Effectiveness of negative air ionization for removing viral bioaerosols in an enclosed space

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ABSTRACT Air ionization is gaining acceptance as a method of choice for the purification of air. The method has been applied successfully to remove non-pathogenic particulates, such as smoke, dust, and pollen, from air. However, little is known on its effectiveness for the purification of air laden with pathogenic microorganisms. This study aimed to investigate the effectiveness of air ionization in removing aerosols loaded with viral pathogens. Reovirus bioaerosols were first generated into an airtight chamber. Subsequently, an air ionization system located within the chamber was activated to generate negative ions. Using biosamplers, samples were taken from within the chamber at predetermined time intervals (5 minutes, 10 minutes, and 30 minutes). A laser particle analyzer was utilized to monitor the size distribution and concentration of the bioaerosols prior and after generation of negative ions. Plaque assays were conducted to determine the infectivity of the sampled air. Preliminary results indicated that the air ionization system significantly reduced the infectivity of reovirus.

Keywords: Bioaerosol; Ionization; Plaque Assay; Reovirus

INTRODUCTION The quality of air within enclosed spaces is critical to the health and welfare of occupants. Clean air is essential for the body's respiratory and metabolic processes. Bioaerosol is one of the most important air contaminants in confined spaces. Shortly after generation, larger aerosols (>30µm diameter) fall off to surfaces; however, fine aerosols (<10 µm diameter) can remain airborne for extended periods of time. Among the methods for air purification, air ionization has gained research attention among air quality investigators. Air ionization involves the generation of ions, which attach themselves to bioaerosols and subsequently drive the charged bioaerosols to grounded or oppositely charged surfaces. The phenomenon occurs when free electrons with high energy dislodge electrons from gas molecule atoms to create more electrons and ions in air. Air ionization can be triggered by corona discharge, which takes place when an electrode supplied with a high potential (greater than 5 kV) causes a partial electric discharge near the electrode (Boghard and Eklund 1998).

Numerous studies have been conducted to investigate the effectiveness of air ionization in the reduction of bioaerosols and airborne particles. Boghard and Eklund (1998) investigated the effectiveness of air ionization in the reduction of NaCl in an enclosed steel chamber and reported a three-fold decrease in the NaCl particles present in the air. Khan et al (2000) utilized an air ionizer to investigate the efficiency of negative air ionization in reducing smoke concentration within 0.5 m^3 enclosure and reported up to a six-fold removal rate. Grabarczyk (2001) conducted studies on a whole room air ionization by hanging a set of electrodes beneath the ceiling of a 50 m^3 room, and reported that 2 hours of whole room air ionization was effective in reducing dust present in the room by 100-fold. Lee et al. (2004) evaluated the efficiency of three commercial air ionizers in reducing aerosols with aerodynamic diameter between 0.04 – 2 μm. They reported that the most powerful device among the three removed about 97% of 0.1 μm particles from air after 30 minutes of continuous operation of the ionizer. In a similar study, Grishpun et al. (2005) evaluated five air ionizers (three stationary and two wearable) to determine their efficiency in reducing aerosol concentration within enclosed spaces. They reported that the more powerful of the two wearable ionizers removed 100% of aerosols (0.3 – 3 μm) after 1.5 hours of uninterrupted operation in the chamber under steady state air conditions. The most efficient of the stationary units removed 100% of the particles (0.3 - 3μm) within 12 minutes.

Clearly, air ionization has been proven to be an effective technology in purifying air that contains non-pathogenic particulates. However, little is known on the effectiveness of air ionization on the purification of air loaded with pathogens. For air ionization to be effective in purifying air laden with pathogens, it must not only remove pathogens from air but in addition kill or inactivate the pathogens. Therefore, the effectiveness of air ionization on airborne pathogens must be determined by laboratory analysis. A technique known as plaque assay can be used to determine the level of infectivity of air borne viral pathogens. It involves infecting host cells with the airborne virus sample and subsequently counting the number of live viruses. The objective of this study was to conduct laboratory experiments to determine the effect of negative air ionization on the infectivity of viral bioaerosols.

METHODOLOGY A bioaerosol chamber (bio-chamber) made from plexiglass and with an internal diameter of 0.8 m, 0.3 m and 0.55 m in length, width and height respectively was built to conduct the experiment (Figure 1). A plastic hose barb, which was installed at the left side of the chamber served as the inlet for introducing the bioaerosols into the chamber. A probe was placed inside the chamber to record temperature and relative humidity levels during experimentation. The bio-chamber was placed under the hood of a biosafety cabinet during experimentation in a virology (Biosafety level 2) laboratory located at the University of Manitoba (Bannatyne Campus).
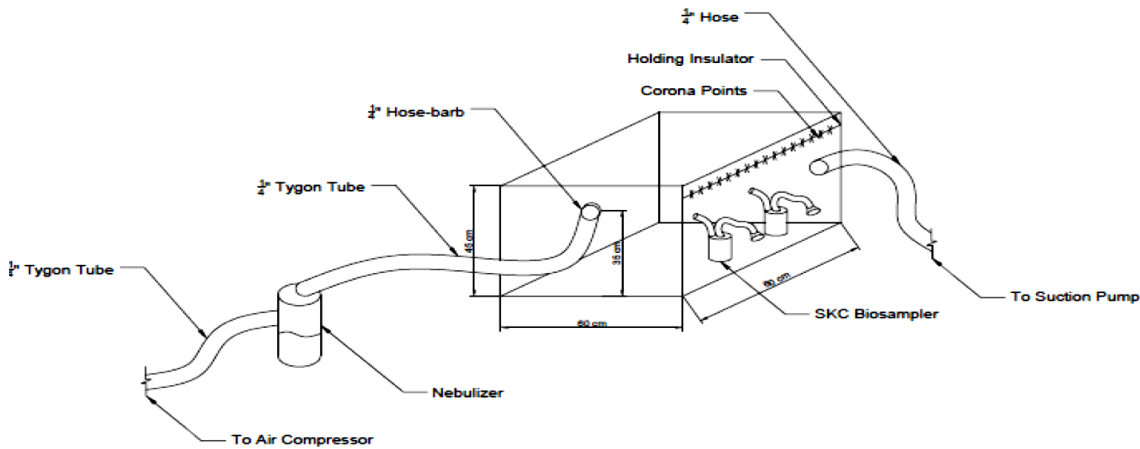
Figure 1. Schematic diagram of experimental chamber used for conducting ionization test.

Aerosol generation An aerosol generation system was used to generate liquid bioaerosols with sizes in the range of 0.2 μm to 5 μm. Through an arrangement of pressure regulators and valves, compressed air was used to drive a non-enveloped virus (mammalian reovirus, MRV) bioaerosols via a 6-jet collision nebulizer (CN25, BGI Inc., Waltham, MA) into the bio-chamber. MRV are pathogens that cause gastrointestinal and respiratory infections (Berard and Coombs 2009). MRV was chosen because it is very stable in air. The MRV virus was grown in L929 cells and subsequently quantified in terms of Plaque forming units (PFU). The virus titers for the stock solution used for the experiments were 10^{10} PFU/mL and 10^8 PFU/mL. Bioaerosols were generated at pressures of 344.7 kPa and 172.7 kPa. Aerosols generation system was turned on for approximately 7 minutes. At a pressure of 344.7 kPa, the aerosol generation rate was 34.3 mL/hr, 17.1 mL/hr at 172.7 kPa. A combination of aerosol generation rate and virus titer was taken as a test and each test was replicated three times.

Table 1. Tests for chamber experiments

Aerosol Generation Rate (mL/hr)	Virus Titer @ 10^{10} PFU/mL (V1)	Virus Titer@10^8 PFU/mL (V2)
34.3 (A1)	A1V1	A1V2
17.1 (A2)	A2V1	A2V2

A1 - Aerosol generation rate (34.3 mL/hr) A2 - Aerosol generation rate (17.1 mL/hr),

V1- Virus Titer @ 10^{10} PFU/mL V2 - Virus Titer@10^8 PFU/mL

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Airflow Two small battery operated fans were placed inside the chamber to circulate the air to enhance the uniformity of bioaerosol distribution. A smoke test was conducted using colored smoke cartridges to determine the pattern of airflow in the chamber. Airflow was video-recorded over a ten-minute period. Video image analysis showed that the air mixed uniformly in the chamber.

Sampling Sampling of the MRV bioaerosols from the chamber was performed using an all glass impinger SKC biosamplers (model no. 225-9594, SKC Inc. Eighty Four, PA). Bioaerosols were sampled at determined time points of 5minutes, 10 minutes and 30 minutes. A tygon tube was fitted to the inlet of the biosampler and in turn connected to the sampling port on the chamber. Each biosampler was filled with 20mL of Phosphate-buffered saline and oriented perpendicular to the chamber inlet during sampling. The biosampler was operated by turning on a suction pump (model no. 228-9605, SKC Inc. Eighty Four, PA), which was connected to the sampling ports of the biosampler. Prior to start of experiments, the biosamplers were calibrated. A particle counter/analyzer (APSS; LAP 322, Topas GmbH, Germany) was used to monitor the bioaerosol concentration in the chamber. Samples from the walls of the chamber were collected using Whatman paper (WHT1005042, Fisher Scientific, Ottawa, Ontario).

Air Ionization System A negative air ionization system (Baumgartner Environics Inc., Olivia, Minn.) was used for this study. The system comprised of several components, namely corona line, corona points (smaller electrode) connected to a large electrode, insulators, high voltage wire, and a power supply. The single corona line was installed parallel to the direction of flow and placed in the centre along the length of the bio-chamber. The corona line and electrode measured 0.6 m in length and was placed at a height of 0.5 m from the bottom of the chamber. The ends of the corona line and electrode were connected to insulators which in turn were attached to the chamber walls. A high voltage wire was used to connect the corona line to a power supply that provided high voltage (30 kV) and low current electricity to the corona points. The ion concentrations within the chamber was measured using an ion counter (AIC 200 million model, AlphaLab Inc., Salt Lake City, Utah). The measured ion concentration in chamber was 10^6 ions per cubic centimeter (ions/cm^3).

Micro and Molecular Biology Work Aliquots of samples collected from the bio-chamber were kept in 1.8 mL cryogenic tubes and stored in a freezer at -80 ^{o}C until all the chamber experiments were completed. Plaque assays were subsequently conducted to determine titers of the air samples. A 100 µL volume of the sample from the biosampler was diluted in 900 mL of gel saline to obtain a tenfold tube dilution. A serial dilution was conducted until a final dilution of 10^{-7} was obtained. A 100 µL volume each from the 10^{-2} to 10^{-6} dilutions was used to infect L929 cells in 6 well-plates. The L929 cells were 85% confluent prior to infection. For the ionization test samples, a 200 µL volume of the initial virus stock from biosampler was used to infect the cells in the first two wells. The rest of the cells were infected with 100 µL volume of serial dilutions up to a final dilution of 10^{-3} for each test. The expected titers from the plaque assays under natural decay were theoretically estimated using criteria shown below;

$$V_{iTC} = \frac{V_{BC} \times V_{iST}}{V_{TC}} \qquad (1)$$

$$V_{AS} = B_R \times T_S \qquad (2)$$

$$V_{iS} = V_{AS} \times V_{iTC} \qquad (3)$$

The quantity of air drawn by the biosampler was diluted in 20 mL of PBS, therefore the new virus titer =

$$V_{iB} = \frac{V_{iS}}{V_{AS}} \qquad\qquad (4)$$

A quantity of 100 µL from biosampler was used for plaque assay, therefore

$$V_{iP} = V_{iB} \times \frac{1mL}{1000\mu L} \times 100\mu L \qquad (5)$$

Where;

V_{iTC} = Virus Titer in whole chamber $\left(\frac{PFU}{mL}\right)$ V_{BC} = Volume of bioaerosols in chamber (mL)

V_{iST} = Virus stock Titer (PFU/mL) V_{TC} = Total volume of chamber (m^3)

V_{AS} = Volume of air sampled (m^3) B_R = Biosampler sampling rate $\left(\frac{L}{min}\right)$

T_S = Sampling time $(mins)$ V_{iS} = Titer of virus sampled (PFU)

V_{iB} = Virus titer in biosampler (PFU/mL) V_{iP} = Plaque assay titer from sample $\left(\frac{PFU}{mL}\right)$

RESULTS Figures 2 through to 4 show results obtained from the chamber experiments. Viral titers from natural decay tests (the ionization system was off) were significantly lower than the theoretical viral titers. Physical losses of bioaerosols occurred at the inlet of the chamber due to condensation and this partly explains the reduction in viral titers for the natural decay test. Furthermore, some larger bioaerosols may have settled on the floor of the chamber and hence contributed to reduction in the expected viral titers of the air samples. In subsequent studies, the quantity of bioaerosol that settled will be quantified and the corresponding viral titers will be determined.

There was significant difference between natural decay viral titers and ionization tests viral titers at each time point for all tests (Figures 2 to 5). The viral titers for the ionization test was significantly lower than natural decay tests. The least reduction in viral titer for the ionization tests occurred in test one (Figure 2) at five-minute sampling time. For most ionization tests, the viral titers had been reduced to zero at ten-minute sampling time (Figures 3 to 5). For all ionization tests, the viral titers had been reduced to zero at thirty-minute sampling time.
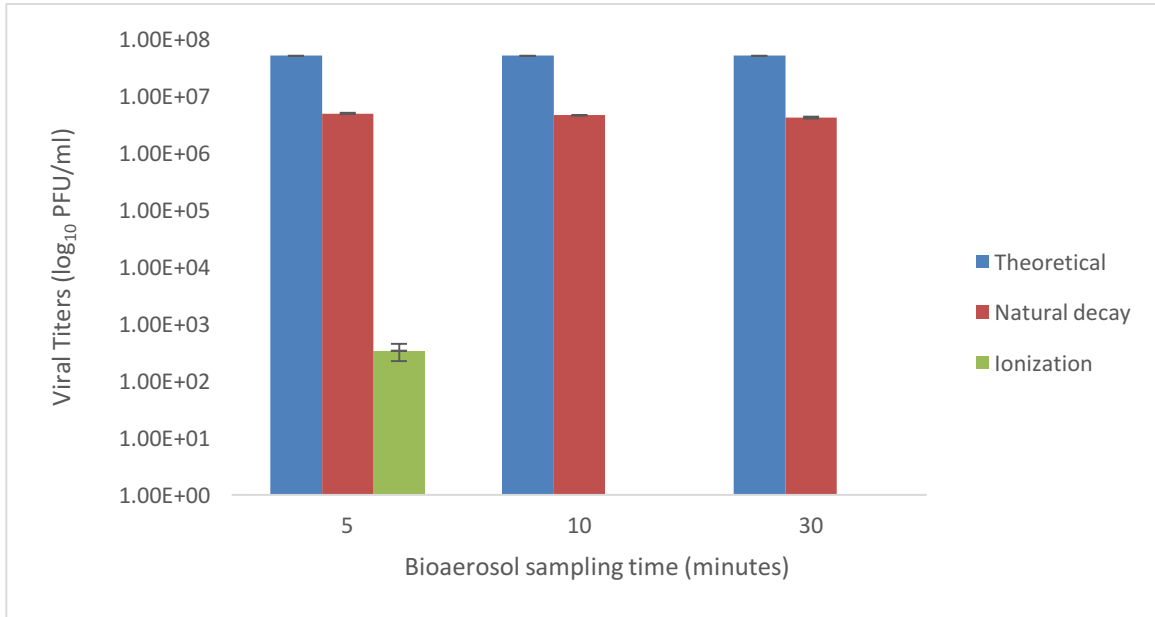
Figure 2. Viral titers as determined by plaque assays at different time points for 10^{10}PFU/mL and 34.3 mL/hr (Test 1).
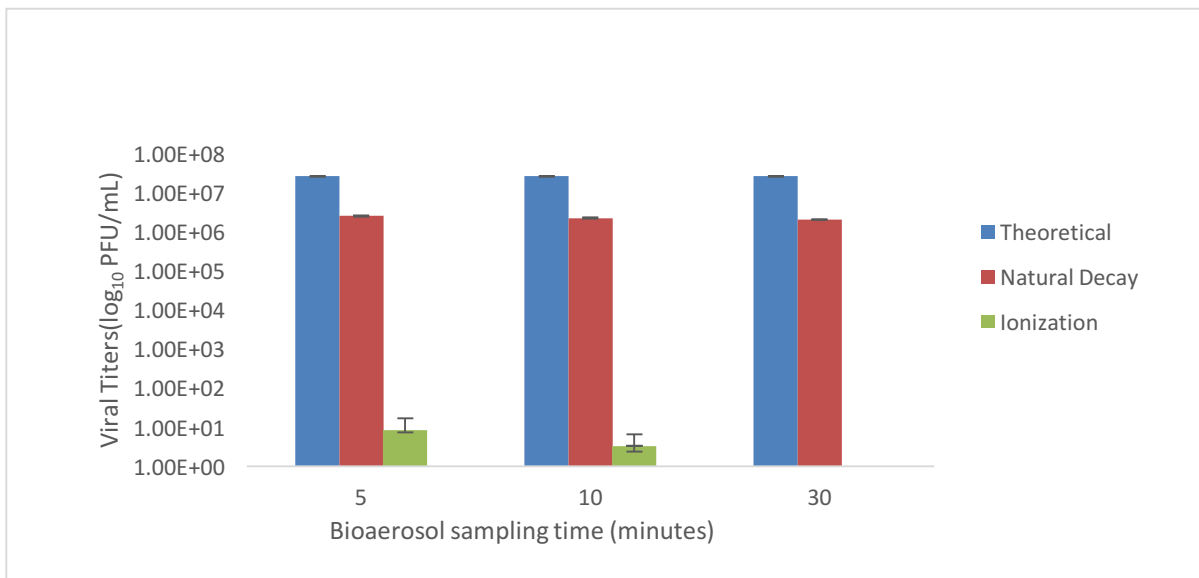

Figure 3. Viral titers as determined by plaque assays at different time points for 10^{10}PFU/mL and 17.1 mL/hr (Test 2).
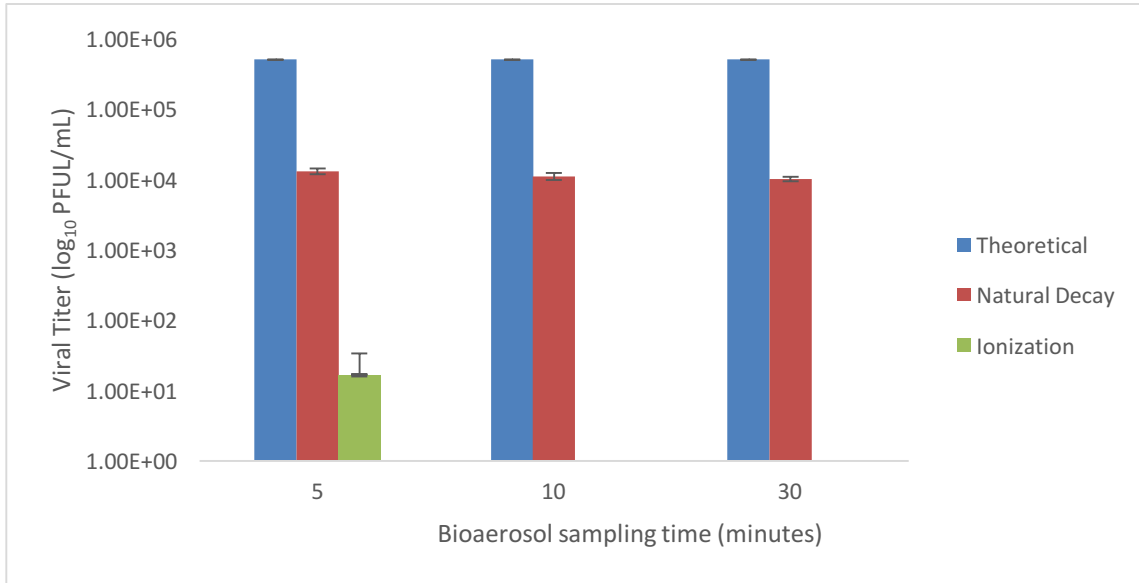
Figure 4. Viral titers as determined by plaque assays at different time points for 10^8PFU/mL and 34.3 mL/hr (Test 3).
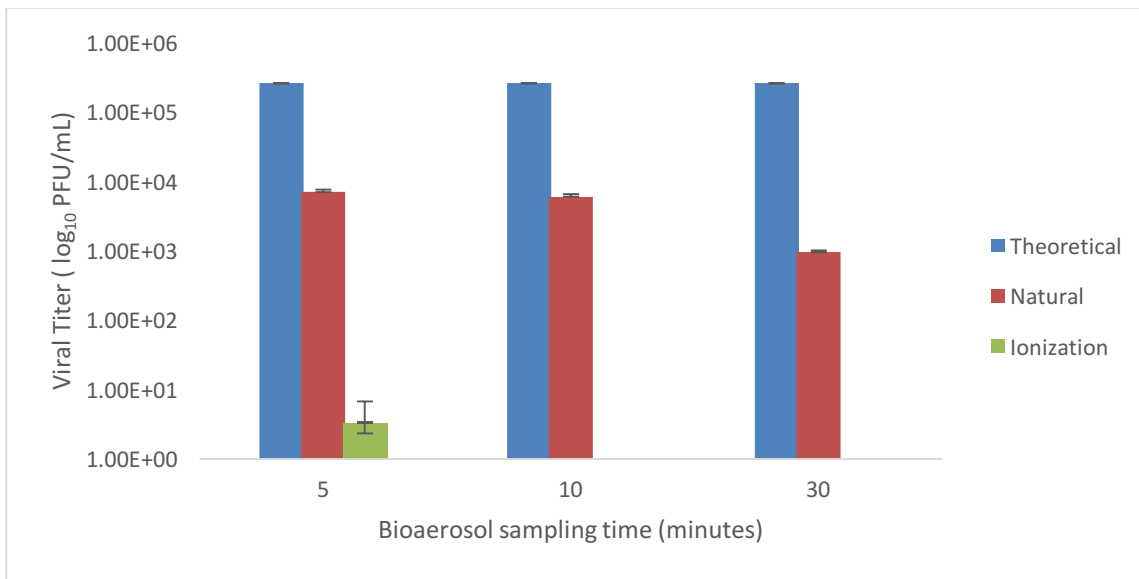

Figure 5. Viral titers as determined by plaque assays at different time points for 10^8PFU/mL and 17.1 mL/hr (Test 4)

CONCLUSION Preliminary results show that air ionization reduced the infectivity of airborne MRV. Specifically, the virus titer was reduced to zero after ten minutes of air ionization at ion concentration of 10^6 ions/cm^3.

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