Characterization of Experimental Influenza Virus-Laden Bioaerosols and Aerosols of Respiratory Origin During Aerosol-Generating Medical Procedures

by

Nathan Doggett

A thesis submitted in conformity with the requirements for the degree of Master of Science
Laboratory Medicine & Pathobiology
University of Toronto

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Laboratory Medicine & Pathobiology
University of Toronto
2017

Abstract

The characterization of infectious bioaerosols is required to identify related determinants of transmission by the respiratory route. The detection and quantification of influenza virus-laden bioaerosols remains a significant challenge in clinical and field settings. Additionally, medical procedures with the potential to generate aerosols may increase the risk of infection for healthcare workers. However, the characteristics and determinants of aerosol production, during potential aerosol generating procedures including intubation and bronchoscopy, remain poorly characterized. Here we assess performance characteristics of four commonly-used bioaerosol samplers for the collection of influenza A virus strains. We also quantify aerosol generation during experimental pig intubations and elective patient bronchoscopies. We show that low-volume filter and cyclone bioaerosol samplers perform best for both the collection of RNA and viable virus under varying environmental conditions. We also detect a non-significant increase in submicron aerosol particles during bronchoscopy and recover oral bacteria from the air during pig intubations.
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<th>Full Form</th>
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<tbody>
<tr>
<td>ACI</td>
<td>Andersen cascade impactor</td>
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<tr>
<td>ARI</td>
<td>acute respiratory infection</td>
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<tr>
<td>ALRI</td>
<td>acute lower respiratory infection</td>
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<tr>
<td>AGPs</td>
<td>aerosol generating procedures</td>
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<tr>
<td>BAL</td>
<td>bronchoalveolar lavage</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Prevention and Control</td>
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<tr>
<td>HA</td>
<td>Hemagglutinin</td>
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<tr>
<td>HCW(s)</td>
<td>healthcare worker(s)</td>
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<tr>
<td>HID</td>
<td>human infectious dose</td>
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<tr>
<td>IAV</td>
<td>influenza A virus</td>
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<tr>
<td>IBV</td>
<td>influenza B virus</td>
</tr>
<tr>
<td>ICV</td>
<td>influenza C virus</td>
</tr>
<tr>
<td>IPC</td>
<td>infection prevention and control</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>matrix assisted laser desorption ionization time-of-flight</td>
</tr>
<tr>
<td>MDCK</td>
<td>Madin Darby canine kidney</td>
</tr>
<tr>
<td>MMD</td>
<td>mass median diameter</td>
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<tr>
<td>NA</td>
<td>Neuraminidase</td>
</tr>
<tr>
<td>NIOSH</td>
<td>National Institute for Occupational Safety and Health</td>
</tr>
<tr>
<td>OPC</td>
<td>Optical particle counter</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PFU</td>
<td>plaque forming units</td>
</tr>
<tr>
<td>PHAC</td>
<td>Public Health Agency of Canada</td>
</tr>
<tr>
<td>pH1N1</td>
<td>influenza A/California/2009 (H1N1)</td>
</tr>
<tr>
<td>PMF</td>
<td>peptide mass fingerprint</td>
</tr>
<tr>
<td>PPE</td>
<td>personal protective equipment</td>
</tr>
<tr>
<td>PR8</td>
<td>influenza A/Puerto Rico/8/1934 (H1N1)</td>
</tr>
<tr>
<td>PSL</td>
<td>polystyrene latex</td>
</tr>
<tr>
<td>PTFE</td>
<td>polytetrafluoroethylene</td>
</tr>
<tr>
<td>qPCR</td>
<td>quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>RSV</td>
<td>respiratory syncytial virus</td>
</tr>
<tr>
<td>SARS-CoV</td>
<td>severe acute respiratory syndrome coronavirus</td>
</tr>
<tr>
<td>TCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% tissue culture infectious dose</td>
</tr>
<tr>
<td>UV-APS</td>
<td>ultraviolet aerodynamic particle sizer</td>
</tr>
<tr>
<td>VPA</td>
<td>viral plaque assay</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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Chapter 1 – Introduction

1.1 Bioaerosols

1.1.1 Generation

Aerosol particles containing organic matter or of biologic origin are referred to as “bioaerosols”. These bioaerosols may pose a risk of infection under certain circumstances if their composition includes pathogenic microorganisms, such as bacteria, fungi, or viruses. Several physiologic functions have been proposed as mechanisms of bioaerosol generation, including breathing, talking, sneezing and coughing. Earlier studies have suggest that aerosol particles are generated during these activities due to turbulent air being expelled at a high rate over mucosal membranes. However, a more recent model of breath aerosol generation, referred to as the “bronchiole fluid film bust” (BFFB) model, suggests that aerosols are produced during breathing as a result of bubbles of respiratory fluid bursting during clearance of fluid closures in the lower bronchioles, as a result of exhalation. Interestingly, several studies have also shown instances of mouth breathing producing more aerosols than other respiratory activities. Nonetheless, there is a dearth of empiric data regarding the determinants of bioaerosols production and characteristics of transmission.

1.1.2 Transmission

Transmission through the air may occur through respiratory droplets alone, and/or though droplet nuclei. Respiratory droplets are comprised of larger particles (>10 µm in diameter) and tend to settle quickly due to inertia, before having a chance to completely desiccate. They are responsible for short-range transmission (<2 m) primarily through direct deposition on a susceptible individual’s mucosal surfaces, or through indirect transmission via contaminated surfaces (fomites). Droplet nuclei are comprised of particles in the respirable range and are responsible for what is classically considered airborne transmission. Airborne transmission involves the inhalation of small bioaerosol particles (<5 µm in diameter), which can penetrate deep into the lower airway and reach the alveolar region. Droplet nuclei can remain suspended in air for prolonged periods, disseminating over extended distances. The settling time for
bioaerosol particles depends on particle size, such that the settling time for a 100 µm droplet to fall from room height (3 m) is 10 s, a 20 µm particle is 4 min, and a 10 µm particle is 17 min, whereas a 1-3 µm particle will remain suspended almost indefinitely\textsuperscript{1,8,9}.

Pathogen transmission via bioaerosols presents a substantial challenge in terms of infection control, and a significant risk to healthcare workers (HCWs) caring for patients with acute respiratory infections (ARIs)\textsuperscript{1,10,11}. Transmission through the air, over both short and long range distances, has been observed in animal models of influenza\textsuperscript{12,13}. Furthermore, a number of studies have shown that the generation of smaller droplet nuclei and larger respiratory droplet from the respiratory tract can occur simultaneously\textsuperscript{14-16}, making it difficult to determine which particles are predominantly responsible for short-range transmission. This may further complicate infection prevention and control (IPC) strategies, particularly when assigning a novel pathogen a specific transmission route, and subsequent determination of appropriate infection control precautions.

1.1.3 Size and Deposition

The size of a bioaerosol particle is the single most important factor in determining its fate within the respiratory tract\textsuperscript{1} and will determine preferential deposition within the respiratory tract. This is an important factor in determining whether an infection will occur, and if so, where; this has implications for clinical manifestations and outcomes of disease. Smaller droplet nuclei particles are preferentially deposited in the lower respiratory tract, where infection is associated with increased severity, morbidity and mortality\textsuperscript{17,18}. Other factors that may affect deposition include particle shape, density, as well as host factors, such as airway physiology and immune status\textsuperscript{18-20}. Additionally, pathogen specific characteristics such as viability in a bioaerosol, virulence, infectious dose, and chemical composition will affect whether an infection will occur at the deposition site\textsuperscript{21,22}.

1.1.4 Deposition and host defenses

The clearance mechanisms for inhaled particles deposited in the respiratory tract depend in part upon anatomic position of deposition. The nasopharyngeal and tracheobronchial regions are protected by a layer of mucus, which is propelled upward by the movement of cilia and can be cleared or swallowed to remove potential pathogens\textsuperscript{20}. However, due to requirements of gas
exchange, the alveolar region in the lower airway is not ciliated or covered by mucus, and particles deposited here must be cleared by macrophages or other immune cells. This means the efficiency of clearance and protection from smaller viral-laden bioaerosols may be affected by an individual’s immune status. Insoluble particles, which cannot be cleared by immune cells, may remain in the lungs causing damage and scarring.\(^1,18,20\)

### 1.1.5 Clinical relevance of bioaerosols

From a clinical perspective, it is imperative to develop a strong understanding of how infectious bioaerosols are generated, their properties, and their risk to HCWs and other patients. While severe acute respiratory syndrome coronavirus (SARS-CoV), is now believed to have been transmitted predominantly through respiratory droplets and contact\(^23\), there is evidence to suggest opportunistic airborne transmission occurred in certain instances. One substantial outbreak in the Amoy Gardens housing complex in Hong Kong was not easily explained by respiratory droplet or contact transmission. A retrospective analysis using computational fluid-dynamics and epidemiological assessment, indicated probable airborne spread, due in large part to the poor design and inadequate functioning of the complex’s sewage system\(^24\). Other reported cases, including one from Canada, were also not easily explained by contact or respiratory droplets, suggesting the possibility of airborne transmission\(^25\). These cases highlight the complexities in determining transmission patterns associated with novel respiratory pathogens, particularly in the early stages of emergence. An improved understanding of the mechanisms underlying droplet and airborne transmission is required in order to better identify transmission characteristics of emerging pathogens and enable rapid and effective interventions\(^26\).

Other respiratory pathogens with a high burden of morbidity and mortality include respiratory syncytial virus (RSV) and seasonal influenza virus. RSV is a relatively contagious respiratory pathogen, and predominantly associated with acute lower respiratory infection (ALRI) in children. Data from 2005 estimated 33.8 million new cases of RSV-associated ALRI in children under 5 years of age, resulting in an estimated 66 000–199 000 deaths, largely in developing countries\(^27\). RSV is also highly contagious within the clinical setting, with an attack rate of up to 40% among hospitalized infants\(^28\). The majority of infection prevention and control (IPC) strategies for RSV are based on transmission through contact and respiratory droplets\(^29\). These policies are supported by an earlier study in a pediatric ward showing evidence that RSV spread between HCWs and infants through larger respiratory droplet and as a result of direct or
indirect self-inoculation after contact with fomites. However, in a subsequent study by Aintablian et al., airborne RSV RNA was successfully collected from 63% of RSV-positive patient rooms, at a distance of up to 7m. Two recent studies detected both RSV RNA and infectious RSV from particles in the respirable size range, indicating the possibility of airborne transmission through droplet nuclei in addition to larger droplets. The transmission characteristics of seasonal influenza virus will be discussed in detail below.

Respiratory pathogens with the potential for airborne transmission present a considerable challenge for HCWs and IPC teams. An airborne pathogen has the potential to infect a much greater number of individuals, including other patients, visitors and HCWs. These pathogens may spread much faster than those transmitted through respiratory droplets or contact. This may be amplified by overcrowding and the lack of adequate ventilation. Inadequate ventilation has been suggested as a possible explanation, or contributing factor, in super-spreader events, such as those observed during the SARS epidemic. Super-spreader events occur when a single individual causes a significant number of secondary infections, which are often nosocomial, as in the case of SARS. Another potential explanation for super-spreader events may be that the index patient emitted more infectious bioaerosols due to their immune status or severity of infection, as systemic infections were observed in index patients during SARS super-spreader events.

Since airborne transmission is primarily contingent upon an infectious dose of a pathogen reaching the host cell receptor within a susceptible individual, infection control is a critical means of reducing transmission through the air in the clinical setting. Infection control is achieved through a combination of prevention (e.g. through vaccination), engineering, policy, and the use personnel protective equipment (PPE). The most effective method to reduce the burden of infectious bioaerosols in the air is through ventilation. Based on the ANSI/ASHRAE/ASHE Standard 170-2008, in order to provide adequate room ventilation sufficient to dilute and remove an airborne pathogen, a ventilation rate of 12 air changes per hour (ACH) is recommended. Additional means of filtration and disinfection, such as the implementation of HEPA filters and ultraviolet germicidal irradiation (UVGI) within the ventilation system, can dramatically enhance the effectiveness of the system. However, ventilation systems cannot completely eliminate the risk of transmission, particularly at close range, and personal protective equipment (PPE) must be used as a physical barrier to prevent infection. Appropriate PPE for airborne and droplet precautions including gloves, gowns, goggles and a fit-tested respirator, such as an N95
mask, are recommended\textsuperscript{38,41,42}. Changes in policy can also help reduce transmission, such as increased surveillance to improve early detection, quarantining or cohorting infected patients, and enhancing HCW education to improve proper PPE use and compliance\textsuperscript{43}. However, in order to implement the correct infection control measures efficiently during an outbreak, the transmission characteristics of a pathogen must be identified.

1.2 Influenza virus

Influenza virus is a negative-sense, segmented RNA virus in the \textit{Orthomyxoviridae} family of viruses. There are four genera (species) of influenza virus; A, B, C, and D, categorized based on the antibody response to their internal antigens, nucleoprotein (NP) and matrix 1 (M1) proteins\textsuperscript{44,45}. Influenza A virus (IAV) is further classified into subtypes based on the presence of two different antigenic surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). There are 18 known subtypes of HA (1-18) and 11 subtypes of NA (1-11) expressed on the surface of IAV. Wild aquatic birds are the main natural reservoir host for of IAV, including alleles of HA 1-16 and NA 1-9\textsuperscript{46}.

1.2.1 Epidemiology

Seasonal influenza results in 3 to 5 million cases of acute viral respiratory infection annually and up to 500 000 deaths, mainly among high-risk groups, including young children, the elderly and immunocompromised individuals\textsuperscript{47}. Seasonal influenza can affect people of all ages, causing illness ranging from asymptomatic to severe disease leading to death. The general incubation period for influenza is 2-7 days and symptoms include fever, dry cough, sore throat, headache, muscle and joint pain, and rhinorrhea. Treatment options for influenza are limited and vaccination remains the best method of prevention. Antivirals are most effective if taken within 48 hours of symptom onset. Widespread resistance has emerged against one of the two available classes of IAV antivirals, the M2 ion channel blockers, amantanes (amantadine and rimantadine)\textsuperscript{47}. In addition, widespread resistance to the neuraminidase inhibitors (oseltamivir), was recently observed during the 2007-2009 influenza seasons\textsuperscript{48,49}.

Seasonal influenza can cause epidemics, which place considerable strain on the healthcare system, and produce a substantial economic burden through healthcare costs, absenteeism and lost wages\textsuperscript{48}. One study in Canada looking at absentee rates among full time
employs in a partially vaccinated population found an average absentee rate of 20 days per year per 100 employees from seasonal influenza, and 40 days per year per 100 employees during pandemic periods\textsuperscript{50}. While the total economic burden associated with influenza is difficult to determine, one study based on data from 2003 determined the direct healthcare costs associated with influenza was an estimated $10.4 billion USDs, with a total annual economic burden of influenza epidemics on the order of $87.1 billion USDs\textsuperscript{51}.

Circulating human seasonal strains of influenza virus include two strains of influenza A virus, influenza A(H1N1)pdm09, which was responsible for the pandemic in 2009, and influenza A(H3N2), as well as two lineages of influenza B virus, influenza B/Yamagata and influenza B/Victoria. The annual influenza vaccine includes both strains of influenza A virus (H1N1 and H3N2) as well as one (trivalent), or both (quadrivalent) strains of influenza B virus, depending on the specific vaccine formula and anticipated strains for the approaching influenza season. Influenza C virus causes mild respiratory infection and is generally not considered to cause human epidemics\textsuperscript{52,53}. Influenza D virus was recently discovered and has only been describe in cattle\textsuperscript{45}.

1.2.2 Transmission

The predominant consensus on influenza transmission is that it occurs primarily through respiratory droplets. However, the relative contributions of direct and indirect contact, respiratory droplets and airborne transmission to the overall burden of influenza remains unclear, and is a topic of considerable debate\textsuperscript{54}. While some recent reviews have suggested that airborne transmission plays a significant role in the total burden of transmission\textsuperscript{9,55}, others maintain the prevailing paradigm of predominantly respiratory droplet transmission\textsuperscript{56}. More recently a number of studies have supported the possibility of airborne transmission in the clinical setting. Blachere et al, collected aerosol particles from an emergency room ward using low-volume two-stage NIOSH cyclone samplers, and found that 49\% of IAV RNA collected was in the respirable zone (1–4 μm)\textsuperscript{57}. A follow-up study in an urgent care center by Lindsley et al, using similar sampling methods, found 43\% of IAV RNA detected in stationary samplers was recovered in the <4.1 μm size range and 48\% of RNA collected from personal samplers was <4.9 μm\textsuperscript{32}. Taken together these studies provide evidence of widespread IAV RNA in the respirable range within the emergency clinic setting.
In a subsequent bedside sampling study, Bischoff et al., measured the concentration of IAV RNA at varying distances from patients during routine care and found that the majority of HCW IAV exposure (89%) was to smaller particles (<4.7 µm)\(^{58}\). IAV RNA was also detected 6 feet from the patients, and HCWs within 1.8 m were exposed to levels or RNA comparable to infectious doses of airborne IAV. These findings were supported by an additional bedside sampling study by Mubareka et al., who detected IAV RNA in air samples collected from rooms of 11 of 12 infected patients at a distance 0.5-1 m, 9 of 12 room at a distance of 1.1-1.5 m and 1 in 5 where sampling was performed outside of the room\(^{59}\). Taken together these studies provide evidence that patients naturally infected with IAV potentially shed virus in the respirable range (< 5 µm) during routine patient care, highlighting possible HCW exposure to potentially infectious bioaerosols. However, these studies did not assess viability, making it difficult to determine if these viral-laden bioaerosols actually pose a risk of infection to HCWs, and transmission was not demonstrated. Considering that seasonal influenza remains a major global health burden, and the threat of an emerging pandemic persists, it remains imperative that we determine the relative contribution of each transmission route to the overall transmission of influenza in order to develop the most optimal, evidence-based, IPC strategies.

The probability of becoming infected by an influenza virus-laden bioaerosol depends primarily on particle size and concentration of infectious bioaerosols inhaled\(^{37}\). Based on early influenza research with human volunteers artificially-infected with clinical isolates of IAV, the human infectious aerosol dose (HID) at which 50% of individuals became infected was determined to be 0.6 to 3 TCID\(_{50}\) or 0.7-3.5 PFU, which was 10 fold less compared to the 30 TCID\(_{50}\) dose of the same virus required through nasopharyngeal inoculation. It was also observed that aerosol transmission produced a more severe infection\(^{60}\). Two subsequent studies found the intranasal infectious dose to be between 89-244 PFU, considerably higher than aerosol infectious dose\(^{61,62}\).

### 1.2.3 Influenza receptor binding specificity and host range

The cellular receptor for influenza virus is a sialic acid, which binds to the HA on the surface of influenza virions\(^{63}\). Salic acids are cellular glycoconjugates, typically present on the terminal end of protein or cellular surface glycans (N- or O-glycans) and glycosphingolipids\(^{44}\). Mammalian influenza viruses preferentially binds to \(\alpha2,6\)-linked sialic acids (Neu5Ac 2-6Gal), which refers to the linkage between the sialic acid and a neighboring galactose residue. Avian influenza
viruses preferentially binds to α2,3-linked sialic acids, which are a major sialic acid present in the intestinal tract of many wild aquatic bird species. Pigs express both α2,3 and α2,6-linked sialic acids in their trachea, and have been posited as the main mixing vessel for genetic reassortment between avian and human influenza strains, enabling the emergence of novel influenza virus strains with pandemic potential.

Highly pathogenic avian influenza (HPAI) A/H5N1 continues to circulate among poultry in the Middle East and Asia, affecting hundreds of millions of domestic birds each year. While cross-species transmission is uncommon, it has been reported in several agricultural species including pigs. In addition, there have been reports of direct transmission of A/H5N1 from birds to human, although sustained human-human transmission has yet to be reported. The WHO has reported over 850 human cases of A/H5N1 since 2003, with a mortality rate of 53%. Perhaps even more concerning, a recent study performed in ferrets showed that as few as two mutations in the HA of A/H5N1 was enough to switch binding preference from α2,3 to α2,6. Furthermore, as few as five mutations were sufficient to enable sustained transmission through the air in ferrets. With ongoing activity in the domestic bird population, and potential for adaptation or reassortment in agricultural settings, avian A/H5N1 remains a substantial concern as a potential cause of a future human pandemic. More recently an additional, potentially more readily transmitted strain of avian influenza has emerged in eastern China. Influenza A/H7N9 was first reported in 2013 and has since caused 1533 laboratory-confirmed human cases with a mortality rate of approximately 40%. Recent studies have shown that H7N9 is already more adapted for replication in the human nasal and tracheobronchial passages than any other avian strain. Additionally, unadapted H7N9 has transmitted through the air amongst ferrets, which has not been observed with any other avian viruses. However, the virus has thus far shown limited, non-sustained, transmission among humans. The main source of human infection appears to be zoonotic, primarily through exposure in live poultry markets. It has been proposed that closing these markets would likely significantly reduce the risk of H7N9 spurring a human pandemic. Continued surveillance will be essential to monitor changes in the virus and protect against a possible pandemic.

1.2.4 Pathogenesis

Influenza viruses predominately infect the columnar epithelial cells that line the respiratory tract, and can cause both upper and lower respiratory tract infections, depending on the site of viral
deposition. Viral infection and the subsequent immune response lead to multifocal destruction of the columnar epithelium in the respiratory tract with marked increases in submucosal edema and congestion. Apoptosis of respiratory epithelial cells can trigger acute inflammatory responses and activation of pro-inflammatory cytokines, including interferons (IFNs), tumor necrosis factor (TNF), interleukins (ILs), and chemokines, which can lead to the recruitment of additional inflammatory cells. Cytokine dysregulation leads to severe immunopathologic cellular and tissue damage, as well as organ dysfunction in severe cases. An additional potential complication of influenza is secondary bacterial pneumonia. Bacterial pneumonia secondary to influenza infection has a mortality rate of 7%, while primary viral pneumonia is less common and has a mortality rate of 10%.

1.2.5 Influenza bioaerosols

It has been shown that naturally infected patients expel infectious virus-laden influenza bioaerosols during coughing and exhalation. The presence of viable virus in droplet nuclei expelled from infected individuals suggests the possibility of airborne transmission. However, the frequency and determinants of airborne transmission of influenza are unknown, in large part due to our limited understanding of how viral-laden bioaerosols behave in the environment.

1.2.5.1 Effect of relative humidity and temperature on viability

Early research in aerovirology determined that relative humidity (RH) has a significant effect on the viability of aerosolized viruses, depending in part on viral structure. It was shown that enveloped viruses, such as IAV, retain infectivity better at lower RH and non-enveloped viruses, such as poliovirus retain more infectivity when aerosolized at high RH. Based on their findings, these early researchers suggested that the effect of RH on viral infectivity may be a driving force for the seasonality of influenza in temperate climates. Since then more recent studies have revisited this idea. One such study using a guinea pig model of IAV transmission, tested transmission under a series of different RH and temperature conditions, and found that transmission was favoured under cold and dry conditions. A follow up study by the same group put forth the hypothesis that seasonal influenza may be predominantly airborne and spread through droplet nuclei in temperate regions during winter months, and by contact transmission in warmer climates or during the summer months.
Despite the early work in influenza aerovirology, there is a lack of recent in vitro experimental data investigating the effect of RH on the infectivity of currently circulating strains of influenza when aerosolized, and whether strain specific differences exist in their ability to cause infection through the airborne route. A better fundamental understanding of how airborne transmission contributes to the spread of current seasonal influenza strains, and how environmental conditions affect the infectivity of influenza-laden bioaerosols may help to develop additional means to reduce transmission.

1.2.6 Bioaerosol samplers

In order to investigate the characteristics of bioaerosols involved in influenza virus transmission, the virus must be collected from the air using a bioaerosol sampler. There are a number of commercially available devices which typically fit into one of four categories: impingers, cyclones, impactors, and filters. Impingers typically collect bioaerosols in a liquid media and cyclones use liquid or dry collection chambers, whereas impactors and filters usually use semi-solid or solid materials for the collection of particles. While each of these samplers has benefits and drawbacks, a number of factors are recommended to consider when choosing a bioaerosol sampler. These include type and size of target pathogen, the sampling environment, budget and the operational considerations in a given setting.

1.2.6.1 Impingers

Impingers collect bioaerosols by pumping a flow of air down an elbow-shaped nozzle and directly through a liquid collection media. This sampling method prevents desiccation, which may help retain viability of certain bacteria and viruses. However, it also subjects the particles to shear forces that may reduce viability. Two of the most widely-used impingers are the AGI-30 and SKC biosampler. Both the AGI-30 and SKC biosampler sample at an intermediate flow rate of 12.5 L/min. Impingers have been used to successfully collect influenza viral RNA and infectious viral bioaerosols from the air.

1.2.6.2 Cyclone samplers

Cyclone samplers use a unique conical shape to force particles against the walls of a collection chamber. The collection media within the chamber swirls around due to its conical shape, and
particles are deposited directly into the liquid collection media or directly against the sides of the collection chamber in a dry cyclone, as a result of centrifugal force. However, because of the centrifugal nature of collection, smaller particles are more likely to pass through the system without being collected. Similar to impingers, some cyclone samplers collect particles directly into a liquid media, which may help to reduce desiccation and maintain viability of some pathogens. However, they also subject particles to the same high shearing forces as impingers, which may reduce pathogen viability. One of the more commonly used liquid cyclone samplers is the Coriolis®, referred to henceforth as the “Coriolis”, a high volume (300 L/min) sampler with a collection efficiency of 49% for particles < 1.0 μm and 92% for particles > 10 μm in diameter. This makes the Coriolis well suited for collection of bacterial bioaerosols. The Coriolis has recently been used in avian IAV surveillance and has been shown to successfully collect viral RNA from the air. Additionally, a two-stage low volume cyclone sampler was developed by the National Institute for Occupational Safety and Health (NIOSH). The NISOH cyclone sampler has two cyclone stages and one final filter stage. It is capable of size fractionated aerosol particles of respirable range, with the first stage collecting particles of >4 μm in a 15 ml centrifuge tube, the second stage collecting particles 1-4 μm in a 1.5 ml microcentrifuge tube and the final filter stage capturing particles <1 μm on a 37 mm polytetrafluoroethylene (PTFE) filter. This sampler is small enough to be worn in the breathing zone for personal sampling, while still allowing for size fractionation of particles.

1.2.6.3 Impactors

Impactors collect aerosol particles on collection plates by inertial impaction. The collection plates are typically filled with liquid, semi-solid, or solid media. Impactors are often designed in a cylindrical cascade involving a series of stages, which are stacked on top of each other, through which air streams flow downward from an opening in the top of the sampler, perpendicular to the stages. Each stage contains a collection plate suspended off the bottom of the stage by a small gap, allowing air to flow around the plate and down to the next stage. Each stage is separated by a series of passage holes, which get smaller as the air moves downward, from stage to stage through the sampler. The same number of holes separate each stage, however as they get smaller the flow rate increases allowing for size fractionation of particles. When a particle’s inertia is large enough it will be forced out of the airstream and will impact on the collection plate in the stage corresponding to its size.
Bioaerosols collected through the use of an impactor are also subject to desiccation and shearing forces which may affect viability, as well as particle bounce which may affect collection efficiency. One of the more commonly used impactors is the Andersen cascade impactors (ACI), which is a 28.3 L/min sampler that can size fractionate particles in up to eight size factions, depending on the model. The ACI has been used recently in the field to collect IAV RNA and viable virus during swine sampling.

### 1.2.6.4 Filter samplers

Bioaerosols can also be sampled using filters of different porosity and material. These filters are often housed in a three-piece plastic casing known as a filter cassette. One of the major advantages of filter samplers is the fact that they are small enough to be worn and can provide an indication of personal exposure to a given pathogen in a particular environment; they may also be disposable, and are thus easy to use in the field since decontamination between samples is not required. After sampling, the filter can be removed for processing by either being placed directly on a culture plate (bacteria) or mixed in a liquid media (virus) and further analyzed. One of the most common types of filter materials is polytetrafluorethylene (PTFE), which is a membrane filter with a complex pore structure, and high collection efficiency, even for particles well below the stated pore size. One of the major limitations of filter samplers is the fact they are constrained to low flow rates, as low as 2 L/min depending on filter pore size and also cause significant desiccation. PTFE filters have been used successfully to collect IAV RNA and viable virus.

### 1.2.6.5 Collection efficiency and sampler choice

The overall collection efficiency will vary considerably between samplers depending on sampler choice, flow rate, specific pathogen characteristics, environmental conditions, and sampling time. There is currently no standardized approach to bioaerosol sampling. While there is a general consensus that one single sampling method is unlikely to be optimal for all situations and pathogens, the wide application of different samplers and approaches to sampling, makes an evaluation of the collection efficiencies of current samplers, based on existing literature, difficult to establish. Therefore, in order to determine an optimal sampler choice for a given pathogen under a certain set of environmental conditions, it is necessary to perform an experimental
comparison in the laboratory, using artificially produced aerosols. Such fundamental aerosol experiments are necessary to facilitate efficient sampling in the clinical and field setting.

1.3 Aerosol generating procedures

1.3.1 Background

Clinical procedures suspected of producing aerosol particles, comprised of droplet nuclei and respiratory droplets, include endotracheal intubation\(^2,100\), cardiopulmonary resuscitation\(^3,8\), bronchoscopy\(^1,101\), sputum induction\(^2,102\), nebulizer therapy\(^3,4,103,104\), surgery and autopsy\(^3,3,105,106\), and bi-level positive airway pressure (BiPAP)\(^3,6\). Other potential AGPs include chest physiotherapy, high-frequency oscillatory ventilation, tracheostomy care and nasopharyngeal swabs\(^4,1\). AGPs may present a risk to exposed healthcare workers (HCWs) and contribute to nosocomial transmission in the setting of influenza or other infections transmissible by the respiratory route. It is unclear whether AGPs create artificial aerosols that are more abundant or finer than what is normally expelled from individuals, potentially representing an artificially amplified risk of transmission, including through the airborne route.

Without well characterized determinants of aerosol generation, HCWs may be at increased risk, especially when dealing with novel respiratory pathogens with unknown transmission characteristics. It is important to note that many of the procedures listed by PHAC as AGPs are classified as such based on expert opinion and retrospective epidemiology analysis, and not empiric data\(^4,1\). Therefore, these procedures are still considered “suspected” AGPs\(^3,7\).

To date only one study has investigated aerosol generation during a suspected AGP. Lavoie et al\(^1,08\) measured aerosol and bioaerosol production during bronchoscopy using an ultraviolet aerodynamic particle sizer (UV-APS). The use of a UV-APS allowed for an examination of the production of both aerosols and bioaerosols, as particles with organic matter fluoresce at a specific wavelength allowing the UV-APS to detect them, although this is not specific to pathogens. It was shown that both aerosol and bioaerosol production increase significantly during the procedure compared to a morning empty-room baseline. However, the use of an empty room for a baseline measurement likely overestimated the number of aerosols produced by the actual procedure, compared to those produced by HCWs moving about while preparing for the procedure.
1.3.2 Limitations in studying AGPs

Research on AGPs remains constrained by technical and operational challenges. A considerable knowledge gap exists, primarily due to the limited availability of practical, well validated, aerosol sampling devices, and the inherent difficulties sampling in the clinical environment. Validation and optimization of sampling protocols will be an essential step in the characterization of the determinants of aerosol production during AGPs. A more comprehensive understanding of aerosol generation during AGPs may help to enhance risk assessment, and reduce the risk of nosocomial transmission to HCWs during AGPs, especially in the presence of patients infected with a respiratory pathogen.

1.4 Rational

Previous pandemic influenza outbreaks, such as the 1918 H1N1 pandemic, were responsible for the deaths of millions of otherwise healthy adults. The 1918 pandemic alone resulted in more than 20 million deaths, enough to significantly reduce the world-wide life expectancy for ten years\textsuperscript{109,110}. More recently, the rapid appearance of SARS in 2003 brought widespread fear among many that it would spread rapidly among the general population, and cause widespread mortality\textsuperscript{111}. Although SARS caused a number of deaths and nosocomial infection among HCWs, sustained transmission in the community was contained. With the ever expanding travel networks globally, increased passenger loads, and exportation, there is substantial concern regarding the potential ease with which a novel virus may spread\textsuperscript{112}.

Establishing performance characteristics for bioaerosol sampling addresses an important knowledge gap in the endeavor to characterize the transmission of bioaerosols emitted by naturally-infected hosts. A better understanding of viral transmission characteristics of currently circulating influenza strains has important implications for the development of more efficient IPC strategies and improvements our ability to plan for pandemics.

In addition to establishing performance characteristics for bioaerosol samplers in the laboratory, determining exposure levels for HCWs to potential infectious aerosols in the clinic, especially during AGPs, may help to further improve HCWs risk assessment ability. However, there is limited empiric data regarding determinants of aerosol generation during suspected AGPs. Without well characterized determinants of AGPs, HCWs may be at an increased risk of infection. In the event of an outbreak of another SARS-like CoV, a more thorough understanding
of aerosol generation during AGPs will help inform risk assessment and reduce the potential for nosocomial transmission to HCWs during AGPs.

**Aim 1:** To determine the effect of RH and strain specific differences on the collection efficiency of four commonly used bioaerosol samplers for the collection of currently circulating strains of season influenza virus.

**Aim 2:** To characterize bioaerosol generation during potential aerosol-generating medical procedures
Chapter 2

Detection of aerosolized influenza virus in an experimental setting: evaluation of bioaerosol sampling devices

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The work in this section has been prepared as a manuscript for future submission.
2.1 Abstract

**Background.** Characterization of infectious bioaerosols involved in the dispersion of influenza virus is required to identify related determinants of transmission by the respiratory route. Here, our goal is to describe performance parameters for different bioaerosol samplers for the recovery of currently circulating seasonal influenza viruses under a range of relative humidities. **Methods.** The sampling efficiency of aerosolized influenza virus A/Puerto Rico/8/1934 (H1N1) (PR8) was compared using four different bioaerosol samplers (NIOSH cyclone, PTFE filter cassette, Andersen six-stage cascade, AGI-30 glass liquid impinger) at three RH conditions, low (30% RH), medium (50% RH), and high (80%). The sampling efficiency of two dry bioaerosol samplers were also assessed for A/H1N1pdm09 and A/H3N2. Influenza virus was aerosolized into a custom-built, biocontained chamber using a Collison nebulizer. Collection efficiency was analyzed by quantitative PCR and viral plaque assays and compared between samplers based on viral RNA copies and viral titres (plaque forming units (PFU) per liter of air sampled). **Results.** *In vitro* sampling with PTFE filter cassette (324.0 copies/L air at 30% RH) and NIOSH cyclone (230.0 copies/L air at 30% RH) samplers showed comparable results with the highest relative collection efficiency and were the only samplers to recover viable virus. The collection efficiency of H3N2 was higher than H1N1 for both dry samplers, while the viability was lower. A trend of decreased collection efficiency and viability with increasing RH was observed for all three viruses. **Conclusion.** Here we show improved efficiency for the ability to detect viral RNA and viable seasonal IAV virus from the air with two low-volume bioaerosol samplers. Establishing performance characteristics for aerosol sampling addresses an important gap in the endeavor to characterize bioaerosols.
2.2 Introduction

Influenza can result in moderate to severe respiratory illness and is a significant cause of annual morbidity and mortality worldwide, with 3 to 5 million cases and up to 500,000 deaths reported, largely among children and older adults. In addition to the annual burden of disease, the threat of an emerging pandemic remains a potential that could cause widespread mortality around the world. Despite the significant global burden of disease and prevailing pandemic threat, knowledge gaps remain with respect to characterizing influenza transmission, specifically the degree to which the airborne route factors in to transmission. This has resulted in considerable debate, with some groups suggesting airborne transmission constitutes a major part of overall transmission, while others maintain that transmission occurs predominantly through large respiratory droplets.

Early bioaerosol research was limited to the use of culture-based systems to evaluate the microbial bioburden. Culture-based systems are often labor intensive processes with poor sensitivity. As such, few studies were performed to assess the microbial content of the air, and even fewer for the presence of viruses, such as influenza virus. However, with recent advancements in molecular technology, such as quantitative polymerase chain reaction (qPCR), the content of bioaerosols can now be assessed through the determination genome copy (RNA or DNA), with greater sensitivity. With this advancement, a number of new bioaerosol samplers were developed to collect smaller pathogens, such as bacteria and viruses.

Low volume bioaerosol samplers have been frequently employed in clinical and field setting to assess exposure to a range of different pathogens. One of the major advantages of these samplers is that they are small enough to be worn in the breathing zone and can be used as personal samplers to assess an individual’s exposure to a particular pathogen. Two commonly used low-volume samplers include the three-stage cyclone sampler developed by the National Institute for Occupational Safety and Health (NIOSH, Atlanta, GA) and polytetrafluoroethylene (PTFE) membrane filter based samplers, which consist of a filter housed in a 3-piece plastic cassette (SKC, Inc. Eighty Four, PA). The PTFE filter cassette was shown to have a collection efficiency of >99% when tested with 0.35 µm polystyrene latex (PSL), and >96% when tested with MS2 bacteriophage virus which ranges from 10-80 nm. Both the PTFE filter cassette and NIOSH cyclone samplers have been used previously to successfully collect influenza viral RNA and infectious virus from the air.
Higher volume bioaerosol samplers, such as the AGI-30 glass liquid impinger (Ace Glass, Inc., Vineland, NJ), and the Andersen cascade impactor (Tisch Environmental, Cleves, OH) allow for a larger quantity of air to be sampled over the same sampling period, providing a more representative account of the actual bioburden, particularly when sampling a larger room or space\textsuperscript{87}. The AGI-30 impacts bioaerosols directly into a liquid medium, which prevents desiccation, potentially improving viability. It was previously shown that the AGI-30 has a collection efficiency of 87\% for bacteria-laden bioaerosols between 0.3 and 1.0 µm in diameter\textsuperscript{118}. The AGI-30 has been used previously to collect influenza RNA\textsuperscript{119} and viable virus\textsuperscript{120}. The Andersen six-stage cascade impactor can size fractionate bioaerosols in six size factions, ranging from 0.85 to 10 µm in diameter, using particle inertia to impact particles in a solid, semi-solid or liquid medium at each stage. The Andersen sampler has also been recently used to collect influenza RNA\textsuperscript{121} and viable virus\textsuperscript{96}.

There are a number of factors that determine the stability of viral bioaerosols, including viral concentration and genotype, as well as environmental factors, such as relative humidity (RH) and temperature\textsuperscript{8,37,57,116,122,123}. Early seminal studies on bioaerosol behavior revealed that RH has a significant impact on the size and stability of viral bioaerosols, with enveloped viruses, such as influenza, maintaining greater viability at lower RH\textsuperscript{83,124--126}. These earlier studies have been mirrored in a recent animal model of influenza transmission, which showed more efficient transmission at low RH and temperature in guinea pigs\textsuperscript{84} and ferrets\textsuperscript{127}. While there is considerable evidence that RH has an effect on bioaerosol transmission, the role of absolute humidity (AH) is less clear. One recent study found that AH had a more substantial effect on bioaerosol transmission of influenza than RH\textsuperscript{128}. However, substantially more work needs to be done to determine the actual degree to which AH modulates influenza transmission.

While it appears that RH significantly affects the stability of viral-laden bioaerosols and the efficiency of influenza transmission, comparatively little is known about the effect of RH on the collection efficiency of commonly used bioaerosol samplers, and whether strain specific differences exist between viruses. Thus our objectives for this study are three-fold. First we seek to determine performance characteristics for four different types of commonly used bioaerosol samplers, with respect to both collection efficiency and retention of viability for influenza A virus (IAV). Secondly, we examine the effect of RH on collection efficiency and viability. Lastly, we investigate whether strain specific differences exist between seasonal H1N1 and H3N2 influenza viruses with respect to sampling. This work will provide information to help inform researchers
when choosing a bioaerosol sampler for specific strains and environmental conditions in the field or clinical setting, as well as enhance our understanding of viral bioaerosol behavior with respect to RH.

2.3 Methods

**Viral strains.** Three different strains of IAV were used for this study. Murine adapted influenza virus A/Puerto Rico/8/1934 (H1N1) stock was propagated in MDCK cells to reach a stock titre of $10^8$ pfu/ml. Clinical isolates of A/H1N1pdm09, and A/H3N2 collected during the 2015-2016 influenza season were also propagated in MDCK cells to obtain a viral stock of $7 \times 10^5$ pfu/ml and $3 \times 10^5$ pfu/ml respectively.

**Bioaerosol Samplers.** The sampling efficiency for the collection of aerosolized influenza viruses A/Puerto Rico/8/1934 (H1N1) (PR8), A/H1N1pdm09, and A/H3N2 was compared using four different sampling devices: a glass liquid impinger (AGI-30, Ace Glass, Inc, Vineland, NJ), a three-stage cyclone bioaerosol sampler from the National Institutes for Occupational Safety and Health (NIOSH, Atlanta, GA), a 3-piece cassette preloaded with a 1.0 µm, 37mm, polytetrafluoroethylene (PTFE) membrane filter (SKC, Inc, Eighty Four, PA), and an Andersen viable six-stage impactor (Tisch Environmental, Cleves, OH). These four samplers were chosen due to their routine use in either field or clinical settings for the recovery of IAV. The AGI-30 was filled with 20 ml of PBS (Wisent Inc, St-Jean-Baptiste, QC) and sampled at a flow rate of 12.5 L/min. The Andersen impactor was loaded with special 100x15mm exact diameter petri plates (Phoenix Biomedical Products, Inc, Bolton, On) filled with 20 ml of PBS to reach the appropriate media height in each stage and sampled with a flow rate of 28.3 L/min. Both the PTFE filter cassette and NIOSH cyclone samplers were run at a flow rate of 3.5 L/min.

**Aerosolization in an experimental chamber.** Influenza virus was aerosolized into a custom-built, pressure-controlled biocontained chamber using a three-jet Collision nebulizer (BGI, Waltham, MA), operating at 20 psi, which generates aerosols between 0.78 µm to 9.0 µm, with a mass median diameter (MMD) of 2.5 µm (Fig 1). The internal airflow through the chamber was 36 L/min during aerosolization, operating at a differential pressure of 0 psi within the chamber. For aerosolizations using PR8, aerosol samples were collected with each sampler during independent 30 minute aerosolizations. PR8 was diluted 1/15 ml in PBS prior to aerosolization. All four
samplers were used during sampling for PR8. Based on results of our initial experiments, only the PTFE and cyclone samplers were used for H1N1 and H3N2 sampling. Both samplers were run concurrently during each aerosolization and sampling time was reduced to 10 minutes. Following aerosolization, pumps for respective samplers were turned off and the system, which has an internal airflow to HEPA filtered exhaust ports of 36 L/min, was allowed run for 5 minutes to clear the air before samplers were removed for processing. The chamber was then cleaned and a blank aerosolization, using sterile Milli-Q purified water, was performed for 25 minutes to ensure clearance of any remaining bioaerosols in the system. The aerosolizing process was repeated for three runs under low (30%), medium (50%) and high (80%) levels of RH. Relative humidity and temperature were recorded with an internal RH meter (Fisher Scientific, Markham, Ontario) with accuracy of ± 2 to 4% RH. Temperature within the chamber was constant at room temperature at 23°C ± 2°C for all aerosolizations. RH was controlled using an ultrasonic humidifier (Bonico 7146, Widnau, Switzerland).

**Biocontainment and PPE.** The aerosol chamber used in this study was housed inside a biosafety cabinet within a HEPA-filtered biobubble. The biobubble was situated within the inner room of a biosafety level 2 plus (BSL-2+) biohazard suite. All personnel wore fit-tested N95 respirators, goggles, gloves, and a Tyvek suit during aerosolizations.
**Figure 1.** Schematic representation of a custom-built, pressure and biocontained aerosol chamber with 3-jet Collison nebulizer. Image created by and used with permission from D. Verreault(2013).
**Processing of samples collected from each sampler.** After aerosolization the Andersen sampler was processed by transferring the collection media from each stage into separate 50 ml centrifuge tubes. These tubes were then mixed by vortexing for 1 minute, before being aliquoted into 1.5 ml microcentrifuge tubes. The AGI-30 was processed in a similar manner, collection media from the collection chamber was transferred to a 50 ml centrifuge tube, mixed by vortex for 1 minute, before being aliquoted in 1.5 ml microcentrifuge tubes. The NIOSH cyclone sampler was processed by stage. The first stage (15 ml centrifuge tube) was processed by mixing 1 ml viral transport media (VTM) directly in the collection tube, vortexing for 1 minute, and then aliquoting the VTM wash media in 1.5 ml microcentrifuge tubes. Stage two (1.5 ml microcentrifuge tube) was processed in the same manner using 0.5 ml of VTM, followed by 1 minute of vortexing. Stage three (1.0 µm PTFE filter) was removed, placed in a 15 ml centrifuge tube with 2 ml of VTM, mixed by vortexing for 1 minute, then aliquoted in 1.5 ml microcentrifuge tubes. The PTFE filter cassette sampler was processed the same as stage 3 of the NIOSH cyclone. The PTFE filter was removed from the cassette, placed in a 15 ml centrifuge tube with 2 ml VTM and mixed by vortexing for 1 minute before being aliquoted. All aliquoted processed samples were then transferred to -80°C for storage.

**Quantification of viral RNA using qPCR.** RNA was extracted using the QIAamp viral RNA mini kit (Qiagen, Chatsworth, CA) from 140 µl of processed sample collected from each sampler to yield 60 µl of extracted RNA, as described by the manufacturer instructions\textsuperscript{129}. All qPCR was performed using the SuperScript® III with Platinum One-Step RT-PCR kit (Invitrogen, Carlsbad, CA) as per manufacturer instructions (Invitrogen, 2016), using primers and probes previously described by Lee et al.,\textsuperscript{131} All reactions were performed in duplicate and averaged.

**Determination of infectivity by viral plaque assay in MDCK cells.** To determine the quantity of viable virus in collected samples, viral plaque assays (VPA) were performed on all samples using Madin Darby canine kidney (MDCK) cells (ATCC CCL-34\textsuperscript{TM}), as previously described\textsuperscript{132}. Briefly, MDCK cells were seeded in six-well tissue culture plates, inoculated with 250 µl of serially diluted sample, which was allowed to adsorb for 45 minutes. After infection, the cells were washed with PBS, covered with an agarose infection media overlay and incubated at 37°C and 5% CO\textsubscript{2} for 48-72 hours. Plaques were then fixed with 4% paraformaldehyde (Sigma Aldrich Inc., St-Louis, MO) and stained with crystal violet. Enumeration of plaques was performed visually.
**Decontamination and internal controls.** The experimental chamber was thoroughly cleaned following each aerosolization using Virox 5 (Virox Technologies, Inc., Oakville, ON), followed by 70% ethanol. The chamber was then purged with a blank (water) aerosolization for 25 minutes to give ample time for residual ethanol to evaporate and ensure clearance of any remaining viral bioaerosols. A PTFE filter was used to sample the air during the purge period as a control for contamination. PBS and VTM were included as negative controls for all extraction and qPCR runs. PR8 was used as a positive control for extraction, qPCR and VPAs.

**Statistical analysis.** A one-way ANOVA test was used to evaluate statistical difference for RNA collection efficiency of the PTFE filter cassette (Fig 5). A two-way ANOVA test was used to evaluate statistical difference for RNA collection efficiency of the NIOSH cyclone sampler and its respective stages (Fig 5). All statistical analyses were performed using GraphPad Prism version 6.0c.

### 2.4 Results

**PR8.** All four samplers collected detectable RNA. The PTFE filter cassette showed the highest efficiency (324.0 copies/L air) compared to the NIOSH cyclone sampler (230.0 copies/L air), Andersen viable six-stage impactor (192.0 copies/L air (six-stages pooled)) and AGI-30 (150.0 copies/L air) (Fig 2). The collection efficiency decreased with increasing RH for the PTFE filter cassette, NIOSH cyclone sampler, and to a lesser degree, the Andersen sampler, while remaining relatively consistent for the AGI-30 (Fig 2). The two samplers that impact particles directly in a liquid media, the Andersen and AGI-30, performed better under higher RH levels, with respect to collection efficiency, than the two dry samplers. The size distribution of viral RNA collected by the Andersen sampler at low RH indicated that most viral RNA was collected on stages 5 (1.1 – 2.1 µm) and 6 (0.65 – 1.1 µm), suggesting that viral particles were associated with smaller aerosols (< 2.1 µm) at low RH (Fig 3). As RH increased, the size distribution of viral particles became associated with larger aerosols in stages 4 (3.3 – 4.7 µm) and stage 1 (>7.0 µm) (Fig 3).

The viability of virus collected using the four samplers was assessed by VPA. Both the NIOSH cyclone and PTFE filter cassette samplers recovered comparable levels of viability, with slightly more viable virus recovered under 50% RH using the PTFE filter cassette (Fig 4). No viable virus was recovered using the Andersen or AGI-30 under any RH condition (Fig 4).
total quantity of viable virus decreased with increasing RH for the PTFE filter cassette and to a lesser degree the NIOSH cyclone sampler.

**H1N1 and H3N2.** Due to the lack of viability recovered from the air during our initial PR8 aerosolizations using the liquid based samplers, the efficiency of collection of subsequent H1N1 and H3N2 influenza virus experiments was assessed using only the NIOSH cyclone and PTFE filter cassette samplers. Both samplers successfully recovered RNA from both H1N1 and H3N2 influenza virus under all three RH conditions, however the same trend of decreasing collection efficiency with increasing RH was observed (Fig 5). Interestingly, the relative collection efficiency for viral RNA was slightly higher for H3N2 than H1N1 for both the NIOSH cyclone and PTFE filter cassette samplers.

Viable H1N1 and H3N2 was recovered using both samplers, however the recovery efficiency was low. Viable virus was only recovered from the first of three sampling runs. Since the three runs for each RH condition for each virus were done back to back on the same day, this lack of viability in the second and third runs may indicate residual decontaminant in the system, a result of the inter-sampling decontamination. For both H1N1 and H3N2, the relative viability ratio decreased with increasing RH for both the NIOSH cyclone and PTFE filter cassette. The relative viability ratio was higher for all positive samples recovered for H1N1 compared to H3N2 (Fig 6). The amount of viable virus compared to RNA copies for the stock solution was also lower (0.00035 PFU/RNA copies/ml for H1N1 and 4.5x10^-16 PFU/RNA copies/ml for H3N2), suggesting that the aerosolization process alone was not responsible for the lower viability.
Figure 2. Quantitative analysis of PR8 collection efficiency across all samplers under 30%, 50%, and 80%, RH. Quantities shown in relative viral RNA copies per liter of air sampled. Viral RNA quantified using qRT-PCR. Data represent mean ± SEM (n=2).
Figure 3. PR8 viral RNA copies collected using an Andersen viable six-stage impactor representing the size distribution of aerosols containing viral RNA with respect to different RH conditions. Viral RNA quantified using RT-PCR. Data represent mean ± SEM (n=2).
Figure 4. Comparison of PR8 viability across all samplers at 30%, 50%, and 80% RH. A) NIOSH cyclone; B) PTFE filter cassette. Data represent mean ± SEM N=2. Nd = no detectible viability.
Figure 5. Quantitative analysis of H1N1 and H3N2 collection efficiency for the NIOSH cyclone sampler and PTFE filter cassette sampler under 30%, 50%, and 80%, RH. The PTFE filter cassette has a collection efficiency of 99% for particles >0.35 µm. Quantities shown in relative viral RNA copies per liter of air sampled. Viral RNA quantified using qPCR. Data represent mean ± SEM (N=3). Statistical significance calculated using a two-way ANOVA (A,B) and one-way ANOVA (C,D).
Figure 6. Comparison of H1N1 and H3N2 relative viability, a ratio of plaque forming units to viral RNA collected per liter air sampled, across 30%, 50%, and 80% RH. N=1.
2.5 Discussion

Bioaerosol samplers have been employed to determine the presence of IAV from air emitted by naturally-infected hosts, both in clinical\textsuperscript{32,57,58,133–135} and field settings\textsuperscript{94,117}. Unless previous research has been performed under the same conditions, it is difficult to draw conclusions from the literature with regard to performance characteristics of a given bioaerosol sampler\textsuperscript{87}. There is limited data on currently circulating seasonal influenza viruses consisting of experimental head-to-head performance characteristic of different bioaerosol samplers under varying environmental condition in a controlled setting\textsuperscript{114}. Therefore we sought to determine the performance characteristics with respect collection efficiency for four types of commonly used bioaerosol samplers, using one laboratory strain (PR8) and two circulating seasonal strains (H1N1 and H3N2) of IAV.

For PR8 sampling all samplers were successful in collecting viral RNA (Fig 2). The low-volume samplers (NIOSH cyclone and PTFE filter cassette) showed the highest comparable collection efficiency (RNA copies/L air), which was highest at low RH and decreased as RH increased. The Andersen sampler also showed a similar decrease in collection efficiency with increasing RH, although to a lesser degree than the low-volume samplers. A shift in the size of particles associated with viral RNA was observed in the Andersen sampler, such that the majority of the viral RNA was associated with particles $<2.1$ µm at 30% RH and increased to $2.1 – 3.3$ µm for 80%, with a spike in the $>7.1$ µm, possibly due to condensation within the sampler (Fig. 3). This agrees with previous clinical studies which have found the majority of airborne IAV RNA in bioaerosols between 1-4 µm\textsuperscript{57,136}. The AGI-30 collection efficiency remained relatively consistent over all three RH conditions. Viability was only recovered using the dry low-volume samplers, which showed increased viability at lower RH for the NIOSH cyclone (Fig 4a) and slightly higher viability at 50% RH for the PTFE filter cassette and substantially lower viability at 80% RH. These results agree with the previous mentioned studies showing decreased viability at higher RH, although the PTFE filter cassette results were somewhat unexpected.

For H1N1 and H3N2 collection efficiency was greatest at lower RH and decreased with increasing RH for both samplers and strains of virus. H3N2 showed higher collection efficiency for both samplers (Fig 5). It is unclear what may be responsible for such strain-specific differences, however it may be related to differences in the physical structure of each virus, their electrostatic properties, or differences in the sensitivity of qPCR for each virus. Viability data showed limited
viability, with only 1/3 of runs, in each condition, yielding viable virus with each strain (Fig 6). As stated previously, this may be due to residual decontaminant in the aerosol setup. Nevertheless, we see significantly lower relative viability ratios for H3N2 compared to H1N1 under the same conditions when viability was detected. This was interesting as the total amount of RNA detected was higher for H3N2, indicating the increased amount of viral RNA recovered did not translate increased viability.

The results of this study should be taken in light of a number of limitations. First, PBS was used as a collection media for the Andersen and AGI-30 samplers to reduce bubbling. PBS has been shown to enhance viral RNA recovery, but has potential to reduce infectivity compared to more protein rich media, such as plasma\textsuperscript{137}. Also, PBS was used to dilute the virus prior to aerosolization. The use of a liquid media in the Andersen may have resulted in evaporation over the course of sampling, changing the effective jet-steam within the sampler, and resulting in a shift in actual size distribution of each stage compared to manufacturer specifications. Additionally, PBS is lacking in components found in respiratory secretions which are likely emitted from infected hosts. Lastly, the seasonal IAV isolates used to propagate our viral stocks were not plaque purified and thus may contain defective interfering particles.

### 2.6 Conclusion

In summary, we assessed the collection efficiency of four commonly used bioaerosol samplers for the collection of seasonal influenza virus strains, H1N1 and H3N2, under low (30%), medium (50%), and high (80%) RH conditions, in the laboratory setting. To our knowledge this is the first experimental head-to-head comparison of H1N1 and H3N2 bioaerosols comparing bioaerosol sampler efficiency at various levels of RH. Overall our results indicate that all four samplers tested in our study are proficient to detect IAV RNA from air. Both the NIOSH cyclone and PTFE filter cassette samplers showed comparative collection efficiencies and were able to capture viable virus from all three strains used. Therefore these low volume samplers provide a good option for IAV surveillance, and may be particularly useful in personal exposure studies. While the higher flow-rate samplers had lower collection efficiency, these remain options for large clinical rooms or field settings, such as swine barns. Our results also confirm earlier studies showing reduced viability with increasing RH. In terms of clinical relevance this study provides evidence to inform the selection of bioaerosol samplers for clinical surveillance or experimental applications, and adds further evidence to suggest controlling RH in clinical or other settings may be used as a means to
mitigate IAV spread. Further research is needed to determine effectiveness and feasibility, as well as how such changes may impact other respiratory viruses.
Chapter 3

Characterization of bioaerosol generation during potential aerosol-generating medical procedures

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The work in this section has been prepared as a manuscript for future submission.
3.1 Abstract

**Background.** Healthcare workers preforming medical procedures with the potential to produce aerosols such as bronchoscopy, intubation or extubation, may be exposed to infectious bioaerosols if the patient has an active respiratory tract infection. However, the determinants of aerosol generation during such procedures and their relative risk to HCWs remain poorly characterized.

**Methods.** Aerosol generation was measured during highly controlled pig intubations (N=16) and elective bronchoscopies (N=34) using an optical particle counter. Additionally the presence of bacteria in the procedure room air was assessed using a low-volume portable personal filter sampler and a high-volume cyclone sampler (pig intubations only). **Results.** Here we show that the concentration of aerosol particles (0.3 µm, 1.0 µm and 5.0 µm) does not significantly change during highly controlled pig intubations. Furthermore, in a series of elective bronchoscopies, we show that the concentration of smaller aerosol particles (0.3 µm) increase measurably (869.5 ± 518 particles/cycle, p=0.10), while the generation of larger particles (1.0 µm and 5.0 µm) decrease significantly compared to baseline (p < 0.01). **Conclusion.** These results support the appropriate use of personal protective equipment for HCWs performing bronchoscopy, while indicating the need for more in-depth investigation to elucidate the determinants of aerosol generation during bronchoscopy and other potential AGPs.
3.2 Introduction

Medical procedures with the potential to produce aerosol particles include endotracheal intubation\textsuperscript{25,100}, extubation and bronchoscopy\textsuperscript{41,101}. Potentially infectious bioaerosols (aerosol particles of biological origin) generated during these aerosol-generating procedures (AGPs) may represent a risk to exposed healthcare workers (HCWs) when patients are infected with a respiratory pathogen. The importance of understanding the risks associated with AGPs was emphasized during the SARS epidemic of 2003, when HCWs suffered a substantial burden of the total SARS cases\textsuperscript{25,38,138,139} despite adherence to enhanced droplet and airborne precautions. Furthermore, retrospective analyses revealed evidence of potential airborne transmission of SARS during AGPs\textsuperscript{24}. Additionally, a subsequent systematic review evaluating the risk of acquiring SARS for HCWs performing AGPs, compared to those providing care, but not involved in AGPs, found that endotracheal intubation was associated with a significant increased risk of acquiring SARS\textsuperscript{107}. However, there remains a dearth of empiric data regarding bioaerosol production and quantification during AGPs, and risks associated with AGPs remain poorly characterized.

Bronchoscopy is a clinical procedure involving endotracheal insertion of a bronchoscope into the lungs for diagnostic or therapeutic purposes. Airway manipulation during bronchoscopy has been shown to trigger the cough reflex in many patients, potentially producing significant aerosols, which may contain pathogenic microorganisms\textsuperscript{101}. Recent small-scale studies have found that a significant number of aerosols were produced during bronchoscopy and identified an increase in aerosol-borne respiratory bacteria during the procedure compared to an empty room\textsuperscript{108,140}. Here, our objective was to measure the quantity and size of aerosols produced during highly controlled pig intubations and elective patient bronchoscopies, relative to a representative baseline immediately before the procedure.

3.3 Methods

An AeroTrak\textsuperscript{®} optical particle counter (OPC Model 9303; TSI Incorporated, Shore- view, MN, USA) was used to quantify aerosol production based on particle number and size. Aerosol production was measured in a highly-controlled experimental setting during pig intubations. The pigs were given a mixture of atropine and ketamine and intubated under mild isoflurane sedation in a fully equipped operating room. Aerosol generation measured during and immediately following intubation (100 sec post-intubation), was compared to a baseline concentration of
aerosols immediately before intubation (100 sec pre-intubation). Particles were captured and counted in three size categories, 0.3 µm, 1.0 µm, and 5.0 µm, which represent particle size ranges with preferential deposition throughout the upper and lower respiratory tract. Bioaerosols within the respirable aerosol size fraction can include bacteria (0.3-10 µm), fungi (0.5-30 µm), viruses (0.02-0.3 µm; often found in clusters or attached to larger particle), as well as smaller cellular debris and biotoxins. In addition, bioaerosol content was assessed using a high-volume (300 L/min) Coriolis air sampler that collects particles in liquid medium (PBS), as well as a low volume 1.0 µm PTFE filter cassette at 3.5 L/min. Both samplers were placed within the breathing zone of the HCW performing the intubation. Samples collected were plated on blood agar and any colony forming units (CFUs) were accurately identified using matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. MALDI-TOF mass spectrometry is a technique that involves irradiation of a sample, followed by ionization. The ionized sample is then sent through a flight tube to determine the mass-to-charge ratio (m/z), which is then used to determine a peptide mass fingerprint (PMF). The PMF is then compared to a database of PMFs of known organisms, which allows accurate identification of an unknown organism.

Aerosol generation was also measured during elective patient bronchoscopies (N=34) and mean aerosol generation during and immediately following bronchoscope removal (100 sec post-procedure) was compared to a pre-procedure baseline immediately preceding the insertion of the bronchoscope (100 sec pre-procedure), with all medical personnel present and donned in PPE. Aerosols were sampled 0.75 m from the patient’s head, at the foot of the stretcher and at a flow rate of 2.83 L/min (Fig 7). In addition, the presence of bacteria in the air was measured during a subset of bronchoscopies using a small portable personal air sampler consisting of a 1.0 µm polytetrafluoroethylene (PTFE) filter cassette worn within the breathing zone of the researcher to assess potential HCW exposure. The collection of bacteria of oral or respiratory origin was used as an indicator of potential exposure for HCWs performing the procedure. Timing of specific procedural events including scope insertion, scope removal, coughing, suction, bronchoalveolar lavage (BAL) and biopsies were recorded for a later analysis of aerosol generation. All bronchoscopies were performed in a negative pressure endoscopy suite with 12 air changes per hour. The primary outcome for this study was aerosol generation during bronchoscopy compared to a baseline immediately before the procedure. A two-tailed Student paired t-test was used to evaluate statistical difference for the primary outcome measure. The secondary outcome of this study was aerosol generation with respect to specific procedural activities including bronchoscope...
insertion, scope removal, coughing, suctioning, bronchoalveolar lavage (BAL), and biopsy. A one-way ANOVA was used to evaluate statistical significance for the secondary outcome. All statistical analyses were performed using GraphPad Prism version 6.0c. Research ethics board approval was obtained from the research ethics board of Sunnybrook Health Sciences Centre and consent was obtained from patients prior to air sampling.
Figure 7. Schematic representation of aerosol sampling in an endoscopy suite during elective bronchoscopy.
3.4 Results

A total of 16 pig intubations were sampled in this study. There was no significant increase in aerosol production in any size category (Fig 8). Coriolis samples were collected during 8 pig intubations, with 1 sample resulting in bacterial growth when cultured. Analysis of bacteria on MALDI-TOF mass spectrometry showed the presence of oral bacteria commonly found in pigs. PTFE filter cassette samples were collected during the remaining 8 intubations. A total of 3 of 8 samples resulted in bacterial growth, which appeared to be *Micrococcus spp*, but were not positively identified by MALDI-TOF.

A total of 34 bronchoscopies were sampled, of which 10 were excluded from analysis for technical reasons (different OPC flow rate setting or battery failure) (n = 4) and interruptions (staff unmasking or the door opening for extended periods of time during sampling) (n = 6). A measurable increase in aerosol production was observed in the 0.3 µm size range (p=0.10), although not statistically significance, while a significant decrease was observed for 1.0 µm (p < 0.01) and 5.0 µm (p < 0.01) sized particles (Fig 9). A considerable amount of inter-patient variation was also observed in all size categories, with a mean difference and SEM between baseline and procedure among patients of 869.5 ± 518 particles/cycle (cycle = 10 sec), -42.4 ± 14.7 particles/cycle, and -8.6 ± 2.0 particles/cycle for 0.3, 1.0, and 5.0 µm sized particles respectively (Fig 9). The data was further analyzed to determine aerosol generation during specific procedural events including scope insertion, scope removal, coughing, suction, bronchoalveolar lavage (BAL) and biopsies. Both suction (p = 0.10) and BAL (p = 0.089) were associated with increased aerosol production in the smaller 0.3 µm sized particles, although neither reached statistical significance (Fig 10). Bacteria was recovered from 3 of 12 bronchoscopies sampled using the portable personal air sampler. MALDI-TOF identification of the bacteria revealed that none of the bacteria were of respiratory origin.
**Figure 8.** Mean particle counts for 0.3, 1.0 and 5.0 µm sized aerosol particles sampled during pig intubations (n = 16). A two-tailed Student paired t-test was used to evaluate statistical difference.
Figure 9. Particle counts for 0.3, 1.0 and 5.0 µm sized particles sampled during elective bronchoscopies (n = 24) compared to baseline. Results are expressed as particle count per cycle (1 cycle = 10 seconds sampling), *p<0.05, **p<0.01. Significance was assessed using a two-tailed Student paired t-test.
Figure 10. Mean particle counts for 0.3 µm sized particles sampled during elective bronchoscopy 30s, 60s, and 180s after each event. A) Suction mean increase of 9.5% compared to baseline (P > 0.05); B) BAL mean increase of 7.7% compared to baseline (P > 0.05). Significance calculated using a one-way ANOVA.
3.5 Discussion

AGPs have been implicated in the transmission of respiratory pathogens\textsuperscript{41,107,142}. While endotracheal intubation and bronchoscopy are listed as a suspected AGPs and are considered high-risk medical procedures for HCWs, this classification has been made primarily based on epidemiological data and expert opinion\textsuperscript{107}. This study is among the first to quantitatively measure aerosol generation during experimental pig intubations and elective bronchoscopy. Here we show that there is no significant change in aerosol generation as a result of experimental pig intubation. This was not entirely unexpected given that the pigs in this experiment were sedated and paralysed prior to intubation.

This also suggests that sedating high-risk patients prior to intubations, when possible, may reduce the amount of aerosols generated, subsequently reducing the risk to HCWs, although more work will need to be done to quantify aerosol generation during intubation in non-sedated patients undergoing various procedures and with different patient health characteristics. Also it is important to note that oral bacteria commonly found in pigs was recovered from the air during intubation, which indicating that a potential risk of exposure still exists, even though aerosol generation does not increase under these conditions.

Additionally, we also provide quantitative evidence of increased aerosol production during bronchoscopy in the submicron respirable aerosol size range (0.3 µm) relative to pre-procedural concentrations, although this increase was non-significant. Fine aerosol particles can travel extended distances and be inhaled deep into the lungs, representing a potential risk of infection if laden with pathogenic microorganisms\textsuperscript{1}. This increase in the aerosol concentration of smaller submicron aerosols was expected given the propensity of bronchoscopy to cause patients to cough\textsuperscript{101}. This finding is also in accordance with another recent study by Lavoie et al\textsuperscript{108}, which evaluated aerosol and bioaerosol generation during bronchoscopy using a UV-APS. To our knowledge this is the only other study to directly assess aerosol generation during bronchoscopy, however, in this study the aerosol generation was compared to an empty-room baseline. The use of an empty room as baseline would have over-estimated the quantity of particles attributed to the procedure, when in fact a proportion is due to the presence of personnel and pre-procedure activities. We used the period immediately preceding the insertion of the bronchoscope as the representative baseline. Incorporation of particles generated by the presence of personnel allowed us to attribute an increase in particle concentration to the bronchoscopy alone.
Furthermore, a significant decrease in the larger 1.0 and 5.0 μm sized particles was also observed, which may indicate their limited ability to reach the sampling device. In addition, obstructions such as the inserted bronchoscope, gauze used around the scope and bite block may have obstructed the release of larger particles during the procedure. Alternatively, the fact that 1.0 and 5.0 μm size range particles were higher during the pre-procedural period may highlight a period of potentially unseen exposure risk, during which HCWs may be unknowingly exposed to an increased concentration of aerosols before donning PPE for the procedure, possibly as a result of particle re-entrainment. If procedures are performed serially, this particle re-entrainment may expose HCWs to potentially infectious bioaerosols from the previous procedure, although this is speculative and more work will need to be done to determine if these particles pose a risk. It is also possible that the increase in pre-procedure aerosol concentration is a result of aerosols generated by the clinical staff themselves, before donning PPE. Finally, interpatient variation was observed, indicating that specific host and procedural factors may be determinants of aerosol generation during bronchoscopy. However, the precise quantity of increased aerosols that constitutes a significant increased risk of infection to HCWs remains unclear.

There are a number of important limitations in the present study. First, the sample size is small. Second, no clinical data was included to address either individual patient or procedural factors. Finally, we examined aerosol generation and did not specifically quantify bioaerosol generation, which would indicate aerosol particles containing biological material, but would also not be specifically sensitive toward pathogenic organisms.

3.6 Conclusion

Fine respirable bioaerosols containing pathogens may thus represent a risk for the transmission of respiratory infections, especially during AGPs\textsuperscript{41,107}. Our results show a measurable increase in small respirable aerosols produced during bronchoscopy. Additionally, our data reveals significant interpatient variation between procedures, which suggests procedural factors, or personal physical or health characteristics, may be significant determinants of aerosol generation. Interpatient variation may also support the idea of some patients being emitters, producing larger quantities of aerosols than other similar patients undergoing the same procedure, and possibly representing an increase risk of infection to HCWs. This data supports the need for ongoing sampling with clinical data collection to identify determinants of bioaerosol production, and further supports the need for appropriate PPE use during bronchoscopy. A more complete understanding of bioaerosol
generation during bronchoscopy will ultimately enhance risk assessment for HCW exposure to respiratory pathogens.
Chapter 4

Overall discussion and conclusion

4.1 Discussion

With the continual emergence of novel avian influenza strains\textsuperscript{67} with pandemic potential representing a major risk to public health, developing a comprehensive understanding of influenza transmission is critical for pandemic preparedness. However, there continues to be considerable debate regarding the transmission of influenza, particularly with respect to respiratory droplet vs droplet nuclei transmission\textsuperscript{54}, with some groups maintaining that larger respiratory droplets account for the majority of transmission\textsuperscript{56}, and others favoring aerosol transmission as the dominant transmission route\textsuperscript{143}. While it remains difficult to distinguish between short-range respiratory droplet vs. droplet nuclei transmission, a number of recent studies have demonstrated that influenza viral RNA can be detected in bioaerosols of respirable range (< 5 µm) during bedside sampling in the clinical environment\textsuperscript{32,58,59,132}. Although transmission of influenza likely occurs across a range of both larger respiratory droplet and smaller droplet nuclei, short-range airborne transmission through droplet nuclei has considerable practical implications for IPC, as standard precautions against contact and droplets, including a surgical mask, may not be enough to stop transmission\textsuperscript{143,144}. However, the transmission characteristics of influenza via droplet nuclei remains poorly understood, as the detection and quantification of influenza virus-laden bioaerosols remains a significant challenge in clinical and field settings.

4.2 Performance of bioaerosol samplers

In this study we sought to determine the performance characteristics of four types of commonly used bioaerosol samplers for their ability to collect viral RNA and infectious virus, under varying RH conditions, using different strains of IAV, including current seasonal IAV strains of H1N1 and H3N2. We show that all four samplers tested were successful in collecting detectable RNA within our experimental setup. Furthermore, we show that only two of the samplers, the low volume NIOSH cyclone and PTFE filter cassette samplers, were able to collect viable virus. As mentioned previously, the lack of viability in the other two samplers may have been a result of the use of PBS
as a collection media. However, we chose PBS because it doesn’t cause excess bubbling in the sampler and is commonly used and widely available.

4.2.1 Effect of RH on collection efficiency

A number of early studies have implicated RH as having a significant effect on the viability of IAV in bioaerosols. These findings were mirrored in more recent transmission studies in guinea pig and ferret models of influenza transmission, which showed increased transmission at low RH and decreased transmission at high RH. Our findings agree with these studies, which show decreasing viability of H1N1 and H3N2 with increasing RH. We also observed a decreased RNA collection efficiency in our two low-volume samplers with increasing RH, which may be a result of changes in size, viral clustering or other changes in the physical properties of the bioaerosols that affect their interaction with the samplers. Alternatively, the decrease in RNA collection efficiency at higher RH may be a result of RNA degradation, as previous research has shown that increased RH can be deleterious toward RNA.

4.2.2 Strain specific differences

To our knowledge this was the first recent experiment to perform a head-to-head comparison of seasonal H1N1 and H3N2 for collection efficiency and viability at various RH conditions using various bioaerosol samplers within an experimental setup. While our results show a slight increase in the collection efficiency of H3N2 for viral RNA, we see increased viability in recovered H1N1. This may indicate that H1N1 is more resistant to the aerosolization process, maintaining more viability while in aerosol form. However, this finding should be taken in light of a number of limitations, including the low sensitivity of VPA to detect infectious virus at extremely low quantities, our relatively small sample size (n=3) for each condition and the possibility of loss of viability as a result of repeated decontamination of the aerosol setup. More work needs to be done to determine if this difference translates to differences in transmission. Establishing performance characteristics for aerosol sampling addresses an important gap in the endeavor to characterize bioaerosols emitted by naturally-infected hosts by helping to inform future researchers in their sampler choice for a given surveillance or research application, as well as providing insight into the bioaerosol behavior of currently circulating strains of seasonal IAV.
4.3 Characterization of aerosol generation during AGPs

In addition to influenza bioaerosols we also assessed aerosols in the clinical setting. Presently there is a dearth of empiric data regarding the circumstances under which significant aerosols are produced during potential aerosol generating medical procedures. Without well characterized determinants of AGPs, HCWs may be at increased risk, especially when dealing with novel respiratory pathogens or emerging pandemic IAV, with potentially unknown transmission characteristics. We sought to determine the quantity of aerosol generation during intubation and bronchoscopy, as well as to assess the bioburden within the air.

4.3.1 Aerosol generation during AGP

This study has revealed a measurable, though non-significant, increase in the production of smaller 0.3 µm aerosols, and a significant decrease in larger 1.0 µm and 5.0 µm aerosols produced during bronchoscopy, compared to a representative baseline concentration. Pig intubation data revealed no significant increase in aerosol production during intubation. However, there was a large amount of interpatient variation observed between procedures, and additional work needs to be done to characterize the procedural and patient specific determinants of aerosol generation, as well as determining the level of increased aerosol generation that constitutes a measurable increase in risk to healthcare workers.

4.3.2 Presence of bacteria in the air during AGPs

While there were no significant differences in aerosol generation during pig intubations, oral flora was recovered, which highlights the fact that HCWs may still be exposed to potentially infectious bioaerosols during AGPs, even though the total number of aerosols generated is not significantly higher than what is present in the air pre-procedure. This data provides support for the use of appropriate PPE, including an N95 mask, while preforming bronchoscopy and intubation, as well as the need for ongoing sampling with clinical data collection to further identify determinants for bioaerosol production.
4.4 Conclusions

While we have shown evidence of successful collection of viral RNA and infectious virus in the respirable range, more work needs to be done to characterize influenza transmission in order to reduce infection and better prepare for future outbreaks. AGPs remain an area of concern for HCWs who perform such procedures. While the results of this study seem to indicate low risk of aerosol generation during AGPs, performing emergency AGPs in a less controlled environment may be associated with an additional degree of risk. The results of this study also raise an important question about potential for room air contamination from particle re-entrainment and serial procedures. Additionally, a large degree of interpatient variation in aerosol generation was observed, indicating that procedural and/or patient factors may be determinants of aerosol generation.

4.5 Impact

The research presented in this study provides a unique head-to-head comparison of some of the most readily used bioaerosol samplers. The results of which may be used to help inform researchers on their choice of sampler when sampling in the field or clinical setting, particularly during surveillance applications or during a pandemic. We also show that despite a non-significant increase in aerosols produced during intubation and bronchoscopy, oral bacteria was culturable from bioaerosol samples taken during the procedures. This indicates a potential risk for HCWs who perform these procedures on a day-to-day bases, and provides further evidence for the appropriate use of PPE during AGPs. This information may be particularly important in the context of a novel or pandemic pathogens, while highlighting important knowledge gaps.

4.6 Future Directions

Moving forward, a number of additional experiments could be performed to expand the in vitro portion of this study. First, additional influenza experiments could be performed to assess whether alternative choices of collection media in the high-volume samplers have an effect on the viability of collected IAV. Also, additional samplers could be tested and animal experiments could be performed to assess transmission in relation to virus viability, environmental conditions and virus type. Reverse genetic techniques could also be applied to determine viral determinants of survival.
This technique allows for specific directed mutations to be applied to the viral genome to determine both the function of various proteins, as well as the effect of mutations on viral replication and infectivity\textsuperscript{146}. Such mutations could be applied, using reverse genetics, to determine which mutations are associated with enhanced survival and infectivity of viral bioaerosols. In addition, different viruses such as RSV, could be used to determine differences between common respiratory viruses. Finally, a rotating drum could be used to test the stability of bioaerosols extended in air. A rotating drum allows bioaerosols to be suspended for extended periods of time in a cylindrical biocontained drum. The drum rotates, which prevents deposition and allows for bioaerosol aging. The drum also allows for control of environmental conditions such as temperature, RH and UV. Static sampling of the aged bioaerosols within the drum can be performed at various time points, under different environmental conditions, to determine the effect of temperature, RH and UV on viral stability, as well as the length of time a viral bioaerosol can remain viable under a given set of environmental conditions\textsuperscript{147}. Rotating drum experiments could be performed to compare different strains of influenza, and other respiratory viruses, for their stability as viral bioaerosols, both over time, and as a function of varying environmental conditions. These experiments would ultimately help expand our knowledge of viral bioaerosol behavior, improve existing control measures and aid in the development of novel mitigation techniques.

The clinical AGP research presented in this study could be expanded by the addition of a patient health questionnaire to assess how individual patient factors and procedural characteristics impact aerosol generation during bronchoscopy. Additionally, AGP related sampling could be performed using many samplers, set up strategically positioned throughout a bronchoscopy suite, or other rooms such as a negative pressure isolation ICU room, to measure aerosol dispersion throughout a room over multiple distances. This work will ultimately enhance risk assessment for HCWs potentially exposed to respiratory pathogens while performing AGPs.
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