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Song Ge[a], Thomas H. Kuehn[a], Martha Abin[b], Harsha Verma[b], Aschalew Bekele[b], Sunil K. Mor[b], Sagar M. Goyal[b], Jessica Appert[c], Peter C. Raynor[c] & Zhili Zuo[a]

[a] Department of Mechanical Engineering, College of Science and Engineering, University of Minnesota, Minneapolis, Minnesota, USA

[b] Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA

[c] Division of Environmental Health Sciences, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA

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Song Ge,[1] Thomas H. Kuehn,[1] Martha Abin,[2] Harsha Verma,[2] Aschalew Bekele,[2] Sunil K. Mor,[2] Sagar M. Goyal,[2] Jessica Appert,[3] Peter C. Raynor,[3] and Zhili Zuo[1]

[1]*Department of Mechanical Engineering, College of Science and Engineering, University of Minnesota, Minneapolis, Minnesota, USA*

[2]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA*

[3]*Division of Environmental Health Sciences, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA*

In order to evaluate the survivability of airborne viruses and the sampling performance of an eight-stage non-viable Andersen impactor in typical indoor environments featuring low viral aerosol concentrations, aerosols of a male-specific bacteriophage (MS2), human adenovirus type 1 (HAdV-1), and avian influenza virus (AIV) were sampled size-selectively using the impactor in an environmental chamber. Live virus titer, total virus RNA or DNA concentration, and intensity of a fluorescein tracer were measured to calculate relative virus recovery and virus survival. Viral aerosols were first sampled for 1 and 6 h at 25°C and 50% relative humidity (RH). Virus inactivation and plate overloading were found to be significant in the impactor. Viral aerosols were then sampled at different temperature and humidity levels. 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. For the tested AH range of 8.8 to 15.2 g/m^3, MS2 and HAdV-1 had the highest survival at the lowest AH while AIV had the highest survival at the highest AH. More than 95% of mass collected was for particles smaller than 4.7 μm, with the mass median diameter of 1.5 μm. In the nebulizer, virus inactivation was not significant at 10 psi (69 kPa) compressed air pressure for up to 6 h of nebulization. Nebulizer analysis also reveals that the use of fluorescein tracer may not always accurately predict the physical loss of virus.

INTRODUCTION

Viruses are important pathogens accounting for about 60% of disease burden among humans (Horsfall 1965). Viral aerosols may travel thousands of kilometers and remain infectious for several days (Li et al. 2008; Memarzadeh 2012). Significant cases involving possible viral aerosol transmission include the outbreak of small pox in the 1960s (Wehrle et al. 1970) and the severe acute respiratory syndrome (SARS) epidemic in the early 2000s (Yu et al. 2004). Studies on viral aerosol transmission have thus become necessary.

Because naturally occurring viral aerosol transmission usually involves low virus concentration, the aerosol sampling method is important for sensitive and accurate field assessment of viral aerosol survival and transmission. Dry impactors, together with cyclones, impingers, and filters are among the most common aerosol samplers. Sampling efficiencies of dry impactors were found to be generally satisfactory in previous studies (Verreault et al. 2008). However, during the sampling process, viruses may be inactivated due to impaction force and desiccation. With the development of real-time quantitative polymerase chain reaction (PCR), however, the inactivated viruses can now be quickly detected and quantified. Dry impactors have been widely used under laboratory conditions but their performance in long-term sampling for low level virus concentration has not been fully tested, leaving their performance as a field sampler unclear. An eight-stage non-viable Andersen cascade impactor (ACI) has been compared with a micro-orifice uniform deposit impactor (MOUDI) (MSP, Shoreview, MN, USA) in a previous study, where the former was capable of size-selectively sampling viral aerosols with better recoveries of virus (Appert et al. 2012). This previous study is related to the current study, but the test facility was changed from a wind tunnel to a larger and more complex environmental chamber. As a result, the test protocols were

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Address correspondence to Song Ge, Department of Mechanical Engineering, College of Science and Engineering, University of Minnesota, 111 Church Street SE, Minneapolis, MN 55455, USA. E-mail: ges@umn.edu

different, and the sampling times in this study were 1–6 h compared to 15 min.

Temperature and humidity are among the most significant factors that affect virus survivability in aerosols. Generally, higher temperature results in lower survival of virus (Mbithi et al. 1991; Zuk et al. 2009; McDevitt et al. 2010), but the effect of high temperature varies for different viruses. Unlike temperature, different viruses react differently to change of relative humidity (RH). Generally, lipid-enveloped viruses, such as influenza viruses, have higher survival at lower RH (< 30%), and non-lipid viruses, such as adenoviruses, are more stable at higher RH (>70%) (Benbough 1971; de Jong et al. 1973). Exceptions to this generalization, however, do exist (Mbithi et al. 1991). Actual response of a virus may also depend on the type of medium it is suspended in (de Jong et al. 1973; Appert et al. 2012; Zuo et al. 2014). Significant interactive effect between RH and temperature on virus survivability has been reported (Tang 2009; Zuk et al. 2009). Some studies on influenza virus found that use of absolute humidity (AH) could eliminate the interactive effect between temperature and humidity, thus virus survival can be fitted linearly on a logarithmic scale versus AH for a given temperature. This means AH should be a better predictor than RH for virus survivability (Shaman et al. 2010; McDevitt et al. 2010). However, whether this conclusion applies to other types of viruses is unknown.

In this study, a male-specific bacteriophage (MS2), human adenovirus type 1 (HAdV-1), and avian influenza virus (AIV) were tested. MS2 is a small (25 to 30 nm), non-enveloped, single-stranded RNA (ssRNA) coliphage. It has been frequently tested as a general surrogate for animal and human viruses due to its innocuousness, high stock titer, and rapid analysis (Woo et al. 2010; Appert et al. 2012). The high stock titer of MS2 compared to AIV and HAdV-1 used in this study gives MS2 an advantage for better virus detection and lower measurement uncertainty. HAdV-1 is a non-enveloped double-stranded DNA virus with a diameter range of 70 to 100 nm (Kennedy and Parks 2009). It is believed that DNA viruses are generally more stable than RNA viruses (Memarzadeh 2012). The AIV, an influenza A virus, is a spherical and enveloped ssRNA virus with a diameter range of 80 to 120 nm. Influenza viruses cause respiratory diseases in humans and animals including domestic poultry, horses, swine, whales, and mink (Lamb and Choppin 1983). Due to physical and genetic similarities, the avian strains have been used as conservative surrogates for the human strains (Mitchell and Guerin 1972).

Viral aerosol transmission in the air is subjected to both physical and biological losses. The physical loss is the percentage of physical viral particles that were generated but not collected. It is usually caused by gravitational settling, convection, diffusion, electrostatic attraction, or dilution (Harper et al. 1958). The biological loss is the percentage of virus particles that were inactivated between generation and collection. The total loss is resulted from both physical and biological losses. By this definition, virus survivability is biological loss. Analysis of live virus (LV) titer gives total loss. Analysis of total virus (TV) concentration or fluorescence intensity (FI) gives physical loss. TV includes both live virus and inactivated virus, and is usually determined using PCR to quantify RNA or DNA concentrations in sample solutions, assuming virus RNA or DNA remains amplifiable even if the virus is inactivated. Therefore, PCR benefits from long sampling durations and performs well with low concentration samples (Cha and Thilly 1993). On the other hand, fluorescein is commonly used as a tracer for virus physical loss measurement. The method has been considered economical, simple, fast, and reliable by several researchers (Ijaz et al. 1987; Agranovski et al. 2005; Verreault et al. 2008).

The objectives of this study were to test the performance of an eight-stage non-viable ACI for short- and long-term size-selective viral aerosol sampling. In addition, the effects of temperature, AH, and RH on the survival of viral aerosols were also measured. An environmental chamber was used to simulate a typical field situation featuring a low level of viral aerosol concentration and a complex flow field.

TEST METHOD

Virus Propagation and Titration

MS2 (15597-B1, ATCC) was propagated and titrated using log-phase *Escherichia coli* C-3000 (700891, ATCC) in tryptic soy broth as described in Appert et al. (2012). The prepared MS2 stock was stored at −80°C until used. The titer of live virus was determined by the double agar layer procedure and the virus titer was expressed as plaque forming units per 100 μl sample (PFU/100 μl). The lower detection limit of this procedure was 1 PFU/100 μl.

HAdV-1 (VR-1, ATCC) was propagated and titrated in A-549 human lung carcinoma epithelial cells (CCL-185, ATCC). The cells were grown in Eagle's minimum essential medium (MEM) (Mediatech, Herndon, VA) as described in Appert et al. (2012). AIV (A/Maryland/2007/H9N9) was propagated and titrated using Madin-Darby canine kidney cells (CCL-34, ATCC) in MEM as described by Zuo et al. (2013). The prepared virus stocks were stored at −80°C until used. Virus titers (expressed in $TCID_{50}$/100 μl) were calculated by the Karber method (Karber 1931). The lower detection limit of this method was 1 $TCID_{50}$/100 μl. If no virus was detected in a particular sample, 0.1 $TCID_{50}$/100 μl was used for data analysis.

Real-Time Quantitative PCR and Fluorescein Tracer

Reverse transcription PCR (or RT-PCR) for RNA viruses MS2 and AIV have been described in Zuo et al. (2013). The PCR procedure for the DNA virus HAdV-1 is described here: the HAdV-1 DNA was extracted from 200 μl of sample using the QIAamp DNA Mini kit and protocol (Qiagen, Valencia,

CA). For PCR amplification of the HAdV-1 DNA, the initial cycle was 50°C preheat for 2 min followed by 95°C heat activation for 15 min. The following thermal amplification cycles were 40 cycles of 95°C for 15 s and 56°C for 45 s. The direct output of PCR or RT-PCR is a cycle threshold, also known as C_t value. The concentration of virus RNA or DNA in a sample was determined by projecting the measured C_t value onto a standard curve, which was determined by measuring standard samples of known concentrations of virus RNA or DNA. TV measured by PCR was thus expressed as projected PFU/100 μl for MS2 or projected $TCID_{50}$/100 μl for AIV or HAdV-1. In addition to the PCR method, a fluorescein tracer was added to the nebulizer fluid and used to measure the physical loss of virus in terms of FI, a dimensionless quantity, for each sample using the procedure described in Appert et al. (2012). The lower detection limit of FI was 0.1. FI is proportional to the concentration of fluorescein in the sample as long as there is no addition or extraction during a test. This property makes fluorescein tracer very useful to track changes of concentration or physical loss with a lower uncertainty than directly measuring virus quantity.

Relative Recovery and Virus Survival

Indicated using relative recovery and survival, virus survivability or biological loss can be calculated if total loss and physical loss are known (Agranovski et al. 2005; Zuo et al. 2013):

$$\text{Relative recovery} = \frac{(\text{Impactor}/\text{Nebulizer})_{\text{LV}}}{(\text{Impactor}/\text{Nebulizer})_{\text{FI}}}, \quad [1]$$

$$\text{Survival} = \frac{(\text{Impactor}/\text{Nebulizer})_{\text{LV}}}{(\text{Impactor}/\text{Nebulizer})_{\text{TV}}}, \quad [2]$$

where "Nebulizer" is the geometric mean of the virus or fluorescence concentrations in the nebulizer fluid before and after a test, namely, "pre" and "post," and "Impactor" is the amount of virus or fluorescence detected on an impactor stage. On the right-hand side of Equations (1) and (2), the "Impactor" value is normalized by the "Nebulizer" value for FI, LV, or TV, and in this study, they are named normalized FI, normalized LV, and normalized TV, respectively.

Concentration and Virus Inactivation in the Nebulizer

Evaporation of the nebulizer fluid during nebulization could gradually increase the concentrations of fluorescein and TV. This effect was evaluated by calculating the concentration ratio (CR) equal to the "post" divided by the "pre" based on either FI or TV. To test whether virus was inactivated significantly during nebulization, CR of LV was divided by that of FI or TV as described in Appert et al. (2012):

$$\gamma = \frac{(\text{LV}/(\text{FI or TV}))_{\text{post}}}{(\text{LV}/(\text{FI or TV}))_{\text{pre}}} = \frac{\text{CR}_{\text{LV}}}{\text{CR}_{\text{FI or TV}}}. \quad [3]$$

Environmental Chamber

Designed as a half-scaled room, the inside dimensions of the environmental chamber are 1.95 m wide, 1.95 m deep, and 1.45 m high. The schematic of the chamber setup is shown in Figure 1. A round supply diffuser with a diameter of 25.4 cm and a square return grill with a side length of 27.9 cm are located in the ceiling. Two glove ports on the door (1.09 m tall by 0.57 m wide) are used to manipulate test instruments inside the chamber during a viral aerosol test. HEPA filters are installed upstream of the supply diffuser, downstream of the return grill, and in other places in the air-handling unit (AHU) to prevent contamination outside the chamber. The AHU is capable of changing the air temperature, humidity, ventilation rate, and negative pressure within the chamber. Heat exchangers running 50% (v/v) ethylene glycol solution in water are used to adjust the temperature of the supply air, the chamber floor, and the chamber walls. Temperatures at various locations in the chamber and AHU are measured with type-T thermocouples (TFE-T-20, Omega Engineering, Stamford, CT, USA). Return air temperature and RH are measured with a hygrometer (HX94C, Omega Engineering). The electrical signals from the sensors are picked up, processed, and stored using a multimeter (2700, Keithley Instruments, Cleveland, OH, USA) and its computer software (Excelinx-1A, Version C04).

Viral Aerosol Generation and Sampling Devices

Viral aerosols were generated from a six-jet Collison nebulizer (BGI, Waltham, MA, USA) using compressed air. An eight-stage non-viable ACI (Thermo Scientific, Franklin, MA, USA) was used to sample viral aerosols in the chamber size-selectively. Detailed descriptions of an ACI can be found in Andersen (1958) and Hinds (1999). The ACI used in this study collected size-selective samples on eight 80 mm aluminum plates. At the standard sampling flow rate of 1 ft^3/min (28.3 l/min), its full size range was from 0.4 to 10 μm with boundaries at 0.7, 1.1, 2.1, 3.3, 4.7, 5.8, and 9.0 μm in between forming eight size bins corresponding to stages numbered from 7 to 0.

Test Setup and Conditions

As shown in the schematic of the chamber test setup (Figure 1), viral aerosol was injected 20 cm below the center of the supply diffuser toward the left chamber wall, and was sampled 80 cm above the floor by an upward-facing sampling probe below the return grille. Flexible tubing of 0.5 inch (1.3 cm) inside diameter, and 0.75 inch (1.9 cm) outside diameter was used to control the locations and directions of viral

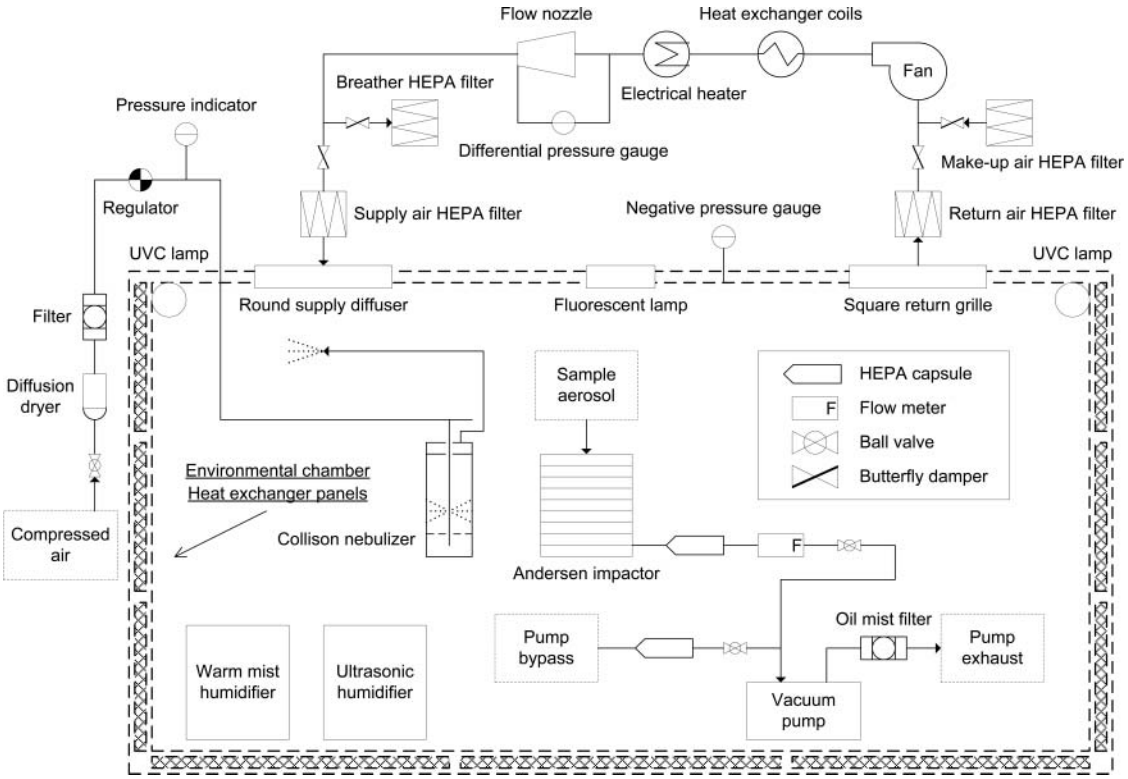

FIG. 1. Schematic drawing of the viral aerosol test setup in the environmental chamber, including a compressed air system, viral aerosol injection and sampling setups, a chamber air-handling unit, and other minor equipment.

aerosol injection and sampling. The length of the tubing used for injection was 140 cm, and that for sampling was 135 cm. A warm mist humidifier (DWM250, Duracraft) and an ultrasonic humidifier (V-5100NS, Vicks) were placed on the chamber floor to increase humidity. A vacuum pump (NT10, Oerlikon Leybold Vacuum, Cologne, Germany) was used to draw air through the impactor. The sampling flow rate was monitored with a mass flow meter (Model 4100, TSI, Shoreview, MN, USA), which was calibrated by a Sensidyne Gilian Gilibrator.

The air change rate for the tests was 15 air changes per hour (1500 l/min), which was similar to some public indoor environment, such as a computer lab or an examination room in a hospital (Atkinson et al. 2009; Blachere et al. 2009). At this ventilation rate, the negative pressure in the chamber relative to the surrounding lab was higher than 25 Pa, which was recommended by the American Institute of Architects to ensure containment of generated viral aerosols (AIA 2001).

Two sets of experiments were conducted to address the study objectives. First, 1 h and 6 h sampling tests were run with all three viruses at 25°C and 50% RH. Second, tests were conducted to evaluate the effects of temperature and humidity. Test conditions for the five test scenarios were: 25°C and 38% RH (8.8 g/m^3 AH), 25°C and 50% RH (11.5 g/m^3 AH), 25°C and 66% RH (15.2 g/m^3 AH), 30°C and 38% RH (11.5 g/m^3 AH), and 30°C and 50% RH (15.2 g/m^3 AH). In order to keep the general particle movement and residence time inside the chamber unchanged for different conditions, the chamber was operated isothermally, which means the heat exchangers in the chamber walls and AHU were run at the same temperature. Variations of temperature and RH were, respectively, kept within 1°C and 5% of the target values. Three replicate tests were conducted for each combination of conditions.

The compressed air pressure for nebulization was 10 psi (69 kPa). The nebulizer fluid was composed of virus stock, 0.05 g/ml fluorescein solution (Fluka, Buchs, Switzerland), and antifoam Y204 (Sigma, St. Louis, MO, USA) with volumetric proportions of 48:1:0.1. The volume of nebulizer fluid was 49.1 ml for all 1 h tests, and 147.3 ml for all 6 h tests. A 1 ml nebulizer sample was taken before and after each test serving as the "pre" and "post" samples. After each test, impactor plates were removed and eluted with cell scrapers in a certified biosafety level-2 cabinet. An additional clean plate was eluted as a negative control. For plate elution, 1 ml of elution buffer, which was a 3% (v/v) solution of beef extract in 0.05 M glycine, was used per plate. All samples were divided into two parts: a 50 μl aliquot for fluorescein measurement, and the rest for live virus and total virus measurement. The fluorescein portion was stored at 4°C, and the virus portion at −80°C until they were analyzed. After each test, the chamber was disinfected by running two UVGI lamps (G25T8, General Electric) and its ventilation system for 3 h then leaving the chamber

idle for at least 15 h. Contaminated objects including the nebulizer and impactor were soaked and washed with 1:32 (v/v) bleach solution in water, and were rinsed three times with deionized and filtered water.

Statistical Analysis

Each test was conducted in triplicate, and the test order was randomized for each virus. Analysis of variance (ANOVA) was performed on a logarithmic scale in R (Version 2.15.0, the R Foundation for Statistical Computing) to assess significance of the factors and their interactions to responses including FI, LV, TV, relative recovery and survival. Specifically, in the 1 h and 6 h sampling tests, ANOVA was carried out using a set of factors consisting of sampling time, particle size, and virus type. While in the temperature and humidity tests, ANOVA was carried out using two sets of factors: one consisted of particle size, virus type, temperature, and RH; the other substituted RH with AH. A factor or an interaction was considered significant if its p-value was smaller than 0.05. ANOVA was performed based on samples collected on the impactor stages 3–7 only, because LV for stages 0–2 for some of the tests was too low to be detected, leading to very high uncertainties.

RESULTS AND DISCUSSION

Concentration and Virus Inactivation in the Nebulizer

CR of fluorescein ($p = 0.481$) or total virus ($p = 0.320$) was not significantly affected by the type of virus according to ANOVA. CR based on fluorescein for the 1 h tests was 1.11 with a standard error of 0.04, and that for the 6 h tests was 1.21 ± 0.06. CR based on total virus for the 1 h tests was 1.27 $\pm$ 0.12, and that for the 6 h tests was 1.32 ± 0.13. As can be expected, CR was higher for longer nebulization durations. In addition, CR based on total virus was higher than that based on fluorescein, suggesting that fluorescein was nebulized at a higher rate than total virus.

Regarding virus inactivation during nebulization, γ did not significantly depend on the type of virus ($p = 0.887$ based on fluorescein or 0.847 based on total virus) or the duration of nebulization ($p = 0.663$ based on fluorescein or 0.634 based on total virus) according to ANOVA. The geometric mean and 95% confidence interval (in square brackets) of γ were found to be 1.12 [0.89, 1.40] based on fluorescein, or 0.98 [0.80, 1.20] based on total virus. Both intervals contain unity, indicating that virus inactivation was negligible for 6 h nebulization using 10 psi (69 kPa) compressed air. In Appert et al. (2012), 20 psi (138 kPa) compressed air was used to nebulize the same solutions for 15 min, and no significant virus inactivation due to nebulization was observed. In another study, viral aerosols were generated from the same nebulizer for 10 min using the compressed air pressure of 26 psi (180 kPa), and no significant inactivation of virus was found as well (Ijaz et al. 1987). Therefore, the compressed air pressure used in this study was very likely not high enough to cause significant virus inactivation due to nebulization stress.

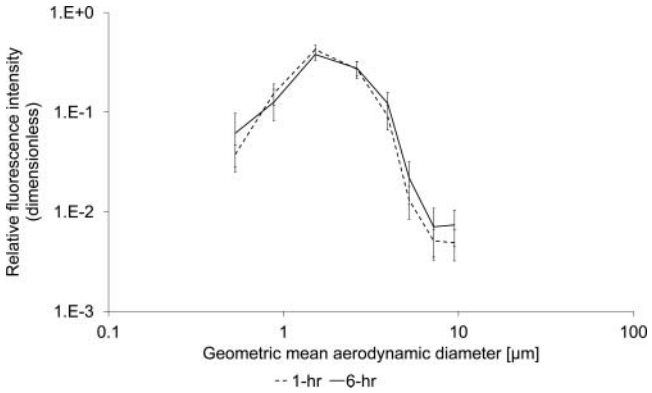

FIG. 2. Relative fluorescence intensity (dimensionless, vertical axis) versus geometric mean aerodynamic diameter of the impactor stages [μm] (horizontal axis) for the 1 h and 6 h tests based on all three viruses with error bars showing standard deviations.

One-Hour and Six-Hour Sampling Tests

Relative FI in the impactor samples for all three viruses for the 1 h and 6 h tests, which were calculated by dividing impactor stage FI with the sum of all stages, are compared in Figure 2. Assuming FI was proportional to aerosol mass, the figure shows that impactor stage 5 (1.1–2.1 μm) collected about 40% of the total mass, and impactor stages 3–7 (0.4–4.7 μm) collected more than 95%. This size distribution was similar to those in Yang et al. (2011) and Appert et al. (2012). The figure also shows that particle size distribution of the 6 h tests was more uniformly distributed than that of the 1 h tests. By ANOVA, the test duration and particle size were both significant factors for normalized FI ($p < 0.001$), their interaction was also significant ($p < 0.001$), which supports the second observation by indicating that the particle size distribution in the impactor samples significantly depended on the test duration. This result suggests that the aerosol collection efficiencies of impactor stages 4–6 could have decreased compared to the other stages. One reason could be the impactor plates of stages 4–6 were overloaded with collected particles in the 6 h tests (Agranovski et al. 2005). Plate overloading was evidenced during the elution process, when unevenness on the collection plates of impactor stages 4–6 for the 6 h tests was observed. The change of the plate surface geometry could have increased particle bouncing, thus affecting the collection efficiency.

The test duration was significant for both relative recovery ($p = 0.001$) and virus survival ($p = 0.014$) indicating significant virus inactivation in the impactor. In order to evaluate this, 6 h to 1 h ratios were calculated for normalized LV, normalized TV, normalized FI, relative recovery, and survival

based on all viral aerosols collected on the eight impactor stages (Figure 3). Ideally, the ratio of normalized total virus or normalized FI should be close to 6, but from Figure 3, these ratios were generally closer to 5, meaning that the aerosol collection rate of the impactor decreased during the tests. The reason could be the decrease of sampling efficiency, aerosol generation rate, or both. Since virus inactivation in the nebulizer fluid could be neglected, and if no significant virus inactivation occurred in the impactor, the live virus ratio should be comparable with the total virus ratio. On the other hand, if all collected virus was inactivated within 1 h, the ratio should be close to unity. In Figure 3, the ratios of normalized LV were lower than those of normalized TV, but higher than unity, suggesting that significant virus inactivation occurred in the impactor, but some of the collected virus remained infectious for longer than 1 h. Virus inactivation in the impactor could also be determined from the ratios of relative recovery and survival, which were similar to each other and smaller than unity.

According to Figure 3, the rate of virus inactivation in the impactor may depend on the type of virus, but the trend among the three viruses is difficult to discern due to the large uncertainty level. The results suggest that when detecting virus collected by the Andersen impactor, PCR can benefit from longer sampling durations, but the cell-culture-based titration for live virus measurement may not. No published study can be found discussing virus inactivation in impactor, but the information can be very useful when designing test setup and protocol for sampling viral aerosol using an impactor. More tests and measurements can be carried out following the procedure used in this study to determine inactivation rates of viruses during operation of impactors.

Temperature and Humidity Tests

Using a corresponding nebulizer concentration (or titer) to divide an impactor stage concentration (or titer), normalized FI for MS2, AIV, and HAdV-1 were calculated for all five test conditions. The standard deviations were not found to be significantly different between different levels of particle size, temperature, or relative humidity. According to the ANOVA results for normalized FI, the type of virus was a significant factor ($p = 0.015$), the interaction between virus type and particle size was not significant, but the p-value was close to the critical value ($p = 0.068$). By further comparing the three viruses, it was found that MS2 was significantly different from HAdV-1 ($p = 0.010$), while AIV was not ($p = 0.707$). The comparison suggested possible effect of suspension medium on particle size distribution. The interaction between virus type and RH ($p < 0.001$), and that between virus type and AH ($p = 0.009$) were both significant, suggesting different hygroscopic properties of different suspension media or viruses. Both RH ($p < 0.001$) and AH ($p < 0.001$) were significant, as well as the interaction between particle size and RH ($p < 0.001$) and that between particle size and AH ($p < 0.001$), suggesting humidity affected both overall particle size and size distribution, in agreement with the findings of some previous studies (Lowen et al. 2007; Verreault et al. 2008). Temperature ($p = 0.166$) was not significant, nor was the interaction between temperature and particle size ($p = 0.753$), showing that neither the total amount nor the size distribution of the collected particles was significantly affected by temperature. This result implies that the isothermal flow field in the chamber was well maintained throughout these tests, and particle trajectories were not significantly different for different temperatures.

Normalized TV was calculated in a similar way to normalized FI. The two physical losses calculated by FI and TV were similar for MS2, but for AIV and HAdV-1, overall normalized TV was significantly smaller than normalized FI. This indicates that using fluorescein underestimated the physical losses of AIV and HAdV-1. Considering the analysis of the nebulizer fluid where TV was found to be concentrated faster than

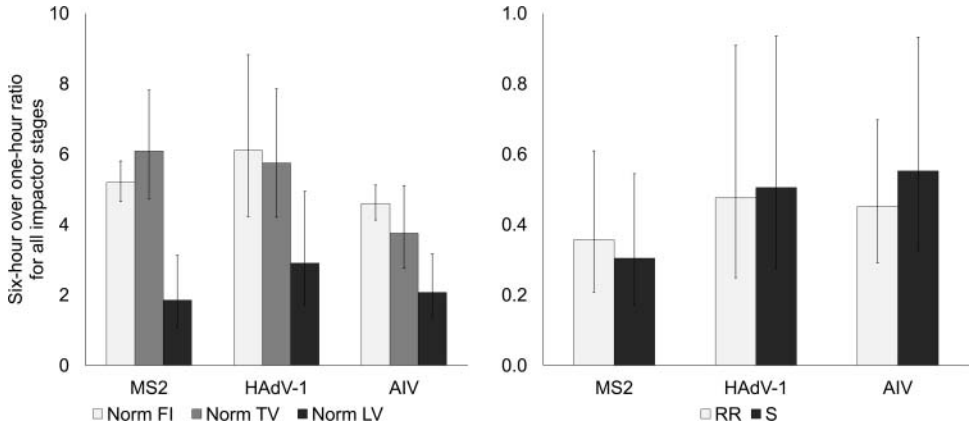

FIG. 3. Dimensionless ratios of 6 h over 1 h test results for normalized fluorescence intensity (labeled as "Norm FI"), normalized total virus concentration (labeled as "Norm TV"), normalized live virus titer (labeled as "Norm LV"), relative recovery (labeled as "RR"), and virus survival (labeled as "S") based on all viral aerosols collected in the impactor with error bars showing standard errors.

fluorescein, therefore, the reason could be that the nebulizer output for virus was slower than that for fluorescein. This difference might be a function of virus size: the very small particles generated by the nebulizer contained only fluorescein but no or very scarce virus.

For biological losses, relative recovery and survival of MS2, AIV, and HAdV-1 for all five test conditions are compared in Figure 4. The plots show that MS2 generally had higher relative recovery and survival at lower RH or AH. In previous studies, MS2 aerosol generally had the highest survival at <30% RH, the lowest survival near 60% RH, and medium survival at >60% RH (Dubovi and Akers 1970; Trouwborst and de Jong 1973; Woo et al. 2010). In Appert et al. (2012), MS2 was found to have the highest relative recovery at 50% compared with results at 15% and 85% using the same suspension as used in current study. AIV had higher relative recovery at 38% or 66% RH than at 50% RH on average, and it had the highest survival at 15.2 g/m^3 AH. Influenza A virus was reported to survive better at RH lower than 40%, and have higher inactivation at higher RH (Harper 1961; de Jong et al. 1973; Zuk et al. 2009; McDevitt et al. 2010). Some other studies showed that influenza A virus had the lowest recovery at about 50% RH, the highest recovery at lower RH, and moderate recovery at higher RH (Hood 1963; Schaffer et al. 1976; Yang et al. 2012). In Appert et al. (2012), the relative recovery of AIV in MEM was the lowest at 50% RH, the highest at 85%, and moderate at 15%. It was also reported that both extremely low and high RH could reduce the survival of influenza A virus (Memarzadeh 2012). The different findings above could be caused by the different virus suspensions. HAdV-1 had significantly higher relative recovery at 8.8 g/m^3 AH than at the other AH levels, and its survival was the highest at 8.8 g/m^3, the lowest at 11.5 g/m^3, and moderate at 15.2 g/m^3. The effect of RH, however, was not clear. Adenovirus was reported to be more stable at high RH than low RH, and was the stablest at RH above 80% (Harper 1961; Miller and Artenstein 1967; Davis et al. 1971). In Appert et al. 2012, HAdV-1 in the same MEM suspension had the lowest relative recovery at 50% RH compared with 15% and 85% RH. The plots also show that MS2 had higher relative recovery than AIV and HAdV-1, but did not have higher survival. Given that the only difference between relative recovery and survival was in physical loss calculation, the lower relative recovery results for AIV and HAdV-1 correspond to the lower physical loss calculated by FI for the two viruses. Because of this issue with fluorescein and the different reactions of the test viruses to the change of humidity, ANOVA was carried out for survival for each virus individually for both individual effects and some of the two-factor interactions.

For MS2 survival, particle size was not a significant factor ($p = 0.885$), meaning that the difference in virus inactivation rate between stages was not large enough to be statistically detected. Temperature was significant ($p < 0.001$). In Figure 4, it is obvious that virus inactivation was faster at the higher temperature. RH ($p = 0.015$) and AH ($p = 0.003$) were both significant. The interaction between temperature and RH was significant ($p = 0.023$), but that between temperature and AH was not ($p = 0.278$). This implies that predicting MS2 survival using AH instead of RH could decouple humidity and temperature effects.

For AIV survival, particle size was also not significant ($p = 0.846$). Temperature was not significant ($p = 0.075$), but the p-value was close to the critical value, indicating increasing temperature from 25°C to 30°C may still negatively affect the survival of AIV. As can be seen in Figure 4, AIV survival was lower at 30°C on average for the same AH. RH ($p = 0.011$) and AH ($p = 0.008$) were both significant for survival. The interaction between temperature and RH was not found to be significant in this case ($p = 0.276$), and that between temperature and AH was also not significant ($p = 0.449$).

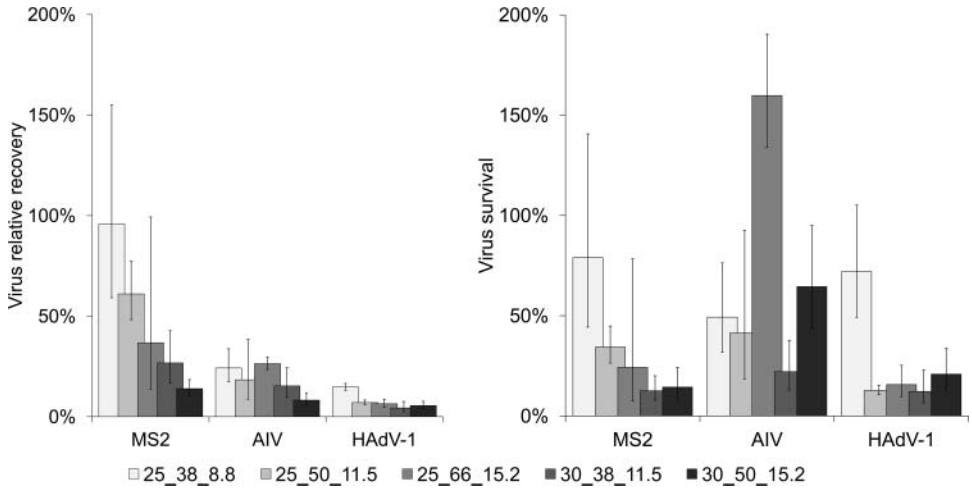

FIG. 4. Virus relative recovery and survival based on all viral aerosols collected in the impactor with error bars showing standard errors. The three numbers in the data series' names are temperature (°C), relative humidity (%), and absolute humidity (g/m^3), respectively.

For HAdV-1 survival, particle size was a significant factor ($p = 0.022$). Decreased virus survival was observed as particle size increased. This is similar to the result presented in Appert et al. (2012). Temperature was not significant ($p = 0.666$), as is also shown in Figure 4. This suggests that HAdV-1 was more resistant to the higher temperature than the other two viruses. RH was not significant ($p = 0.178$), but the interaction between RH and temperature was significant ($p = 0.002$). On the other hand, AH was a significant factor ($p = 0.002$), but the interaction between AH and temperature was not ($p = 0.619$). The four p-values for humidity suggest that when predicting HAdV-1 survival with AH instead of RH, the interaction term between temperature and humidity could be eliminated. This agrees with the MS2 results and some of the published studies on AIV (McDevitt et al. 2010; Shaman et al. 2010).

In general, within the test conditions of this study particle size did not significantly affect virus survival, temperature was found to be significant for MS2. According to published data, higher temperature generally results in lower survival, and the vulnerability to high temperature can be different for different viruses (Harper 1961; Mbithi et al. 1991; Zuk et al. 2009; McDevitt et al. 2010; Memarzadeh 2012). For adenovirus, its survival was found to be stable between 4 and 36°C, and was significantly lower at higher temperatures (Ginsberg 1956), another study found that its infectivity dropped dramatically above 29°C (Li et al. 2008). AH played a more important role than RH in predicting virus survival not only for AIV. Interaction between temperature and RH has been discussed for viral aerosol survival and transmission (Tang 2009; Zuk et al. 2009). Some studies have also suggested that AH might be a better predictor than RH for the survival, transmission, and seasonality of influenza viruses, because using AH could eliminate the interaction term between temperature and humidity (McDevitt et al. 2010; Shaman et al. 2010; Memarzadeh 2012). In general, humidity effects can be complex, and can depend on suspension ingredients, especially the content of salts, proteins, and polyhydroxy (Dubovi and Akers 1970; de Jong et al. 1973; Trouwborst and de Jong 1973; Appert et al. 2012). Some exceptions may be due to different virus structures, test errors, or limitation of test conditions. Future tests are recommended especially for greater ranges of temperature and humidity levels to further explore the exceptions.

CONCLUSIONS

MS2, HAdV-1, and AIV aerosols were size-selectively sampled using an eight-stage non-viable ACI for different sampling durations, temperatures, and humidities. Collected viruses were significantly inactivated in the impactor, suggesting that if using dry impactors, a longer sampling duration may not lead to a better detection for live viruses in a field measurement. Instead, the PCR method for virus RNA or DNA detection is recommended. In addition, longer sampling durations may cause the collection plates in the impactor to be overloaded, reducing the collection efficiencies of these stages. For airborne virus survivability, HAdV-1 is more resistant to high temperatures than MS-2 and AIV. AH is a better predictor than RH for virus survival. This agrees with the previous studies on AIV, and suggests that the same conclusion could be applied to other viruses as well. It is therefore recommended that the AH effects should be further tested in future viral aerosol research. In this study, using fluorescein overestimated the nebulizer output of AIV and HAdV-1, suggesting that using fluorescein may not always accurately represent the physical loss of viruses in a testing process depending on sizes of viruses and particles. Using 10 psi (69 kPa) compressed air for up to 6 h nebulization did not significantly inactivate the viruses in the nebulizer. Future research should further investigate the accuracy of using the fluorescein tracer. It is also recommended that similar tests are conducted for a broader range of temperature and humidity.

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