Bioaerosol sampling for the detection of aerosolized influenza virus

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Background Influenza virus was used to characterize the efficacy of a cyclone-based, two-stage personal bioaerosol sampler for the collection and size fractionation of aerosolized viral particles.

Methods A Collison single-jet nebulizer was used to aerosolize the attenuated FluMist® vaccine into a calm-air settling chamber. Viral particles were captured with bioaerosol samplers that utilize 2 microcentrifuge tubes to collect airborne particulates. The first tube (T1) collects particles greater than 1.8 μm in diameter, while the second tube (T2) collects particles between 1.0 and 1.8 μm, and the back-up filter (F) collects submicron particles. Following aerosolization, quantitative PCR was used to detect and quantify H1N1 and H3N2 influenza strains.

Results Based on qPCR results, we demonstrate that aerosolized viral particles were efficiently collected and separated according to aerodynamic size using the two-stage bioaerosol sampler. Most viral particles were collected in T2 (1-1.8 μm) and on the back-up filter (< 1 μm) of the bioaerosol sampler. Furthermore, we found that the detection of viral particles with the two-stage sampler was directly proportional to the collection time. Consequently, viral particle counts were significantly greater at 40 minutes in comparison to 5, 10 and 20 minute aerosol collection points.

Conclusions Due to a lack of empirical data, aerosol transmission of influenza is often questioned. Using FluMist®, we demonstrated that a newly developed bioaerosol sampler is able to recover and size fractionate aerosolized viral particles. This sampler should be an important tool for studying viral transmission in clinical settings and may significantly contribute towards understanding the modes of influenza virus transmission.

Keywords Airborne virus, bioaerosols, bioaerosol sampler, influenza, viral detection, qPCR.

Introduction

Influenza infections are a public health concern accounting for more than 30 000 deaths and 100 000 hospitalizations annually.[1] The primary populations at risk for infection include young children, elderly adults, and immunocompromised subjects. During influenza outbreaks or pandemics, healthcare workers are at an elevated risk for acquiring influenza infection due to the prolonged periods of exposure to influenza viruses within healthcare settings. The mechanisms of influenza virus dissemination and transmission are poorly understood. Experimental studies have shown that influenza viruses can be transmitted via contact with respiratory secretions, large droplets, and aerosolized small particles/droplet nuclei.[2] Mucous membrane contact of large droplets expelled from the respiratory tract are thought to be the predominant mode by which influenza infection is transmitted, but small particle aerosols and droplet nuclei are also of concern due to the potential for prolonged airborne suspension.[3,4]

Given the epidemic potential and public health concern of newly emerging diseases such as avian influenza (H5N1) and severe acute respiratory syndrome (SARS),[5,6] it is important to develop methods to study aerosolized viral particles. Such measures would not only enable improved monitoring and detection of viruses, but more importantly, would help prevent widespread transmission. To date, several bioaerosol samplers are available that use impaction or impinger sampling methodologies for viral aerosol detection.[7,8] However, limitations exist within each of these

methods such as poor collection efficiency, limited sampling time, and inability to separate particles based on size. Further complicating the study of viral aerosols is that in many environments where it would be desirable to sample for viral aerosols (e.g., hospitals, airports), the level of other biologically relevant particles such as bacteria, fungi, and pollens can also be very high.

Based on a one-stage, cyclone-based bioaerosol sampler developed at the National Institute for Occupational Safety and Health (NIOSH),[9] a two-stage, cyclone-based bioaerosol sampler was recently fabricated.[10] The two-stage bioaerosol sampler is a lightweight device that can be used either as an area sampler (e.g., in a hospital room) or as a personal breathing zone air-sampling device worn on the lapel of the subject (e.g., a healthcare worker). This bioaerosol sampler contains two microcentrifuge tubes and a back-up filter, which separate airborne particulates based on their aerodynamic diameter.[10] The first tube (T1) of the sampler collects particles that are greater than approximately 1.8 μm in diameter, while the second tube (T2) collects particles from 1.0 to 1.8 μm in diameter and the back-up filter (F) collects submicron particulates. Because the sample is deposited directly in microcentrifuge tubes, the sampler facilitates the direct processing of samples for downstream diagnostic applications such as quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay. The bioaerosol sampler potentially eliminates several limitations associated with other air sampling devices such as sample loss, contamination, and degradation. In this proof of concept study, we characterized the utility of the two-stage bioaerosol sampler for fractionating viral particles and separating them from other particulates.

Materials and methods

Virus suspension

The Influenza Virus Vaccine FluMist® 2005–2006 formula was purchased from MedImmune Vaccine, Inc. (Gaithersburg, MD, USA). FluMist® is a live, trivalent vaccine, composed of the A/New Caledonia/20/99 (H1N1), A/California/7/2004 (H3N2), and B/Jiangsu/10/2003 (B/Shanghai/361/2002-like) strains. These strains are genetically altered to attenuated, cold-adapted, and temperature-sensitive phenotypes, which limits viral replication to the nasal pharynx. Each 0.5 ml dose has been formulated to contain approximately 10^7 $TCID_{50}$ ($10^{6.5}$–$10^{7.5}$ median tissue culture infectious dose) per viral strain.

Viral aerosolization and collection

Viral aerosols (FluMist®) were collected using 4 two-stage bioaerosol samplers and two vertical reference samplers placed at the bottom of a calm air aerosol settling chamber, as described previously.[11] The bioaerosol samplers were connected to personal air sampling pumps (Model 224-PCXR4; SKC, Eighty Four, PA, USA), while the vertical reference samplers were connected to a central vacuum line through a rotameter (Matheson Gas Products, Montgomeryville, PA, USA). The airflow through each sampler was calibrated to 3.5 l/minute before each experiment using a flow calibrator (DryCal DC-Lite; Bios International, Butler, NJ, USA). The bioaerosol samplers collect material in two polypropylene microcentrifuge tubes (no. 506-624, PGC Scientifics Corporation, Frederick, MD, USA) and on a 37 mm 2 μm polytetrafluoroethylene (PTFE) filter (P/N 225-17-07; SKC), 37 mm gelatin filter (P/N 225-9552; SKC), 37 mm glass fiber filter type A/E (P/N 225-7; SKC), or a 37 mm 5.0 μm polyvinyl chloride filter (P/N 225-8-01; SKC). Reference samplers used a 25 mm 0.5 μm PTFE filter (P/N P5PQ025, Pall Corporation, East Hills, NY, USA).

For aerosolization experiments, one dose (0.5 ml) of FluMist® was diluted with 49.5 ml of saline (0.9% NaCl). One milliliter of this solution was initially drawn for assay, and the remainder placed in a Collison single-jet nebulizer (BGI Incorporated, Waltham, MA, USA). The solution was aerosolized at 138 kPa (20 lbs/in²) air pressure, passed through a diffusion drier (Model 3062; TSI Incorporated, Shoreview, MN, USA), and mixed with 26 l/minute dry filtered air. The dry aerosol then flowed into the top of the calm air chamber. To avoid possible degradation of the virus by ozone, an unavoidable by-product, the bipolar ionizer employed in earlier studies[10] was not used. Instead, all conducting lines and the settling chamber itself were metal and grounded. Previous tests with the calm air chamber indicated that these precautions were sufficient to avoid electrostatic aerosol deposition.[11] The concentration and size distribution of the diluted aerosol was recorded using an aerodynamic particle sizer (APS Model 3321; TSI), which drew air from a vertical probe placed at the same height as the bioaerosol sampler inlets.

At the start of each experiment, the nebulizer was operated for 10 minutes to allow the aerosol concentration to reach equilibrium. After 10 minutes, the sampling pumps and vacuum source were activated simultaneously for all samplers. For aerosol collection studies, sampler pumps were switched off after 5, 10, 20, and 40 minutes, while reference samplers were switched off after 20 and 40 minutes. After aerosol collection, the exterior of each sampler was disinfected (Conflikt; Decon Labs, King of Prussia, PA, USA) and the collection tubes and filters removed for analysis. A 1 ml aliquot of the solution remaining in the nebulizer was also removed for analysis.

The particle size-segregation characteristics of the two-stage sampler were reported previously for a range of particle sizes.[10] Using this information, the particle size and

number data from the APS, an estimate was made of the relative amounts of viral material that should have collected in the first and second tubes and on the back-up filter. This estimate was compared to the actual proportions of viral material found in each stage of the sampler as measured by qPCR (below) to indicate if the two-stage sampler was separating the aerosol particles based on size as expected.

RNA extraction

Viral RNA was extracted using the MagMAX™ Viral RNA Isolation Kit (Ambion, Austin, TX, USA). Briefly, the supplied lysis/binding solution supplemented with carrier RNA was used in the extraction of viral RNA from either the neat FluMist® (qPCR standards) or experimental samples (T1 and T2). The back-up filters (F) from the bioaerosol samplers were aseptically removed, immersed in 1 ml of the supplemented lysis/binding solution and vortexed at moderate speed for 2 minutes. Viral lysis, RNA binding, magnetic capture, washing, and drying of the RNA-bound beads were all carried out according to the manufacturer's instructions.

Reverse transcription (RT) PCR

cDNA was generated by reverse transcription of the isolated viral RNA using TaqMan™ Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA). In brief, 40 μl of viral RNA was added to the RT-PCR mixture containing 10 μl of 10X RT buffer, 22 μl of 25 mm MgCl$_2$, 20 μl of 10 mm dNTP mix, and 5 μl of 50 mm random hexamers. RNASe inhibitor (2.0 μl, 20 U/ml) and 2.5 μl of MultiScribe Reverse Transcriptase (50 U/ml) were added finally, for a final volume of 100 μl. Samples were briefly centrifuged, placed in a Thermo Hybaid thermocycler (Ashford, UK) and run under the following conditions: 25°C for 10 minutes, 48°C for 30 minutes, and 95°C for 5 minutes. A control without template was run for each experiment.

Viral and fungal aerosolization

As a pilot study to simulate the more biologically complex aerosols that one might encounter in field situations, FluMist® was mixed with fungal spores and then aerosolized and collected. *Aspergillus versicolor* (ATCC 44408, American Type Culture Collection) was grown on malt extract agar for 16 days at 24.9°C and the spores isolated as previously described.[10] Spore concentrations were determined by hemacytometer count. For co-aerosolization, one dose of FluMist® was diluted with 49.5 ml of 0.9% NaCl containing 10^7 *A. versicolor* spores and 2 ml of RNAsecure Reagent (Ambion) to inhibit viral RNA degradation. The aerosol was generated using a Collison single-jet nebulizer, passed through a diffusion drier and mixed with dry filtered air

using the same experimental procedure as described above. The aerosol was collected with 4 two-stage samplers and two reference samplers. Two of the two-stage samplers and one reference sampler collected the aerosol for 10 minutes, while the remaining samplers collected the aerosol for 40 minutes.

DNA extraction

Genomic DNA was extracted from *A. versicolor*-containing samples as described by Griffin *et al.*[12] Briefly, 400 μl of AP1 buffer supplemented with 4 μl RNase A (DNeasy Plant Mini Kit; Qiagen, Valencia, CA, USA) was added to 10^7 spores (qPCR standard) or experimental samples. Filters were eluted in 1 ml of the supplemented AP1 buffer, vortexed for 2 minutes, and transferred to a microcentrifuge tube. Approximately 250 mg of 0.1 mm Zirconia/Silica Beads (BioSpec Products, Inc., Bartlesville, OK, USA) was added to each sample. Samples were bead-beaten at maximum speed in a Mini-BeadBeater-8 (BioSpec Products, Inc.) for 1 minute and chilled on ice for 2 minutes. The bead-beating/cooling steps were repeated twice. Samples were centrifuged for 10 minutes at 11000 ***g*** and protein precipitation, washing, and drying of fungal DNA was carried out according to the manufacturer's instructions.

Primer/probe design and optimization

Sequence information for A/New Caledonia/20/99 (H1N1), A/California/7/2004 (H3N2), and B/Jiangsu/10/2003 (B/Shanghai/361/2002-like) was obtained from the Influenza Sequence Database at Los Alamos National Laboratories, Los Alamos, NM.[13] Real-time PCR primers and probes were specifically targeted against influenza surface glycoproteins and designed using Primer Express software (Applied Biosystems). Sequence information for *A. versicolor* was obtained from the U.S. Environmental Protection Agency (http://www.epa.gov/microbes/moldtech.htm). Synthesis of all primers and probes was performed by Applied Biosystems. Table 1 lists the sequence and dye labels for primers and probes used in qPCR detection. To determine the optimal primer concentrations for qPCR analysis, an optimization qPCR matrix was performed for each primer/probe combination (singleplex). Optimal primer and probe concentrations used in qPCR detection ranged from 250 to 900 nm.

qPCR reactions

For detection of influenza or *A. versicolor* in aerosol samples, 10 μl of the generated viral cDNA or fungal genomic DNA, respectively, was added to 25 μl Applied Biosystem's TaqMan™ Universal PCR Master Mix. The appropriate concentration of primers and TAMRA-labeled probes was added, and brought to the final reaction volume of 50 μl with PCR-grade water. All reactions were run using the

Table 1. Real-time PCR primers and probes

Influenza strain	Gene	Sense primer	Antisense primer	TaqMan TAMRA probe
H1N1 (A/NC/20/99)	HA	GGAACATCCCATCCATTCAATC	CCACCCCCTTCAATGAAA	VIC-AGAGGTTTGTTTGGAGCCATTGCCG
H3N2 (A/C/7/2004)	NA	CAAAGCCGCAATGTGACATT	CCACCAGCGGAAAGCCTAA	VIC-CAGGATTTGCACCTTTTCTAAGGACAATTCG
B (B/J/10/2003)	HA	AGGAACAAGGACCAGAGGGAAA	CCCAAGGCCACATCCAGAT	6FAM-TGTCCAGACTGTCTCAA
Fungal isolate				
Aspergillus versicolor	rDNA	CGGCGGGGAGCCCT	CCATTGTTGAAAGTTTTGACTGATCTTA	VIC-AGACTGCATCACTCTCAGGCATGAAGTTCAG

Applied Biosystem's 7500 Real-Time PCR System at the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 60°C for 1 minute. In order to quantify the relative amount of viral particles or fungal spores collected at each stage of the bioaerosol sampler, qPCR was performed in parallel using either serial 10-fold dilutions of cDNA generated from a single dose of non-aerosolized FluMist® containing approximately 10^7 $TCID_{50}$ per influenza strain or genomic DNA isolated from 10^7 spores. A negative control without template was included in all qPCR reactions.

Statistical analysis

Statistical analysis was conducted on the experimental setup as repeated-measures anova, with main effects of stage and time. Stage is a three-level categorical variable with factors tube 1, tube 2, and back-up filter. To determine the effect of time, measurements were taken at 5, 10, 20, and 40 minutes. Proc Mixed in SAS v9.1 (SAS Institute, Cary, NC, USA) was used to analyze the data for significance and to calculate regression parameters. Proc Mixed was also used to insure similarity of experiments by testing variability between replicates. Results were considered significant if $P < 0.05$.

Results

Optimization of qPCR

Standard curves were generated for influenza strains H1N1 and H3N2 using serial 10-fold dilutions of cDNA isolated from a single dose of FluMist® (10^7 $TCID_{50}$/viral strain). For qPCR detection of *A. versicolor*, a standard curve was generated using genomic DNA isolated from 10^7 spores and serially diluted 10-fold. The standard curves for H1N1, H3N2, and *A. versicolor* were linear over a 4-log range of 1.0×10^3–1.0×10^6 particles. We were unable to generate a reproducible signal for the B strain virus using the specific primers that were designed. Standard curves were used to extrapolate relative viral or spore numbers in unknown samples using the 10^7 titer reported by the manufacturer or hemacytometer counts, respectively.

Optimization of aerosol detection

When FluMist® was aerosolized the virus-laden aerosol in the calm air chamber typically contained about 17 000 particles/cm³ with a median aerodynamic diameter of 0.8 μm and a geometric standard deviation of 1.33. As part of methods development, the optimal back-up filter composition for virus collection was determined. Four bioaerosol samplers were fitted with different types of filters – gelatin, glass fiber, PTFE, and polyvinyl chloride. Following a 40 minute collection, several gelatin filters were found to
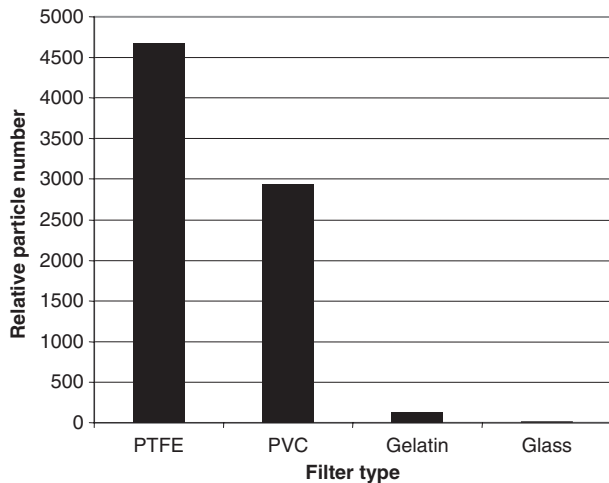
Figure 1. Relative number of H3N2 viral particles collected on the back-up filters. FluMist® was aerosolized in the calm air chamber, samples were collected on filters of different composition after 40 minutes and analyzed using qPCR.

be fractured. During RNA extraction, the glass fiber filters released a high number of particulates that presumably interfered with qPCR detection. Based on an initial experimental run, the PTFE filter was found to be optimal for the recovery of viral particles (Figure 1). All subsequent aerosol experiments were performed with the PTFE filters.

Kinetics of aerosol collection

To characterize the collection efficiency of the bioaerosol sampler, three identical experiments were performed. Figure 2(A, B) illustrates the collective results for viral aerosols collected at 5, 10, 20, and 40 minutes. For both H1N1 and H3N2, collection of viral particles with the

two-stage sampler was linear up to 40 minutes. Viral particle counts were significantly greater at 40 minutes in comparison with the 5, 10, and 20 minute collection times ($P < 0.05$). Furthermore, as predicted by the aerodynamic particle size data, the greatest proportions of the viral particles were detected in T2 and the back-up filter F, regardless of the collection time. For strain H1N1 at 40 minutes, 75% of the viral particles were detected in stage T2 and F, while only 25% of particles were detected in T1. Likewise, with strain H3N2, 77% of the particles were detected in stages T2 and F with 23% of the particles detected in stage T1. These results were consistent throughout all experiments and demonstrate the ability of the bioaerosol sampler to separate particles based on aerodynamic size. While a similar distribution of H3N2 particles was recovered, for unknown reasons the absolute particle numbers were significantly lower.

Kinetics of co-aerosolization collection

To examine the effectiveness of the bioaerosol sampler to separate a mix of aerosolized particles, we co-aerosolized FluMist® with *A. versicolor* spores (aerodynamic diameter ~2.3 μm). Following 40 minutes of aerosolization (Figure 3), the bioaerosol sampler was able to separate these particles of differing size. Both qPCR and spore counts confirmed that stage T1 retained 96% of the *A. versicolor* spores while stage T2, with 2% of the spores, was found to contain the greatest amount of viral particles (48%). An overall shift in the deposition of viral particles toward T1 was observed when co-aerosolized with *A. versicolor* spores (24%–42%). Significantly fewer viral particles were detected on the back-up filter F (10%) in comparison with the back-up filter F using FluMist® alone (38%), suggesting some interaction or aggregation of the particles during aerosolization.
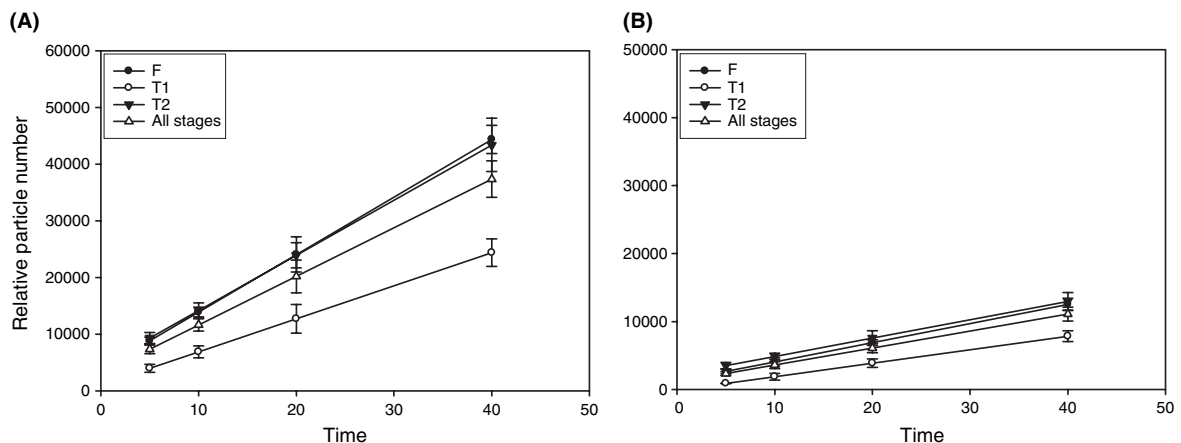

Figure 2. Regression analysis of influenza particle accumulation over time. Values from three replicate experiments were combined and the average relative number of particles for each stage is presented. (A) H1N1; (B) H3N2 viral particles.
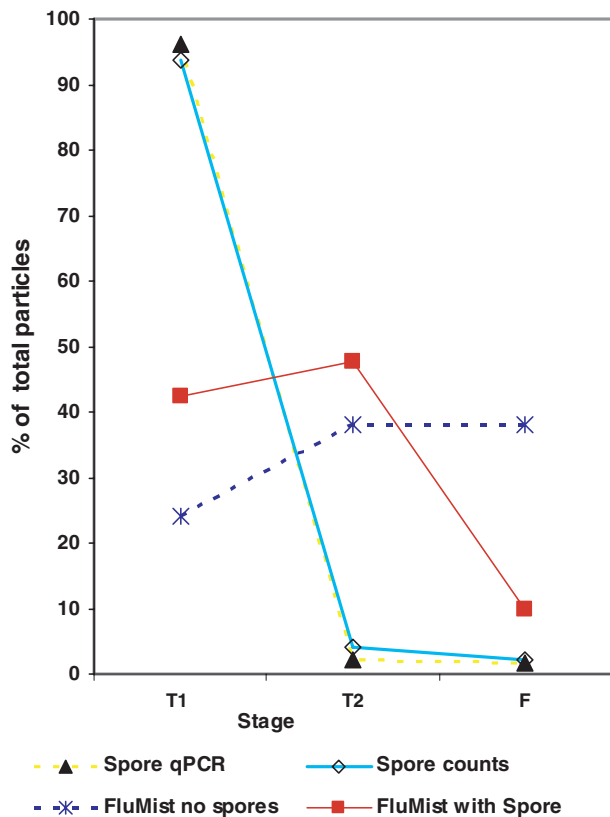
Figure 3. Separation of aerosolized particles by stage. Spores of *Aspergillus versicolor* (10^7) and FluMist® were co-aerosolized together into the calm air chamber. Samples were collected at 40 minutes and analyzed for influenza viruses using qPCR and *A. versicolor* using qPCR and hemacytometer counts. Data for each stage is presented as the percentage of total number of particles collected in the three stages. Spore counts were the average of eight replicate hemacytometer counts. Values for the FluMist® with no fungal spores were taken from the previous experiment presented in Table 2 and represent the combined average values for H1N1 and H3N2 strains.

Discussion

The need for rapid and accurate methods for detecting airborne viruses has increased in recent years, particularly following the reported outbreaks of avian influenza (H5N1) and SARS. Various aerobiological monitoring studies have shown a high degree of variability with capturing, recovering, and detecting aerosolized viral particles in environmental and clinical samples.[7,14–16] Using the NIOSH bioaerosol sampler, we were able to overcome some common viral particle sampling limitations and fractionate aerosolized influenza particles from an artificially generated aerosol.

Bioaerosols vary in size, concentration, composition, and settling times.[3,17] An aerodynamic particle sizer was used to monitor the concentration and size distribution of the FluMist® aerosol within the settling chamber. Using the bioaerosol sampler, it could be demonstrated that capture, recovery, and subsequent detection for each sampler stage (T1, T2, F) were consistent with the expected values based on particle size. As anticipated, qPCR results confirmed that a significant number of the aerosolized viral particles were localized within stages T2 and F. These results are in agreement with the sampler cutoff size of 1.0 and <1 μm for stages T2 and F, respectively. The small number of aerosolized particles detected in stage T1 can be attributed to the collection efficiency of the bioaerosol sampler.

The engineering design of the bioaerosol sampler is critical in the size-fractionation of bioaerosols, but sampling time must also be taken into consideration. qPCR results of aerosolized viral samples collected at 5, 10, 20, and 40 minutes confirms that a sampling time of 40 minutes yields the highest quantity of viral particles; however, this does not necessarily represent an ideal environmental sampling time as the quantities of aerosolized influenza virus expelled from patients have not yet been evaluated. In aerobiological studies using different viral strains,[18,19] it was found that prolonged sampling periods resulted in decreased viral recovery. Likewise, because influenza viruses are stress sensitive, the possibility exists that lengthier sampling times may result in the desiccation of viral-laden aerosol and consequently compromise stability. Future studies will aim at addressing the effects of prolonged sampling time on viral recovery and stability.

Using the bioaerosol sampler, the collection of aerosols within disposable microcentrifuge tubes limits sample loss and contamination. Moreover, samples are directly processed within the respective tubes and further analyzed by different diagnostic methods including immunoassays or molecular detection techniques such as qPCR. Currently qPCR is the preferred methodology for the rapid and sensitive detection of viruses;[20] however, several limitations still exist. The sensitivity and specificity of qPCR detection is limited to the targeted template. In this study, primers and probes were designed to selectively amplify the HA and NA genes from strains H1N1 and H3N2, respectively. Interestingly, the results showed a considerable difference in the number of viral particles that were detected for strains H1N1 and H3N2. The FluMist® vaccine is formulated so as to contain an equivalent concentration of each viral strain using the median culture infectious dose ($TCID_{50}$) assay[21] but does not account for non-viable viral particles. qPCR can detect viral cDNA from both viable and non-viable viral particles, and perhaps explain this discrepancy. As for the qPCR detection of strain B, erratic results were obtained. As qPCR detection was successful for both strains H1N1 and H3N2, this may not be due to the presence of inhibitors in the reaction but instead is more likely due to poor primer or probe design or the presence of secondary

Table 2. Relative number of viral particles recovered

Influenza strain	Time course (minutes)			
	5	10	20	40
H1N1 (A/New Caledonia/20/99)				
Tube 1	5451 ± 2078*	7169 ± 3100*	9760 ± 7596*	25 624 ± 7329
Tube 2	8647 ± 2861*	14 345 ± 3983*	24 970 ± 6314*	42 962 ± 14 161
Backup filter	7576 ± 1869*	13 924 ± 2530*	26 286 ± 11 716*	43 412 ± 7507*
Reference filter	n/a	n/a	58 279 ± 9174	100 964 ± 21 043
Collection efficiency	n/a	n/a	104.7%	110.9%
H3N2 (A/California/7/2004)				
Tube 1	1106 ± 144*	2603 ± 1441*	2419 ± 1855*	8366 ± 2426
Tube 2	3113 ± 1398*	4618 ± 1414*	8793 ± 2964	12 530 ± 3829
Backup filter	2405 ± 1162*	4104 ± 1847*	7228 ± 1254*	12 369 ± 2716
Reference filter	n/a	n/a	15 961 ± 6017	29 225 ± 9331
Collection efficiency	n/a	n/a	115.5%	113.8%

*Significance of time course where $P < 0.05$.
n/a, not applicable.

structures in the influenza B RNA, resulting in poor reverse transcription and insufficient target template.

Environmental bioaerosols vary considerably and may consist of a number of different microorganisms including viruses, bacteria, and fungi. Recent findings by Lindsley et al[10] demonstrated that the two-stage bioaerosol sampler was effective at collecting and separating aerosolized fungal spores and fragments. Results of the current study provide supporting evidence that the bioaerosol sampler is able to successfully recover and size-fractionate viral-laden aerosols. The culmination of these results suggests that the bioaerosol sampler would be an ideal air-sampling device for the aerobiological monitoring of various microorganisms within occupational environments. However, preliminary findings from a co-aerosolization study suggest fractionation of environmental samples may be more complex. Namely, an overall shift in the viral particle deposition in the presence of A. versicolor spores was observed. The shift in viral particle deposition may be attributed to the attachment of viral particles to A. versicolor spores. APS data (not shown) further suggest that particles adhere to one another. Given that the viral and spore-laden solution is pre-mixed prior to aerosolizing, it remains unclear as to whether the binding may occur before, during or after aerosolization within the calm-air settling chamber. Future studies will further elaborate on the use of the bioaerosol sampler to capture and effectively size-fractionate co-aerosolized particles of different species.

Currently, there are conflicting views as to whether influenza viruses are spread by direct contact with secretions, large droplet transmission, or aerosol transmission. Due to the lack of virtually any environmental data, aerosol transmission of influenza viruses is often overlooked as a possible mode of transmission. In this study, by aerosolizing FluMist®, we demonstrate the recovery of aerosolized viral particles using the bioaerosol sampler and detection of influenza by qPCR. Whether aerosolized influenza particles are a significant contributor to influenza transmission in work environments and the community remains to be determined. Future experiments will focus on studying the dissemination of viral-laden aerosols utilizing an artificial cough generator to simulate cough dispersal of influenza viruses within a room-sized aerosol chamber. These findings would significantly contribute toward understanding the transmission of aerosolized influenza viral particles. Furthermore, we will test the bioaerosol sampler in a healthcare setting to monitor the prevalence of airborne influenza viral particles and to study the transmission of influenza via the inhalation of aerosolized viral particles. Such studies will help to further elucidate the routes of transmission of influenza and would ultimately contribute to better patient management as well as improve infection control guidelines and decrease worker health risk.

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