned on or off individually using a power strip with individual switches in the chamber accessible through the glove ports.

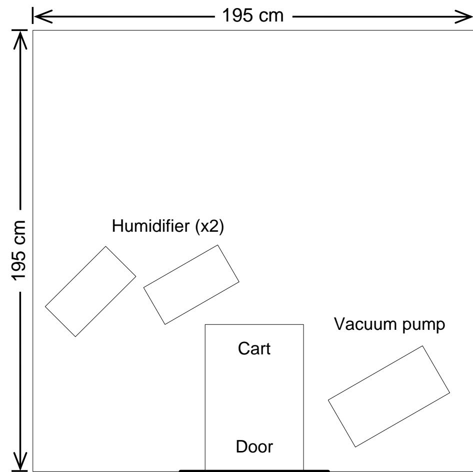

Fig. 9. Chamber floor plan with cart, vacuum pump, and two humidifiers.

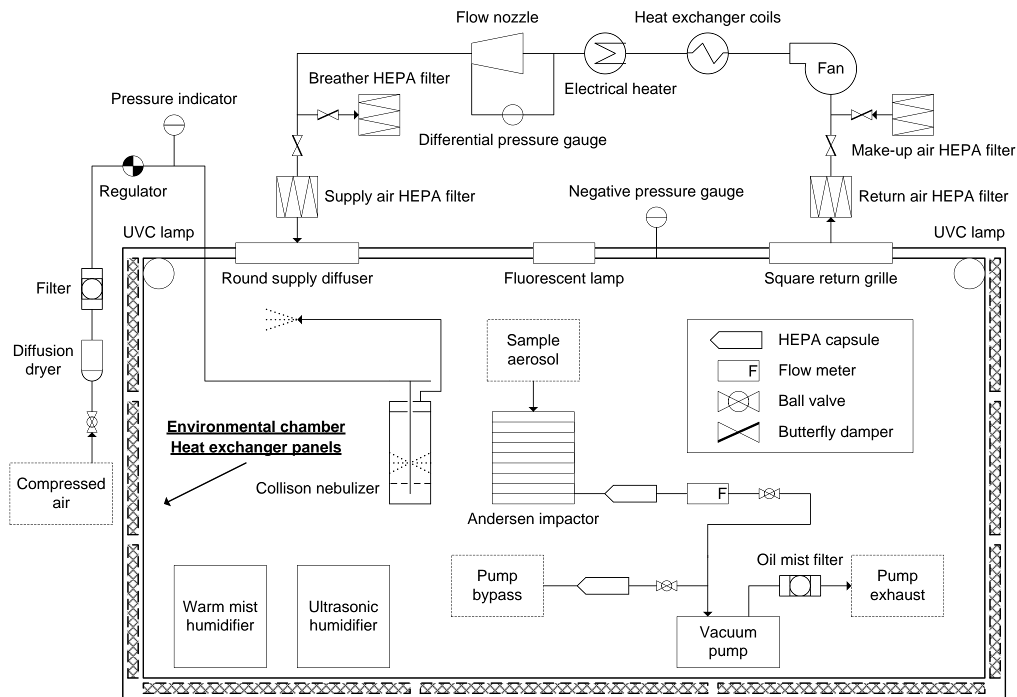

Fig. 10. Schematic drawing of the test setup in the environmental chamber.

7. Publications

Dissertations:

Ge S:[2014] Viral Aerosol Survivability, Transmission, and Sampling in an Environmental Chamber, Ph.D.Dissertation, University of Minnesota.

Zuo Z: [2014] Measurement and Filtration of Virus Aerosols,Ph.D.Dissertation, University of Minnesota.

Articles in peer-reviewed journals:

Appert J, Raynor PC, Abin M, Chander Y, Guarino H, Goyal SM, Zuo Z, Ge S, Kuehn TH. [2012]. Influence of Suspending Liquid, Impactor Type, and Substrate on Size-Selective Sampling of MS2 and Adenovirus Aerosols. Aerosol Sci Technol. 46:249-257.

Zuo Z, Abin M, Chander Y, Kuehn TH, Goyal SM, Pui, D.Y.H.[2013]. Comparison of spike and aerosol challenge tests for the recovery of viable influenza virus from non-woven fabrics. Influenza Virus Other Resp Viruses. 7:637-644.

Zuo Z, Kuehn TH, Verma H, Kumar S, Goyal SM, Appert J, Raynor PC, Ge S, Pui DYH. [2013].Association of airborne virus infectivity and survivability with its carrier particle size.Aerosol Sci Technol. 47:373-382.

Ge S, Kuehn TH, Abin MC, Verma H, Bekele AZ, Mor SK, Goyal SM, Appert J, Raynor PC, Zuo Z. [2014]. Airborne Virus Survivability During Long-Term Sampling Using a Non-Viable Andersen Cascade Impactor in an Environmental Chamber. Aerosol Sci Tech. 48:1360-1368.

Zuo Z, Kuehn TH, Bekele AZ, Mor SK, Verma, H, Goyal SM, Raynor PC, Pui DYH. [2014]. Survival of airborne MS2 bacteriophage generated from human saliva, artificial saliva, and cell culture medium. Appl Environ Microbiol. 80:2796-2803.

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Appert J, Raynor PC, Abin M, Chander Y, Goyal SM, Zuo Z, Ge S, Kuehn TH. [2015]. Size selective aerosol sampling of swine influenza virus, avian influenza virus, avian metapneumovirus, and transmissible gastroenteritis virus. Virus Res, in prep.

Presentations:

Peter C. Raynor, Jessica C. Appert, Sagar M. Goyal, Martha C. Abin, Yogesh Chander, Helena Guarino, Thomas H. Kuehn, Zhili Zuo, and Song Ge, "Relative Effectiveness of Impactor Sampling Among Six Airborne Viruses", 2011 American Industrial Hygiene Conference and Exposition, Portland, OR, May 2011.

Peter C. Raynor, Jessica C. Appert, Sagar M. Goyal, Martha C. Abin, Yogesh Chander, Helena Guarino, Thomas H. Kuehn, Zhili Zuo, and Song Ge, "Virus Recovery Using Cascade Impactors", Aerobiology in Biodefense IV, Glen Allen, VA, June 2011 (poster).

Zuo, Z, Martha de Abin, Yogesh Chandler, Thomas H. Kuehn, Sagar M. Goyal, Jessica Appert, Peter C. Raynor and David Y. H. Pui, "Comparison of Eluent and Aerosol vs. Liquid Spike Challenge Tests for Influenza Virus Recovery from Nonwoven Fabrics", Presented at the 30th annual AAAR Conference, Orlando, FL, October 4-7, 2011.

Jessica C. Appert, Peter C. Raynor, Sagar M. Goyal, Martha C. Abin, Yogesh Chander, Thomas H. Kuehn, Zhili Zuo, and Song Ge, "Effect of Relative Humidity on Sampling of Airborne Viruses", 2012 American Industrial Hygiene Conference and Exposition, Indianapolis, IN, June 2012.

Zuo, Z, Kuehn, T. H., Kumar, S., Harsha, V., Goyal, S. M., Appert, J., Raynor, P. C., Ge, S., Pui, D. Y. H. "Association of Virus Content with its Carrier Particle Size." Platform 8. HA.6 American Association for Aerosol Research (AAAR) 31st Annual Conference, Minneapolis, MN, October 8-12, 2012.

Zuo, Z., Kuehn, T. H., Bekele, A. Z., Harsha, V., Goyal, S. M., Raynor, P. C. and Pui, D. Y. H., "Infectivity and Survivability of Airborne Viruses Generated from Human Saliva, Artificial Saliva, and Cell Culture Media." Platform 6 HA 1. American Association for Aerosol Research (AAAR) 32nd Annual Conference, Portland, Oregon, September 30-October 4, 2013.

Zuo, Z., Kuehn, T. H., Bekele, A. Z., Mor, S. K., Verma, H., Goyal, S. M., Raynor, P. C. and Pui, D. Y. H., "Comparison of Human Saliva, Artificial Saliva, and Cell Culture Medium on the Survival of Infectious Airborne MS2 Bacteriophage." Platform OP63-2. International Aerosol Conference, Busan, Korea, August 28-September 2, 2014.