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Analysis of Generation and Sampling Methods for MS2 Virus Aerosols

Bryan Christensen
Meera Kesavan
Ana Rule

JOHNS HOPKINS UNIVERSITY
Baltimore, MD 21205-2103

Jana Kesavan

RESEARCH AND TECHNOLOGY DIRECTORATE

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This report provides methodology and results from experiments conducted to characterize generation and sampling methods for enterobacteria phage *Emesvirus zinderi* (MS2) viral aerosols. Three aspects were evaluated: (1) the aerosol generation methods, (2) the sampling and analysis methods, and (3) the environmental conditions and growth media on the viability of a viral aerosol. Different methods for aerosol generation were also evaluated, including the Collison nebulizer, bubble generator, noncirculating bubbler, spinning-top aerosol generator, and ultrasonic nebulizer. Various methods exist for sampling and analyzing viral aerosols, which have specific requirements because of their size and biological properties. Bioaerosol sampling methods assessed include filter collection, impingement into fluid, and impaction onto agar. Viral aerosol quantification methods that were evaluated include an ultraviolet aerodynamic particle sizer and an electrical low-pressure impactor. Viral viability can be affected by temperature, humidity, and media. Viability was evaluated within a 20 to 50 °C temperature range and at 30 and 65% humidity. Five sampling media were used: phosphate-buffered saline, tryptone (15 g/L), glycerol (0.1%), skim milk (3 g/L), and albumin (1 g/L).

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PREFACE

The work described in this report was started in January 2010 and completed in December 2014. At the time this work was performed, the U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD) was known as the U.S. Army Edgewood Chemical Biological Center (ECBC).

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ANALYSIS OF GENERATION AND SAMPLING METHODS FOR MS2 VIRUS AEROSOLS

1. INTRODUCTION

Viruses are the most common causes of infectious diseases acquired within indoor environments, particularly for respiratory and gastrointestinal infections. Common virus types that cause respiratory infections include influenza viruses, rhinoviruses, coronaviruses, respiratory syncytial viruses (RSVs), and parainfluenza viruses. Other viruses of concern are those thought to be used as bioterrorism agents that ultimately cause smallpox, viral hemorrhagic fevers, and viral encephalitis.

The purpose of this report is to characterize generation and sampling methods for viral aerosols. The research had three components; namely, to evaluate (1) the aerosol generation methods; (2) the sampling and analysis methods for viral aerosols; and (3) the effects of temperature, humidity, and sample collection media on the viability of a viral aerosol. Five aerosol generation systems were evaluated: the Collison nebulizer (BGI, Inc.; Waltham, MA), the recirculating Willeke bubble generator (recreated at DEVCOM CBC), the noncirculating bubbler (recreated at DEVCOM CBC), the spinning-top aerosol generator (STAG; BGI), and the ultrasonic nebulizer (Sono-Tek Corporation; Milton, NY). The systems varied with respect to dispersion mode and particle size distribution. Various methods exist for sampling and analyzing viral aerosols. Consideration must be given when choosing these methods because of the size and biological properties of the generated viral aerosols. Bioaerosol sampling techniques assessed for this work included filter collection, impingement into fluid, and impaction onto agar. Viral aerosol quantification systems that were evaluated included an ultraviolet aerodynamic particle sizer (UV-APS; TSI; Shoreview, MN) and an electrical low-pressure impactor (ELPI; Dekati; Kangasala, Finland). The viability of viruses can be affected by temperature, humidity, and media. The effects of these factors on airborne virus viability were evaluated.

2. BACKGROUND

2.1 Bioaerosol Size and Shape

Bioaerosols are airborne particles that may either contain living organisms or are released from living organisms. They can be artificially generated or naturally occurring, and they may be diffused in the air or present in another gaseous phase (Gorny et al., 1999). Bioaerosols can be composed of many different components, as single cells or clusters:

- Bioaerosols composed of single cells or aggregates of cells may include fragments of bacterial cells; spores of bacilli, actinomycetes, and fungi; parts of actinomycetal and fungal hyphae; endotoxins; exotoxins; enzymes; glucans; mycotoxins; high-molecular-weight allergens; pollen; and plant fibers (Gorny et al., 1999; Douwes et al., 2003).

- Bioaerosol conglomerations exist (usually in great numbers) with small dust particles and water or saliva droplets. Typically, bioaerosols are in the form of bacteria, fungi, or viruses. Bacteria are single-celled organisms with sizes ranging from 0.3 to 10 μm and densities ranging from 1000 to 1500 kg/m^3, depending on the degree of hydration (Hinds, 1999). Fungi are mostly dispersed as spores ranging in size from 0.5 to 30 μm. They are resistant to environmental stresses and are usually adaptable to airborne transport. Viruses range in size from 0.02 to 0.3 μm and are typically found as part of droplet nuclei or attached to other airborne particles (which can have a wide range of sizes) (Hinds, 1999).

Viruses in the Orthomyxoviridae family include those associated with influenza, such as the Avian flu virus; they range in size from 80 to 120 nm (Mandell et al., 2005). Viruses in the Coronaviridae family, including severe acute respiratory syndrome (SARS), range in size from 80 to 150 nm (Mandell et al., 2005). Although most viruses are on the order of 25–400 nm in characteristic length (Madigan et al., 1997), they often associate with larger particles and aggregate in natural systems. This leads to a virus particle size distribution that spans the ultrafine or nano scale (<100 nm), submicrometer (<1 μm), and micrometer (>1 μm) size ranges (Hull et al., 1970; Hirst and Pons, 1973; Aller et al., 2005).

Bioaerosol sizes follow an approximate log-normal distribution (Kowalski et al., 1999). Log-normal distributions have proven useful to describe the size distributions of aerosol particles or droplets (Hinds, 1999). Bioaerosol particles have diverse shapes, including spheres, dodecahedrons, needles, and flakes. Many viruses are pleomorphic and change their shapes (Kowalski et al., 1999). Because of their similar physical properties, bioaerosols are comparable to nonbiological aerosols in terms of size and shape. Particle shape is a fundamental property and is important for assessing health hazards and interpreting data from sampling methods.

2.2 Bioaerosol Dynamics

Ambient air can contain transient populations of microorganisms, but none actually live in the air regardless of type of dispersal. Microbe viability varies and is affected by sunlight, temperature, hydration, oxygen, and pollution. The controlled climate of an indoor environment favors the survival and transmission of contagious human pathogens (Utrup and Frey, 2004).

The behavior of a bioaerosol depends on its physical and biological attributes. Physical parameters mainly influence the dispersal method and particle size distribution, deposition location, and amount. Particle deposition methods include diffusional, gravitational, inertial, thermal, and electrostatic field effects as well as effects due to temperature and humidity. Thus, knowledge of the physical characteristics of the particle and the media through which it travels is essential to understand the characteristics and the magnitude of the dispersal.

Bioaerosols vary in size from 0.01 to 100 μm; accordingly, their behavior is governed by principles of gravitation, electromagnetism, turbulence, and diffusion (Hinds, 1999). Transport of particles smaller than 1 μm is principally governed by diffusion, whereas larger particle transport is driven by inertial and gravitational mechanisms. Electrical forces,

thermal gradients, and electromagnet radiation can affect bioaerosol fate and transport. Upon generation, airborne particles are invariably charged unless they are purposely neutralized. In addition, dry dissemination of an aerosol usually generates much higher charges than wet dissemination (Cox, 1995). Highly charged particles lead to rapid aerosol mass depletion due to increased surface deposition and aggregation. Although the overall bioaerosol may have a neutral charge, the bioaerosol particles themselves are invariably charged. The impact of electrical forces on bioaerosol viability has not been well studied. Thermal gradients can be responsible for aerosol movement. When a particle is warmer on one side than the other, the resultant force causes particle motion. Aerosol particles interact with electromagnetic radiation primarily through reflection, refraction, absorption, and scattering, thereby leading to changes in particle motion (Cox, 1995).

Although the fate and transport of bioaerosols are understood, the development of risk management measures has been hampered by a lack of valuable methods for bioaerosol generation and sampling.

2.3 Bioaerosol Generation Methods

Various methods exist for the generation of an aerosol. However, bioaerosols require particular attention because of viability concerns. The Collison nebulizer has long been recognized as a common technique for efficient aerosolization of liquids. First, a distinction must be made between an atomizer and nebulizer. In an atomizer, a gas is used to aspirate the liquid into a sonic velocity gas jet, wherein it is sheared into droplets. In a nebulizer, this liquid–gas is impacted against a barrier (the inside of the jar) to remove the larger fraction of the droplets. During nebulization, the remaining smaller droplets are propelled from the nozzle of the nebulizer, thereby creating an aerosol. Nebulizers are constructed with multiple jets (e.g., 1, 3, 6, 12, and 24 jets) and often are operated at 20 psi.

The Collison nebulizer causes a lot of shear stress during aerosol generation. Klaus Willeke designed a recirculating bubble generator that requires less pressure than the Collison nebulizer (Ulevicius et al., 1997). This generator combines bubbling and centrifugal motion. Liquid is placed in the bottom of the jar, similar to a nebulizer. Pressurized air is pushed into a fritted disc near the bottom of the jar. The air causes bubbles to form and then break. Two additional airflows enter the jar and cause a swirling motion, which also dries the particles. The particles then exit through the nozzle as an aerosol. The main goal of the bubble generator is to minimize shear forces and stresses. The Willeke bubble generator was further adapted into a noncirculating generator. The adapted version incorporates two major changes: (1) the liquid is injected (using a syringe pump) and dropped onto the fretted disc; and (2) the second airflow is removed; thus, there is no recirculation within the jar. The noncirculating bubbler is thought to further reduce stresses and shear forces.

The STAG creates particles by placing liquid droplets onto a rotating disc. The disc is rotated in a horizontal plane while liquid flows onto the disc center. Centrifugal force causes the liquid to uniformly spread outward to the periphery of the disc. Ligaments will form at the edge of the disc if the liquid flow is constant and occurs at the proper rate. The ligaments will stretch until the surface tension is overcome. At that point, the ligament will snap off, and a

droplet will be formed. Approximately four satellite droplets will form from the tail of the ligament. The diameter of the satellite droplets will be about 25% of the diameter of the primary droplet. The airflow within the instrument is sufficient to entrain and remove the satellite droplets while not affecting the primary droplet's trajectory. This extraction effect is further enhanced by the addition of an air ejector on the instrument body.

Another commonly used aerosol generator is the ultrasonic nebulizer (Sono-Tek Corporation; Milton, NY), which primarily operates through vibrational energy. Liquid introduced onto the atomizing surface (through a nozzle) absorbs some of the vibrational energy. When a liquid film is placed on a smooth surface that is set into vibrating motion such that the direction of vibration is perpendicular to the surface, the liquid absorbs some of the vibrational energy, which is transformed into standing waves. These waves, or capillary waves, form a rectangular grid pattern in the liquid on the surface with regularly alternating crests and troughs extending in both directions. When the amplitude of the underlying vibration is increased, the wave amplitude increases correspondingly. Critical amplitude is ultimately reached, at which point, the height of the capillary waves exceeds that required to maintain their stability. The result is that the waves collapse, and tiny droplets of liquid are ejected from the tops of the degenerating waves normal to the atomizing surface. The ejected liquid drops flow from the nozzle and disperse as an aerosol.

Although these methods are commonly used for general aerosol generation, they have not been systematically tested for the generation of viral aerosols. These aerosol generation methods are summarized in Table 1.

Table 1. Summary of Bioaerosol Generation Techniques

Method	**Mechanism**	**Advantages**	**Disadvantages**
Collison nebulizer	Atomization using pressure	High output, ease of use, commonly referenced	Stresses organisms
Willeke bubbler	Bursting of bubbles on fretted disc	Reduces stresses and shear forces; drier, uniform particles	Requires additional airflow
Noncirculating bubbler	Bursting of bubbles on fretted disc	Reduces stresses and shear forces	Less-uniform particles
STAG	Centrifugal force breaking liquid	Limits stresses	Difficult to control particle size, low output
Ultrasonic nebulizer	Vibrational energy	Limits stresses	Difficult to control particle size

2.4 Bioaerosol Sampling Methods

Bioaerosol sampling methods can be distinguished based on their ability to assess particle number, size, and viability. The Dekati ELPI and the TSI UV-APS are two real-time particle counters that are often mentioned in the literature. The ELPI enables real-time particle size distribution and concentration measurements within the size range of virus particles (i.e., from 30 nm to 10 µm). In previous studies, the ELPI was used when a wide size range and fast response times were required (e.g., combustion aerosol studies, filter testing, and general aerosol research). The ELPI combines the accuracy of impactor size classification and the rapidity of electrical detection in the same device. With use of an ELPI, it is possible to measure transient particle size distributions for a wide range of particle sizes and concentrations. Because the ELPI is a collecting device, it supports the subsequent application of chemical, biological or gravimetric analysis on the collected size-classified samples.

The ELPI functions by first passing the aerosol around a unipolar corona charger to charge the particles in the air. The charged particles then pass into a low-pressure impactor that has electrically isolated collection stages. The electric current produced by charged particle impaction onto each impactor stage is measured in real time by a sensitive multi-channel electrometer. Particle collection into each impactor stage is dependent on the aerodynamic size of the particles. Measured current signals are converted to (aerodynamic) size distributions using particle size-dependent relations that describe the properties of the charger and the impactor stages.

The UV-APS measures the aerodynamic diameter, scattered-light intensity, and fluorescence intensity of individual airborne particles in real time. The UV-APS provides extremely rapid measurements of aerodynamic diameter and scattered-light intensity for particles between 0.5 and 15 µm. It uses a double-crest optical system, which enables precise aerodynamic diameter and scattered-light intensity measurements. Additionally, the UV-APS spectrometer measures the fluorescence properties of individual particles. Particles are excited by a pulsed-UV laser, and the emitted light is then collected in real time with a photomultiplier tube.

Although particles can be sampled according to number and size distributions, bioaerosols can also be sampled and cultured. Three often-used bioaerosol sampling methods are the BioSampler system (SKC, Inc.; Eighty Four, PA), the Andersen N6 single-stage impactor (SKC), and filters. The BioSampler system is a highly efficient glass collection device that requires a high-volume flow pump to trap airborne microorganisms for subsequent analysis. It resembles an all-glass impinger (e.g., the AGI-30 impinger [Ace Glass, Inc.; Vineland, NJ]), but it is considered to be more effective in maintaining microbial viability and decreasing reaerosolization during sampling. Air is drawn through the BioSampler inlet at a flow rate of 12.5 L/min. The inlet is designed to function like the human nose and has comparable particle-removal efficiency. Within the BioSampler, the collection liquid is swirled upward to wet the inner walls. The incoming particles impact the walls at an angle and are retained by the collection liquid. Collecting particles in liquid simulates the passage of particles through the nose to the lower respiratory system. This gentle particle collection procedure preserves viability, prevents agglomeration, and reduces bounce and reaerosolization. A variety of analytical techniques (described herein) can be used to quantify the biological particles that are collected.

The single-stage inertial impactor is commonly used for sampling indoor and outdoor air for viable microorganisms including bacteria, fungi, and actinomycetes. A sample pump draws air through the sampler at 28.3 L/min, where 400 air jets direct airborne particles toward the surface of the agar plate containing the collection medium. The agar plates are then incubated, and the colonies are counted. The impactor design requires that a hole-correction factor be applied to allow for more accurate assessment of the collected bioaerosol.

In addition to the impingement and impaction sampling methods, filter collection is also recognized as a suitable method for sampling bioaerosols. The literature indicates that with notable limitations, polycarbonate track etch (PCTE) membrane filters, glass fiber filters, and gelatin filters are all suitable for sampling bioaerosols. PCTE filters contain precisely controlled cylindrical pores for maximum particulate capture. Several properties of PCTE filters make them suitable for bioaerosol collection, including a smooth, flat surface (less then 0.1 μm) for microscopic work; a nonspecific binding membrane; a narrow pore size distribution; negligible adsorption or absorption characteristics; and low levels of extractable components. Because biological particles are collected on the surface of the filter matrix, the filters can be readily analyzed under a microscope or vortexed in a medium and subsequently cultured. These filters can be easily overloaded and are subject to sample loss during handling.

Glass fiber filters can also filter a wide range of particulate loads and viscous solutions; various filter thicknesses are available from which to choose. The filters are composed of binder-free borosilicate glass fiber, which has no added extractable components. Glass fiber filters are typically placed into buffer, such as phosphate-buffered saline (PBS) or deionized (DI) water and vortexed to remove particles from the filter. The suspension containing the disintegrated filter material, media, and sample can be cultured for quantification. For glass fiber filters, the pore size is usually 3.0 μm, and the required flow rate may vary. A limitation of glass fiber filters is that particles are collected and tightly held within the complex filter matrix. This makes them difficult to remove and results in uncertain recovery efficiencies. Collection of biological particles on PCTE and glass fiber filters can desiccate particles, which results in potentially significant bias when viable particles are cultured.

Compared with PCTE and glass fiber filters, gelatin filters are water soluble, have a pore size of 3 μm, have a thickness of approximately 250 μm, and can be employed to sample a bioaerosol at 2.7 L/min. However, this flow rate may be increased with a decrease in sample time. Gelatin filters maintain a high retention rate (Burton et al., 2007). Because the filters maintain a moist collection environment, the viability of the collected microorganisms is retained for a relevant and meaningful sampling time. Thus, microbes in one sample can be cultivated in or on different nutrient media. Because gelatin filters are readily dissolved, recovery efficiency is not an issue.

Although these methods are commonly used for fungi and bacteria aerosol sampling, they have not been systematically tested for the sampling of viral aerosols. These aerosol sampling methods are summarized in Table 2.

Table 2. Summary of Bioaerosol Sampling Methods

Method	Mechanism	Advantages	Disadvantages
ELPI	Direct-reading particle sizer and low-pressure impactor	Real-time particle size distribution and concentration measurements from 30 nm to 10 µm; collects samples onto impactor stages	Limited to 12 particle size bins; requires high-flow pump
UV-APS	Direct-reading particle sizer	Measures aerodynamic diameter, scattered-light intensity, and fluorescence intensity	Limited particle size range of 0.5 and 15 µm; limited to 20 particle size bins
BioSampler	Impingement	Collects into liquid medium for ease of analysis; decreases evaporation and reaerosolization	Fragile; requires calibration
Andersen N6 single-stage impactor	Impaction	Collects directly onto medium; commonly used	High flow rate; potential sample overloading; requires hole correction
Glass fiber filter	Interception	Ease of use; inexpensive; variable flow rate	Uncertain extraction efficiency; desiccation
Polycarbonate membrane filter (PCM)	Interception	Ease of use; variable flow rate; narrow pore size; negligible adsorption and absorption	Low extraction efficiency; desiccation
Gelatin filter	Interception	Readily dissolves in liquid medium; moderate extraction efficiency	Fragile; lower flow rates

2.5 Airborne Viruses

Viruses have been identified as the most common cause of infectious diseases acquired within indoor environments. This particularly applies to viruses causing respiratory and gastrointestinal infection. The most common types that cause respiratory infections include influenza viruses, rhinoviruses, coronaviruses, RSVs, and parainfluenza viruses. Additional viruses of concern include those thought to be bioterrorism agents, such as smallpox, viral hemorrhagic fevers, and viral encephalitis.

Virus transmission can occur in various ways. Typically, viruses spread through person-to-person contact or airborne transmission. Person-to-person transmission includes direct contact with an infected person or indirect contact through intermediate objects such as hands or fomites. Airborne transmission occurs via the spread of droplets and contact with droplet residues, skin flakes, and fungal spores. Aerosol droplets are generated and released while individuals speak, cough, sneeze, and vomit, and aerosolization from feces occurs during sewage removal and treatment. Table 3 summarizes the potentially airborne viruses that are current public health concerns.

The degree to which a virus causes a hazard is determined by many factors: the type of virus and its potential health effects; the virus's mode of exit from the body; the virus concentration levels; the size distribution of the aerosol containing the virus; physical characteristics of the environment (temperature, humidity, oxygenation, UV light, and medium); and air circulation patterns (Morawska, 2006). These factors have not been systematically evaluated.

Table 3. Airborne or Potentially Airborne Viruses that Carry Public Health Concern

Virus	Transmission	Symptoms	Protection
Influenza type A	Respiratory droplets	Fever, body aches, cough, sore throat	Isolation, droplet precautions, vaccine
Avian flu	Contact with infected poultry	Fever, body aches, cough, sore throat	Isolation
SARS	Person to person, respiratory droplets, potentially airborne	Fever, cough, gastrointestinal symptoms, myalgia, lethargy	Isolation, droplet precautions
Smallpox	Person to person, contaminated bodily fluids	High fever, body aches, macules or rash, malaise	Isolation, droplet precautions, vaccine
Viral hemorrhagic fevers	Contact with infected host	Severe multi-system syndrome	Isolation

2.6 Viral Aerosol Exposure Assessment

Given the understanding of bioaerosol size, shape, and movement, bioaerosol exposure assessment techniques can be used for risk assessment and management purposes. Particular attention should be paid to viral aerosols (because they have been less studied than bacterial aerosols) and to the growing concern over airborne dissemination of viral particles, including coronavirus, influenza virus, and bioterrorism agents. Viral assessments are complicated by the fact that particles are in the nanoscale size range (<100 nm) (Oberdörster et al., 2005). Viruses range from 20 to 400 nm and typically exist as droplet nuclei or attached to other airborne particles (Reponen et al., 2001). Although most viruses are on the order of 25–400 nm in characteristic length (Madigan et al., 1997), they often associate with larger particles and aggregate in natural systems leading to a virus particle size distribution that spans the ultrafine (<100 nm), submicrometer (<1 μm), and micrometer (>1 μm) size ranges (Hull et al., 1970; Hirst and Pons, 1973; Aller et al., 2005).

Airborne virus particle size distributions are difficult to measure and are rarely reported. Part of the problem is that the samplers commonly used to collect virus particles were designed for collecting micrometer-sized particles (Grinshpun et al., 1997; Willeke et al., 1998). Therefore, virus aerosol studies were limited to investigations of micrometer-sized virus-containing particles (Trouwborst and Kuyper 1974; Trouwborst et al., 1974; Ijaz et al., 1994; Brooks et al., 2005; Tseng and Li, 2005). In ambient air, it is unknown as to whether the majority of virus particles are in the micrometer size range, or if a substantial fraction of virus particles are in the submicrometer and ultrafine size ranges. Submicrometer and ultrafine particles containing viable viruses would be especially damaging because particles in these size ranges can penetrate deep into the respiratory system, diffuse through alveolar membranes, and rapidly enter the blood stream and cause infection. Research has shown that the size of the inhaled particles greatly determines the toxicological and immunological effects of the particles, and in general, the effects are much greater for submicrometer and ultrafine particles (Cassee et al., 2002; Esmen et al., 2002; Daigle et al., 2003). Knowledge of the size distribution of airborne virus particles in environmental studies is important; however, the capability to characterize and control the size distribution of aerosolized virus particles in laboratory studies is essential. In a

study by Hogan et al. (2005), a constant-output atomizer produced 25 nm virus particles from an MS2 suspension. This implies that despite the presence of a high concentration of particle-forming solutes, some virus particles exist as single viruses in the air. For both MS2 and T3 bacteriophage aerosols, Hogan and coworkers found the size distribution of the total generated particles was several orders of magnitude greater than the size distribution of the individual viable virus particles. This implied that the addition of viruses to suspensions for aerosolization would have little effect on the resulting size distribution due to the negligible size of the viruses (Hogan et al., 2005). At present, there is no published systematic evaluation of the impact of different aerosol generation techniques on particle size or viability.

Techniques that were traditionally used for the collection of bioaerosols include centrifugal scrubbing, electrostatic precipitation, filtration, liquid impingement, and impaction (Otten and Burge, 1999; Sattar and Ijaz, 2002). The collection efficiency of different bioaerosol samplers differs significantly. Microbial collection and survival in bioaerosol samplers strongly depends on the type of sampler, microorganism hardiness, sampling time, and sampling flow rate (Macher and Willeke, 1992; Nevalainen et al., 1993; Lin and Li, 1998, 1999). Previous evaluations of collection efficiencies of bioaerosol samplers for virus aerosols have employed harmful human and animal viruses, such as poliovirus, coronavirus, rotavirus, and adenovirus (Tseng and Li, 2005). Because of safety concerns during experiments, the research has evolved to use bacteriophages (e.g., MS2, T3, T7, and φ6) as a substitute for pathogenic viruses (Harstad, 1965; Hatch and Warren, 1969; Trouwborst and de Jong, 1972). However, a single bacteriophage cannot accurately represent all types of viruses because of the wide range of structures and nucleic acids. Thus, selection of the bacteriophage is an important issue.

Using an Andersen six-stage impactor, Ijaz and coworkers (1987) demonstrated that 87% of aerosolized viruses have a particle size smaller than 2.1 μm. Studies show that the collection efficiency of impingers for infective viruses is greater than that of filters, and that relative humidity (RH), sampling stress, and sample extraction strongly influence the collection efficiency for a virus (Harstad, 1965; Hatch and Warren, 1969; Dubovi and Akers, 1970; Trouwborst, et al., 1972; Ijaz et al., 1987). RH, temperature, wind, light, irradiation, as well as suspending medium influence the infectivity of airborne viruses (Benbough, 1971); therefore, these factors must be considered when generating or sampling viral aerosols. Nonlipid viruses are stable at higher RH (>75%), whereas lipid viruses are stable at lower RH (<40%) (Benbough, 1971). In addition, a virus loses its infectivity in the presence of an NaCl- or peptone-containing medium, whereas phenylalanine protects a virus from aerosol inactivation at various RHs (Dubovi and Akers, 1970; Benbough, 1971; Trouwborst and de Jong, 1973). In previous studies, Collison nebulizers were almost exclusively used for laboratory-scale evaluations and animal respiratory challenges for biological aerosols (Ijaz et al., 1994; Lin et al., 2000; Agranovski et al., 2002; Bray et al., 2002; Mainelis et al., 2002; Tseng and Li, 2005).

Airborne biological particles have been most commonly sampled using liquid impingers, which rely on inertial collection mechanisms (Terzieva et al., 1996; Tseng and Li, 2005). Liquid impingers offer a distinct advantage in biological particle collection, given that most analytical methods require samples to be contained in liquid media (Terzieva et al., 1996; Lin et al., 2000). The disadvantage associated with most commercial impingers derives from loss of sampling liquid through evaporation and reaerosolization of collected particles. Thus, the

collection efficiency and viability of particles can be greatly reduced (Lin et al., 1997, 1999, 2000). The BioSampler system was designed by Willeke and colleagues (1998) to prevent loss of sampling liquid during operation and to prevent damage to bacterial cells during collection. Along with other commercial impingers, the BioSampler system has been characterized with respect to the collection of airborne bacterial vegetative cells and spores (Lin et al., 1999, 2000). Despite full-scale evaluation for the collection of micrometer-sized particles, the physical collection efficiency of liquid impingers has not been thoroughly evaluated for ultrafine and submicrometer particles with diameters <300 nm.

Comparing the AGI-30 impinger, the BioSampler system, and the frit bubbler, Hogan and coworkers (2005) showed that collection behaviors were similar, and that collection efficiency increased with increasing particle diameter for submicrometer particles (at a fixed flow rate of 12.5 L/min). According to the authors, all tested samplers had a 50% cutoff sampling diameter of approximately 300 nm. This agrees well with the results of Willeke and colleagues. (1998). For particles <30 nm in diameter, collection efficiency increased with decreasing particle diameter; diffusion was most likely the mechanism of collection for these nano-sized particles. To improve collection of submicrometer-sized components of viral particles using these samplers, viruses must be incorporated into or attached to larger carrier particles (Hogan et al., 2005). When sampling for MS2 and T3 bacteriophages, the BioSampler system showed the lowest but the most linear collection over time (Hogan et al., 2005). The AGI-30 impinger showed consistent collection for T3 bacteriophages; however, MS2 phages appeared to lose viability within the AGI-30 impinger after a 30 min sampling period (Hogan et al., 2005). Similarly, the frit bubbler showed an increase in phage collection with time. This sampling performance was the result of increased collection efficiency with time within the frit bubbler and large sampler volume losses (up to 70% of the sampling liquid); thus, the virus titer increased with time (Hogan et al., 2005).

Similar to the study by Hogan and colleagues (2005), Tseng and Li (2005) examined collection efficiencies for viral aerosols using four different bioaerosol samplers (the Andersen impactor, AGI-30 impinger, gelatin filter, and Nuclepore filter). For their study, they used four test viruses (φX174, MS2, T7, and φ6) and employed a Collision three-jet nebulizer to aerosolize the bacteriophage suspensions. The study revealed that the collection efficiencies of these samplers for airborne viruses strongly depended on virus morphology, hydrophilic nature, and RH. In terms of preserving greater virus infectivity, the Andersen impactor, AGI-30, and gelatin filter were found to be more effective than the Nuclepore filter for collecting virus aerosols (Tseng and Li, 2005). The unsuitability of the Nuclepore filter was possibly due to sampling stress during filtration and dehydration during the sampling and extraction processes. Additionally, results indicated that storage temperature and collection medium were the most significant factors in the storage of collected virus samples. This implies that the loss of virus infectivity could be minimized by adjusting the storage temperature and the composition of the collection medium (Tseng and Li, 2005). Thus, airborne virus samples collected using an impingement method should be processed as soon as possible to avoid decreased virus infectivity.

Relative recovery values for two hydrophilic viruses (MS2 and φX174) were similar for three of the samplers (the Andersen impactor, AGI-30, and gelatin filter) (Tseng and Li, 2005). In addition, the relative recovery values for hydrophobic viruses (such as φ6) were

lower than those for hydrophilic viruses (such as MS2, φX174, and T7) because lipids are extremely sensitive to sampling stress. Virus recovery values for MS2, φX174, and φ6 do not depend on RH, whereas those for T7, which has a tail fiber, strongly depend on RH (Tseng and Li, 2005). These results are similar to those previously reported for bacteria, endospores, and fungal spores (Lin and Li, 1999).

Tseng and Li (2005) demonstrated that both the morphology of the virus particles and the presence or absence of a lipid envelope significantly affected the collection efficiency of the four evaluated bioaerosol samplers and also affected the infectivity of the collected virus. Additionally, the Andersen impactor, impinger, and gelatin filter were superior to the Nuclepore filter for sampling hydrophilic viruses.

Koller and Rotter (1974) looked at several issues concerning the use of gelatin filters for collecting airborne bacteria. They found that gelatin filters had a collection efficiency of >99.95% for particle sizes of 0.5–3.0 μm. Jaschhof (1992) used the gelatin filter to collect laboratory-generated T1 phage and influenza type A virus particles. The investigators found a retention rate of 99.76% for the T1 aerosol and were able to culture influenza type A virus collected during air monitoring in the room of a patient with influenza type A. Myatt and colleagues (2003) used 2.0 μm pore size polytetrafluoroethylene (PTFE) filters with cassette samplers to collect airborne rhinovirus. PTFE filters were found to be efficient for collecting submicrometer and nanoscale aerosol particles, including bacteria and viruses (Burton et al., 2007). In addition, the 0.3 μm PTFE filter used with the 37 mm three-piece cassette exhibited the highest physical collection efficiency for *Bacillus atrophaeus* and MS2 particles. The tested gelatin filter also had fairly high physical collection efficiency, but it may not be suitable for long-term sampling because of the potential for desiccation (Burton et al., 2005; Tseng and Li, 2005). The other PTFE filters also showed very good physical collection efficiencies across the size range of 10–900 nm with relatively low pressure decreases. PTFE filters exhibited good recovery of aerosolized bacteria when used in button samplers (Burton et al., 2005). Thus, filter collection methods can be effectively employed not only for bacteria and spores, but also for viruses. Issues regarding viability during collection remain.

3. METHODS

3.1 Selection of MS2 Bacteriophage

The type of test organism to select for this study was an important consideration. The test organism's size and shape are important because the organisms are aerosolized as surrogates for a variety of airborne infectious agents. These organisms vary naturally in size and shape, and the selected organisms must reflect that diversity. Individual virus particles are in the submicrometer size range; however, the test particles were expected to span a range of sizes. Particle shape was not considered in this study because the direct-reading instruments that were used do not measure this characteristic.

For this work, the viral simulant had to be easy to work with, be relatively inexpensive, and require protection at biosafety level (BSL) 2. In addition to these issues, the size, shape, host, available molecular techniques, and culturability were also taken into account.

Given these requirements, the MS2 bacteriophage was selected as the viral simulant. MS2 has the following features: it is a member of Leviviridae family; has a size range of 0.02–0.03 μm; has an icosahedral shape; is not enveloped; has a linear, single-stranded RNA genome with 3569 nucleotides; has *Escherichia coli* as its host; contains important noteworthy proteins (capsid coat protein, replicase, lysis protein, and attachment protein); and can be analyzed by polymerase chain reaction (PCR) and enzyme-linked immunoassay (ELISA). The MS2 bacteriophage is also easy to culture, very hearty, and inexpensive. The characteristics of MS2 are very similar to those of the hemorrhagic fever viruses, which are enveloped RNA viruses with a 0.08–0.13 μm size range.

Baculovirus and vaccinia virus were also considered as potential virus simulants in this study. They were not chosen because we had more experience working with MS2. Both are representative of smallpox (variola major). Baculoviruses have a 250–300 × 30–60 μm, rod-shaped (circular) DNA genome; they have an insect host; and they can be analyzed with PCR and ELISA. Vaccinia is rod-shaped and has a size range of 200–400 μm. Major concerns with vaccinia are it must be protected at BSL 3, and it has been shown to cause infection in some experiments. Thus, vaccinia was not recommended as a viral simulant for this work. Because of its size, hardiness, low cost, and culturability, MS2 was considered to be an appropriate simulant for a worst-case scenario incident; therefore, it was used in this study. Future studies will evaluate additional viral simulants to assess the generalizability of findings based on using MS2.

3.2 MS2 Stock Preparation

For these experiments, MS2 was grown according to *EPA Method 1602: Male-Specific (F^+) Somatic Coliphage in Water by Single-Agar Layer Procedure*. This method is an adaptation of the double agar layer procedure that is also described in EPA Method 1602.

The agar for the bottom layer was 1.5% tryptic soy agar (TSA), and the top (or soft) agar layer was 0.7% TSA. A bacterial host is required for the MS2 to culture. *E. coli* Famp was grown to log phase by adding a loop full of frozen culture in 25 mL of tryptic soy broth (TSB) and shaking the solution overnight at 37 °C and 110 rpm. On the next day, 1 mL of overnight culture was transferred into 25 mL of TSB and shaken for 4 h at 37 °C and 110 rpm. The bacterial host (100 μL) and MS2 stock (100 μL) diluted to about 10^8 plaque-forming units (PFU)/mL were added to 5 mL of soft agar. The 5 mL of soft agar with the host and MS2 was poured over the bottoms of the agar plates. The plates were incubated at 37 °C without inversion (to increase water condensation on the plate surfaces) for 16–18 h. The MS2 was harvested by gently scraping the top agar layer into a 50 mL centrifuge tube using a cell scraper. PBS was added to achieve a total volume of 23 mL, and then 23 mL of chloroform (or Vertrel specialty fluid [Chemours; Wilmington, DE]) was added. The tube was vortexed very well for approximately 5 min, and then the mixture was centrifuged at 4,000 ×*g* for 30 min. Upon centrifugation, the supernatant was carefully removed into a 15 mL tube. Lastly, the MS2 stock was filtered. Filters were pretreated by passing 3 mL of 0.1% Tween 80 surfactant and then 3 mL of PBS through 0.22 and 0.1 μm filters. The MS2 stock was first filtered through the 0.22 μm filter and then through the 0.1 μm filter. The final step was to titer the MS2 stock to determine its concentration in plaque-forming units per milliliter. For these experiments, the titer of the original stock was 5.85×10^{13} PFU/mL. However, for generation and sampling purposes, the stock was diluted with PBS to a titer of ~10^{10} PFU/mL.

3.3 Analysis of Generation and Sampling Methods

Aerosol testing using MS2 bacteriophage as the viral simulant was conducted in a Plexiglas chamber (Figures 1 and 2). Controlled environments, such as aerosol test chambers, are necessary to contain and uniformly distribute the generated aerosol, optimize the collection efficiency, compare bioaerosol sampling methods, evaluate accuracy and bias, and determine sources of error and variability. The use of an aerosol test chamber limits environmental factors, which is important for developing a systematic method. A 0.140 m^3 Plexiglas test chamber was used for this study. It contained a fan to ensure proper mixing and dispersal of the aerosol throughout the chamber space. This chamber was completely sealed, but contained a high-efficiency particulate air (HEPA)-filtered opening to maintain pressure equilibrium. For safety purposes, the chamber was placed inside a biological safety cabinet.

Figure 1. Chamber design showing points of generation, mixing, and sampling.

Figure 2. Complete experimental setup showing pumps, Collison nebulizer, ELPI, UV-APS, BioSampler system, and sampling filters.

Five methods of aerosol generation were studied: a BGI six-jet Collison nebulizer, a Willeke recirculating bubbler, a noncirculating bubbler, a BGI STAG 2000 system, and a Sono-Tek 60 kHz ultrasonic nebulizer. The generation media was ~10^{10} PFU/mL of MS2 in PBS. The MS2 concentration was verified for each experimental run by plating the generation media (plaque assay). Each generation method was used for 2.5 min to create an aerosol in the chamber. After 2.5 min, aerosol generation was stopped, and sampling was conducted for 10 min. During generation and sampling, a mixing fan operated constantly to ensure proper mixing and distribution throughout the chamber. The chamber temperature and RH were recorded and carefully monitored during all experiments. Each generation method was tested using sampling methods (described as follows).

During the 10 min sampling period, two real-time particle sizers were used: the Dekati ELPI and the TSI UV-APS. The ELPI enables real-time concentration measurements and particle size distributions ranging from 14 nm to 10 µm. The ELPI data were recorded as number of particles per cubic centimeter across the full size range. The data from the ELPI were provided in real time, which was used to monitor the chamber concentration during generation and sampling.

The UV-APS measures the aerodynamic diameter, scattered-light intensity, and fluorescence intensity of individual airborne particles in real time and provides extremely rapid measurements of particles between 0.5 and 15 µm. The values for the ELPI data were recorded as number of particles per cubic centimeter.

In addition to the real-time particle counting and sizing methods, a BioSampler system, an Andersen N6 single-stage impactor, and filters were used to sample the MS2 aerosol. The BioSampler system was operated at 12.5 L/min concurrently with the ELPI, UV-APS, and impactor. PBS was used as the collection fluid to ensure maximum viability of the MS2. The BioSampler system requires 20 mL of collection fluid. After sampling, the collection fluid containing MS2 was plated using the double agar layer method described in Section 3.2.

Efficiency tests were performed on the BioSampler system before it was used with MS2. Efficiency was determined using Osmonics 0.22 µm polycarbonate filters (GVS; Bologna, Italy) as the reference to the BioSampler system. Solutions of green and blue fluorescing polystyrene latex beads were aerosolized into the chamber using a Collison nebulizer. The filter and the BioSampler system were operated simultaneously at approximately the same flow rate (~12.5 L/min). The filters were placed into DI water and vortexed for 5 min to release the particles; the sample liquid was then analyzed using a fluorometer. The collection fluid from the BioSampler system was also analyzed with the fluorometer. From these results, the efficiency of the BioSampler system was determined by applying the formula:

$$\text{Percent efficiency} = \frac{(\text{fluorescence} \times \text{volume/flow rate})_{\text{BioSampler}}}{(\text{fluorescence} \times \text{volume/flow rate})_{\text{filter}}} \times 100$$

During sampling, the single-stage Andersen impactor sampled the aerosol in parallel with the BioSampler system. The impactor operated at 28.3 L/min. Because of the double agar layer method requirement of MS2, the impactor method was slightly modified. Typically, a microbe-free agar plate is placed into the impactor before sampling. However, this was not possible with MS2, given that MS2 requires *E. coli* as its host. Alternatively, 5 mL of soft agar containing 100 µL of log-phase *E. coli* was placed on the bottom layer (35 mL) of agar. The 400-hole jets of the impactor impacted onto the soft agar, where MS2 had the chance to grow. The agar plates were incubated at 37 °C as described in Section 3.2.

Filter collection was performed to compare the PCTE, glass fiber, and gelatin filters. The flow rates for all of the filters were approximately equal and were similar to those for the BioSampler system (12.5 L/min). After aerosol generation for 2.5 min, the filters collected the aerosol for 10 min. After collection, each filter was placed into a 50 mL centrifuge tube that contained 20 mL of PBS. The PCTE filters were vortexed for 50 s and hand-shaken for 10 s, and this sequence was repeated for 5 min. The resulting sample fluid was plated as previously described (Section 3.2). The glass fiber filter was vortexed for 2 min, stored overnight at 4 °C, vortexed again for 5 min, and then plated using the double agar layer method. The gelatin filter was vortexed until it dissolved, which was usually less than 1 min. The sample liquid was then plated using the double agar layer method.

3.4 Analysis of Effects of Temperature, Humidity, and Media on Viability

Using the optimal generation and sampling methods determined from a previous set of experiments, the effects of temperature, humidity, and sampling media on the viability of MS2 were evaluated. Following the procedures described herein, test aerosol was generated using the Collison nebulizer for 2.5 min. The samplers were operated for 10 min after generation. The samplers included the ELPI, UV-APS, BioSampler system, and gelatin filter.

From the literature, it appears that the effect of temperature on viral aerosols has not yet been evaluated. Typically, laboratory temperatures are ~27 °C. In addition to 27 °C, these tests were conducted at chamber temperatures of 20, 30, 40, and 50 °C. A hot plate was placed in the chamber to increase the air temperature. Conversely, the temperature was decreased using an air conditioning unit; it was modified so that its airflow was channeled through a duct into the chamber. For each test, the chamber temperature and RH were carefully monitored.

Humidity has been shown to drastically affect the viability of airborne viruses (Yang and Marr, 2012; Morris et al., 2021). The humidity was increased by adding a second Collison nebulizer that produced an aerosol of water droplets. The water nebulizer was constantly operated during generation and sampling to maintain elevated humidity within the chamber. The low humidity was 30%, and the high humidity was 65%.

DI water has been shown to be somewhat harsh on MS2 due to osmotic properties. Thus, it was important to consider the impact of certain media that could enhance MS2 infectivity. In addition to PBS, tryptone (15 g/L), glycerol (0.1%), skim milk (3 g/L), and albumin (1 g/L) were used as the generation and sampling media to assess their ability to enhance infectivity. Each additive was analyzed separately by applying optimal generation and sampling methods as described in Section 3.3.

3.5 Statistical Methods

Stata 8.0 software (StataCorp; College Station, TX) was used to apply descriptive statistics as part of the exploratory data analysis. Two-way analysis of variance (ANOVA) was employed to determine the statistical significance of the results. Specifically, ANOVA was used to compare the means for each generation method and the various sampling methods. Sample size was calculated using preliminary data because there is no known population mean for this type of research.

4. RESULTS

4.1 Generation and Sampling Methods

The first set of experiments jointly examined the characteristics of the generation and sampling methodologies. The results, summarized in Table 4, illustrate that the methods vary significantly in terms of particle number, particle size, and virus viability.

Table 4. Values from 10 Runs of Each Generation Method*

Generation Method	BioSampler System		Andersen Impactor		UV-APS			ELPI
	Total PFU	PFU/cm^3 Air	Total PFU	PFU/cm^3 Air	GM (μm)	GSD (μm)	Particles/cm^3	Particles/cm^3
Collison nebulizer	2.26E+04 (2.52E+03)	3.62E-01 (0.0404)	2.35E+03 (42.8)	1.66E-02 (3.02E-04)	0.917	1.306	4.53E+03 (2.00E+02)	1.07E+05 (2.13E+04)
Willeke bubbler	1.38E+04 (2.83E+03)	2.21E-01 (0.0455)	2.34E+03 (43.3)	1.66E-02 (3.06E-04)	0.788	1.346	6.53E+02 (4.64E+01)	7.77E+03 (3.29E+03)
STAG	3.05E+03 (7.13E+02)	4.90E-02 (0.0115)	1.94E+01 (6.15)	1.37E-04 (4.35E-05)	0.781	1.519	8.29E+02 (2.43E+02)	9.02E+03 (2.04E+03)
Ultrasonic nebulizer	5.62E+03 (1.79E+03)	9.03E-02 (0.0287)	6.04E+01 (52.9)	4.27E-04 (3.74E-04)	1.122	1.618	5.12E+01 (1.14E+01)	7.34E+02 (1.54E+02)
Noncirculating bubbler	5.38E+04 (5.69E+03)	8.65E-01 (0.0915)	2.38E+03 (28.0)	1.68E-02 (1.98E-04)	1.120	1.416	1.11E+03 (4.43E+01)	2.62E+04 (1.89E+04)

*Statistically significant differences between generation methods ($p < 0.0001$). Values are means; values in parentheses are standard deviations.
GM, geometric mean; GSD, geometric standard deviation.

4.2 Particle Concentration and Size

The first set of experiments focused on examining the differences between each of the generation methods. Given the mechanism of dispersion, it was expected that the resultant aerosols would vary by generation method. These differences are clearly evident when particle concentrations are examined (Figures 3 and 4). From the ELPI and UV-APS, the Collison nebulizer has the highest particle output at 1.07E+05 and 4.53E+03 particles/cm^3, respectively. The difference in particle concentration was expected between the ELPI and UV-APS, as they measure different size bins. The noncirculating bubbler generated slightly lower concentrations of particles as compared to the Collison nebulizer. The ultrasonic nebulizer produced a substantially lower particle concentration as compared with the other methods.

The generation methods produced similar aerosols in terms of geometric mean (GM) and geometric standard deviation (GSD) (Figures 5 and 6). The GM ranged from 0.781 μm to 1.122 μm with a GSD of 1.306 to 1.618. The STAG had the lowest GM, which could have been caused by the satellite particles produced during aerosol generation. The noncirculating bubbler and the Sono-Tek ultrasonic nebulizer had the highest GM at 1.12 μm. The Collison nebulizer produced the most consistent aerosol (GSD of 1.306), and the Sono-Tek ultrasonic nebulizer generated the least consistent aerosol (GSD of 1.618).

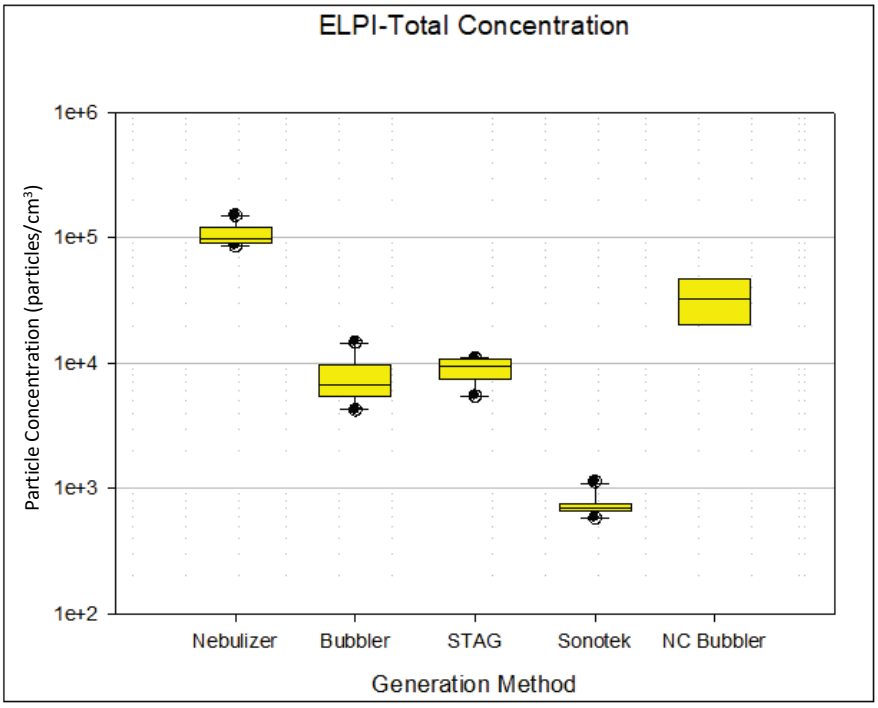

Figure 3. Particle concentration from the ELPI by each generation method.

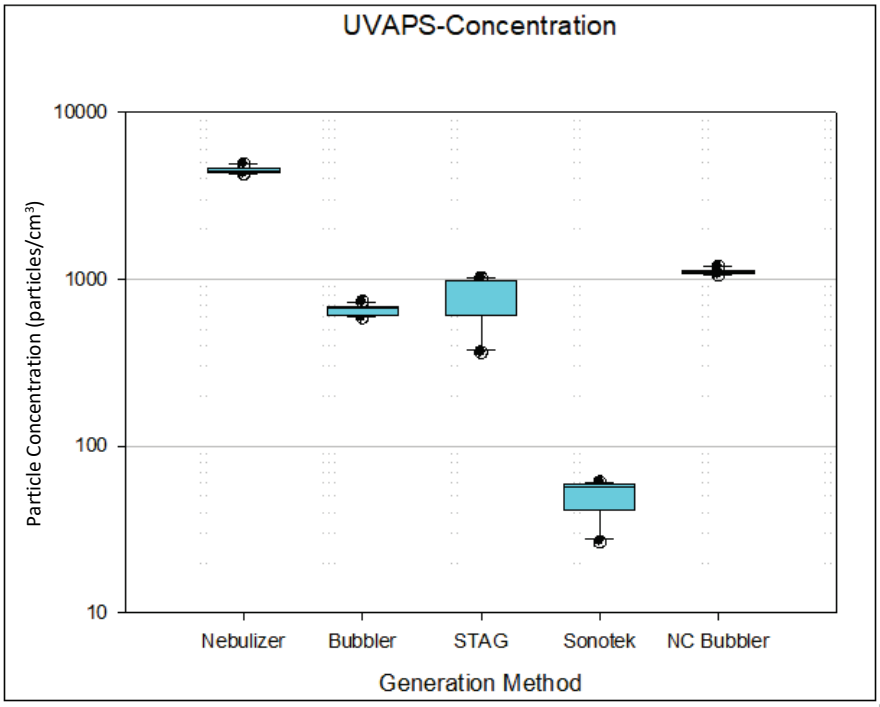

Figure 4. Particle concentration from the UV-APS by each generation method.

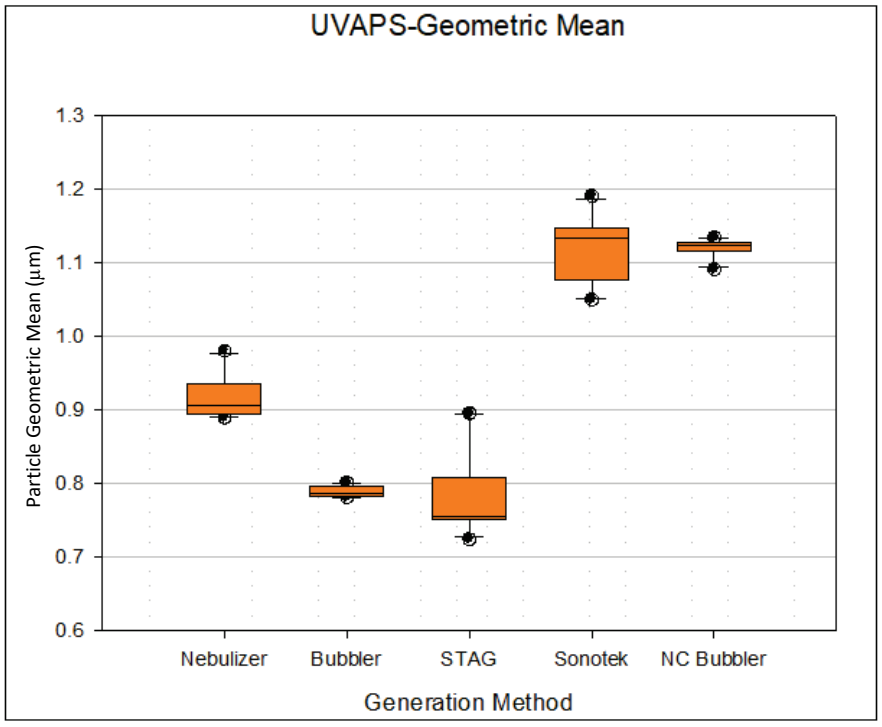

Figure 5. Particle GM from the UV-APS by each generation method.

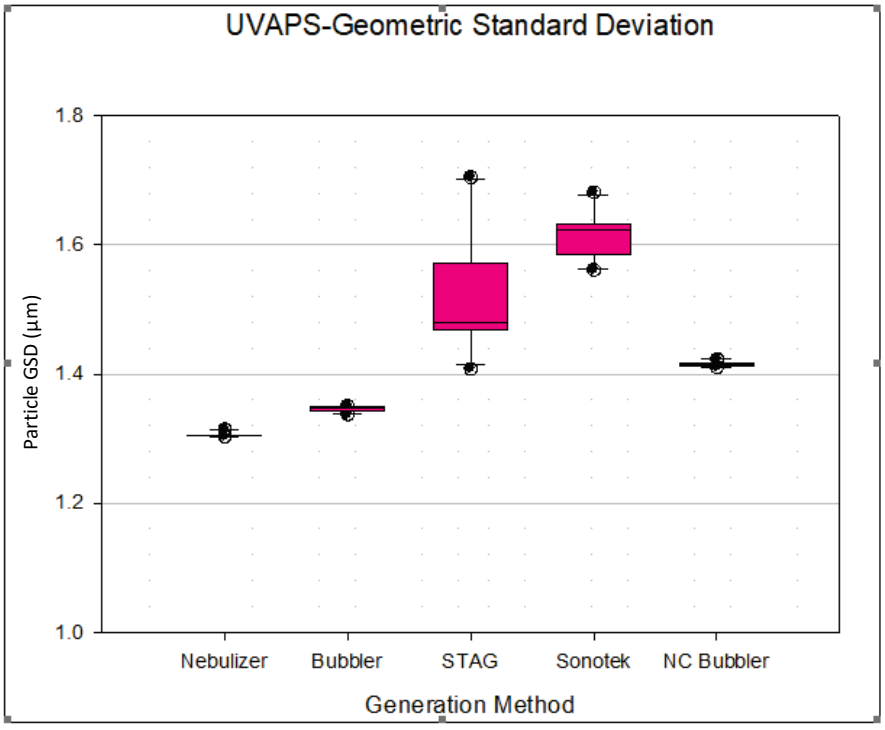

Figure 6. Particle GSD from the UV-APS by each generation method.

4.3 MS2 Aerosol Sampling

In the second phase of this study, the sampling methods were examined to determine viability for an MS2 aerosol. Because of flow rates, available biological safety cabinet space, and laboratory supplies, this phase was performed in two parts: (1) with the BioSampler system and the Andersen impactor; and (2) with the BioSampler system and filters (glass fiber, polycarbonate membrane, and gelatin). The aerosols were collected by impingement, impaction, or filtration and then cultured according to the prescribed method. Figure 7 shows the cultured MS2 sample from the gelatin filter. The BioSampler system provided similar results. Thus, the plaques were easily counted, especially as compared to the impactor. Figure 8 confirms the use of the Andersen impactor for sampling MS2. From the picture, it is evident that overloading was a common problem with the impactor.

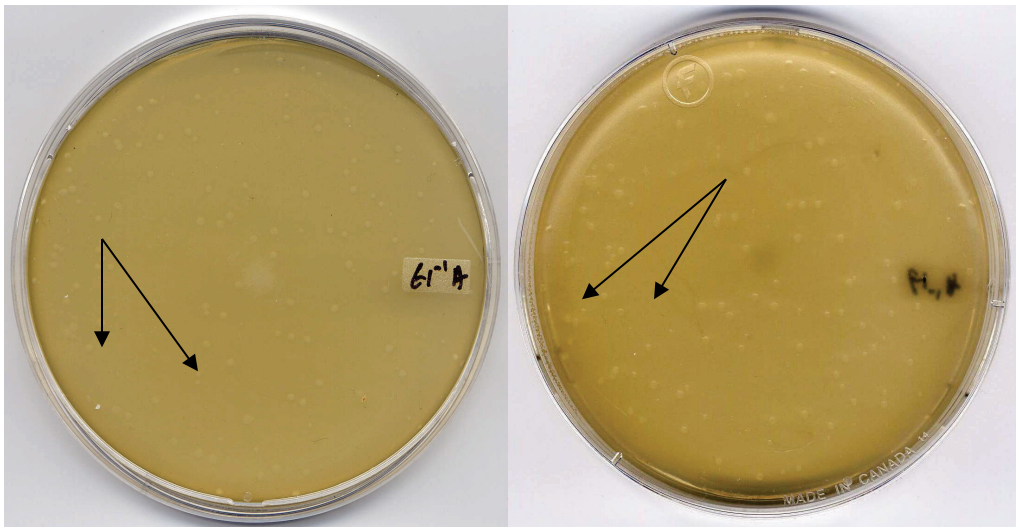

Figure 7. Cultured MS2 from aerosol samples obtained using a gelatin filter.

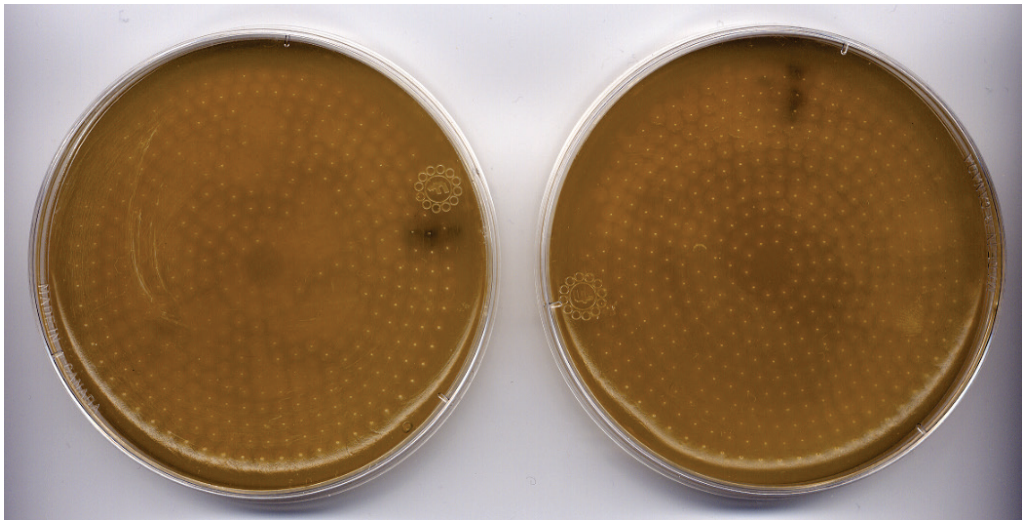

Figure 8. Cultured MS2 from aerosol samples obtained using the Andersen single-stage impactor.

The data clearly show that the generation methods affected MS2 viability as well as particle size and concentration. From Figure 9, it is clear that the noncirculating bubbler affected MS2 viability the least when sampled with the BioSampler system. However, the variability was also greatest with the noncirculating bubbler. The Collison nebulizer and the Willeke bubbler were ranked second and third, respectively, in terms of MS2 viability. When the Andersen N6 single-stage impactor was examined, the Collison nebulizer, Willeke bubbler, and noncirculating bubbler produced similar results in terms of MS2 viability, as shown in Figure 10.

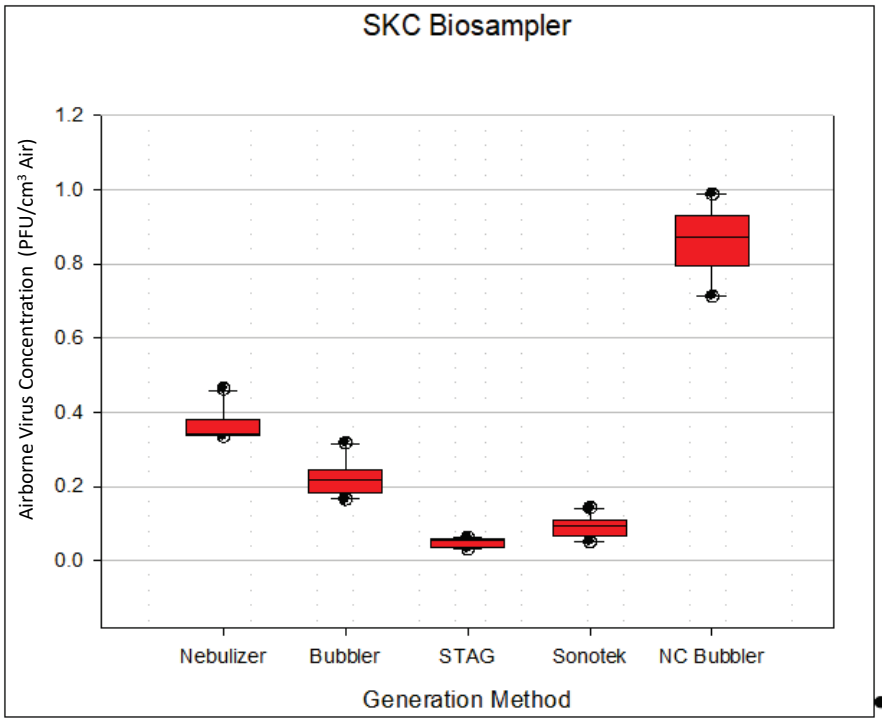

Figure 9. Airborne virus concentrations for the BioSampler system by each generation method.

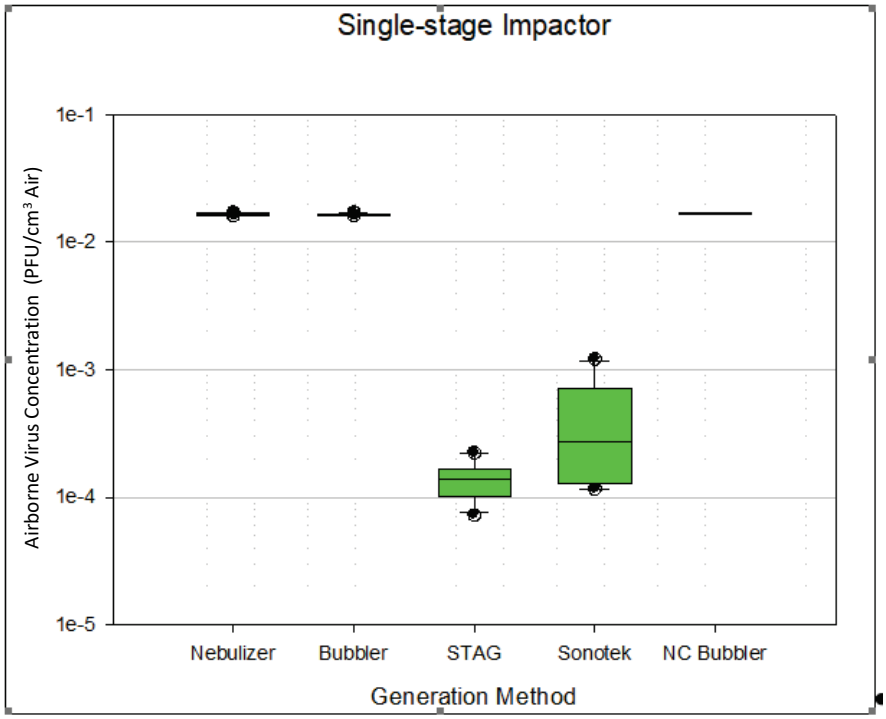

Figure 10. Airborne virus concentration for the Andersen single-stage impactor by each generation method.

In an effort to standardize the data, the number of plaque-forming units per particle was calculated. The calculation was made by dividing the BioSampler system data by the ELPI data for each generation method. This calculation took into account the differences in particle output by each generation method. The Collison nebulizer generated the highest particle concentration and also provided the second-highest viability with the BioSampler system (Figure 11); however, the number of plaque-forming units per particle was lowest for the nebulizer. On the other hand, the Sono-Tek ultrasonic nebulizer produced the highest number of plaque-forming units per particle. It is important to note that the Sono-Tek ultrasonic nebulizer also generated the largest particle, which could influence this calculation.

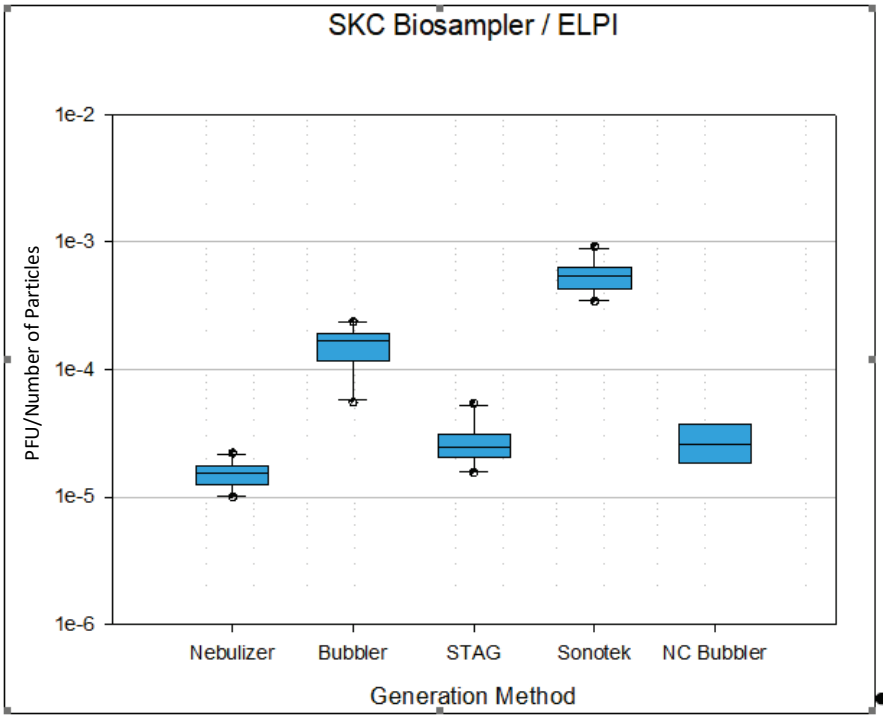

Figure 11. Calculated values of plaque-forming units per number of particles, which were determined by dividing BioSampler system data (PFU/cm^3) by ELPI data (particles/cm^3) for each generation method.

In addition to the BioSampler system and Andersen single-stage impactor, various filters were used in a follow-up experiment. This addition was sparked by the discovery of the applicability of using gelatin filters for viral aerosol sampling. Polycarbonate membrane filters (PCMs), glass fiber filters, and gelatin filters were compared to the BioSampler system with respect to MS2 viability. In Figure 12, the airborne virus concentration for each filter is compared to that for the BioSampler system. The gelatin filter had approximately 3 times less airborne virus concentration than the BioSampler system. The PCM and glass fiber filters had at least 10 times less airborne virus concentration than the BioSampler system.

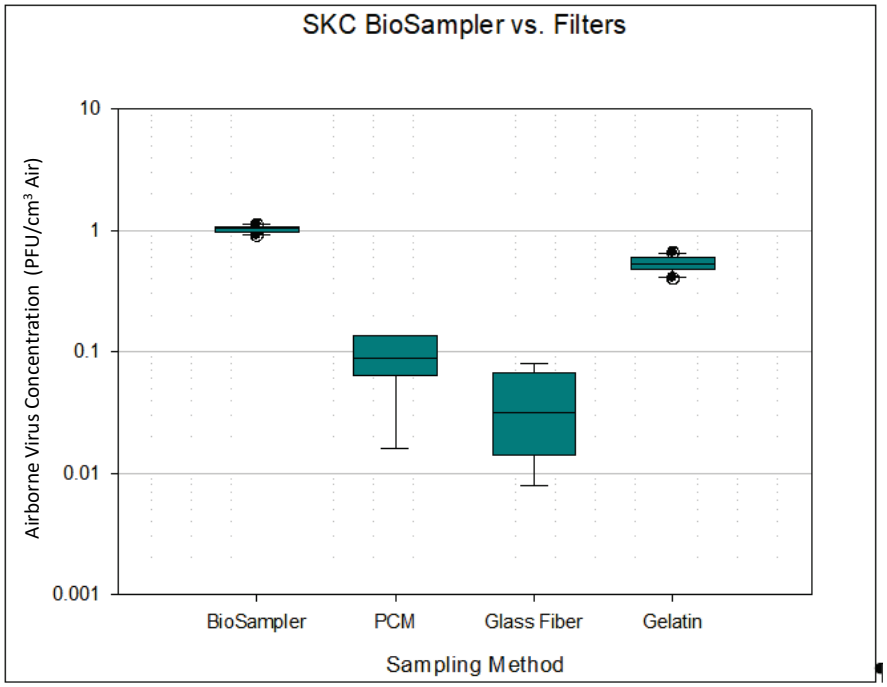

Figure 12. Airborne virus concentration for the BioSampler system, PCM filter, glass fiber filter, and gelatin filter generated by Collison nebulizer.

4.4 Effects of Temperature, Humidity, and Sampling Media

In the final stages of this study, the effects of temperature, humidity, particle charge, and sampling media were examined. Careful analysis revealed that particle charge did not affect MS2 viability. Therefore, the results regarding particle charge are not discussed. Previous studies have shown that temperature and humidity substantially affect airborne virus viability (Chan et al. 2011; Morris et al. 2021).

When the BioSampler system was used at temperatures from 20 to 40 °C, there was little change in the airborne virus concentration (Figure 13). However, at 50 °C, there was a noticeable decrease in virus concentration as compared with the cooler temperatures. Temperature seemed to affect viability less with use of the BioSampler system as compared to the gelatin filter, as shown in Figures 13 and 14. When the gelatin filter was used, temperature had a much greater, linear effect on virus viability. For the BioSampler system, airborne virus concentrations ranged from 1.01 PFU/cm^3 at 20 °C to 0.769 PFU/cm^3 at 50 °C. For the gelatin filter, concentrations ranged from 0.778 PFU/cm^3 at 20 °C to 0.0658 PFU/cm^3 at 50 °C.

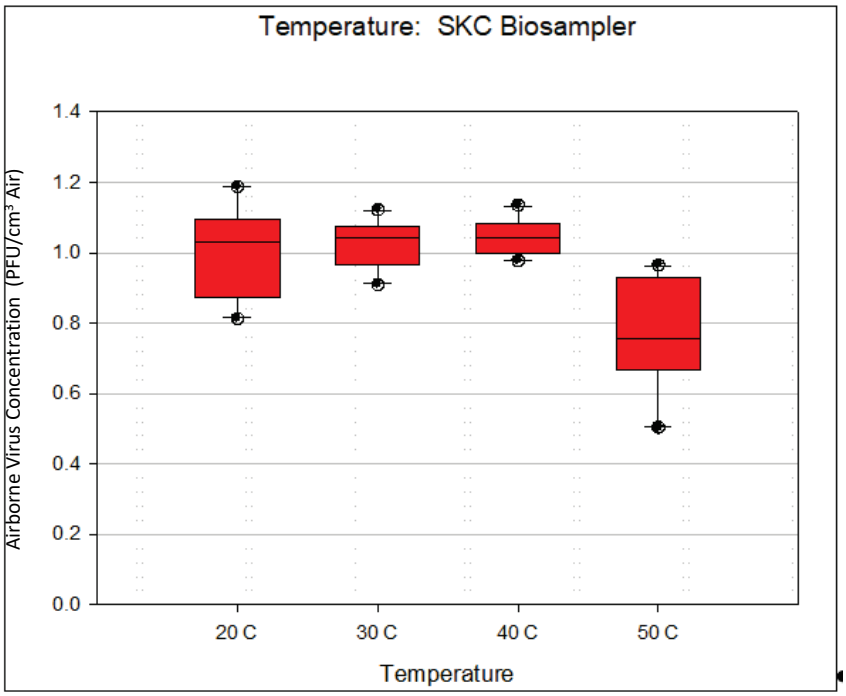

Figure 13. Airborne virus concentration by chamber temperature for the BioSampler system.

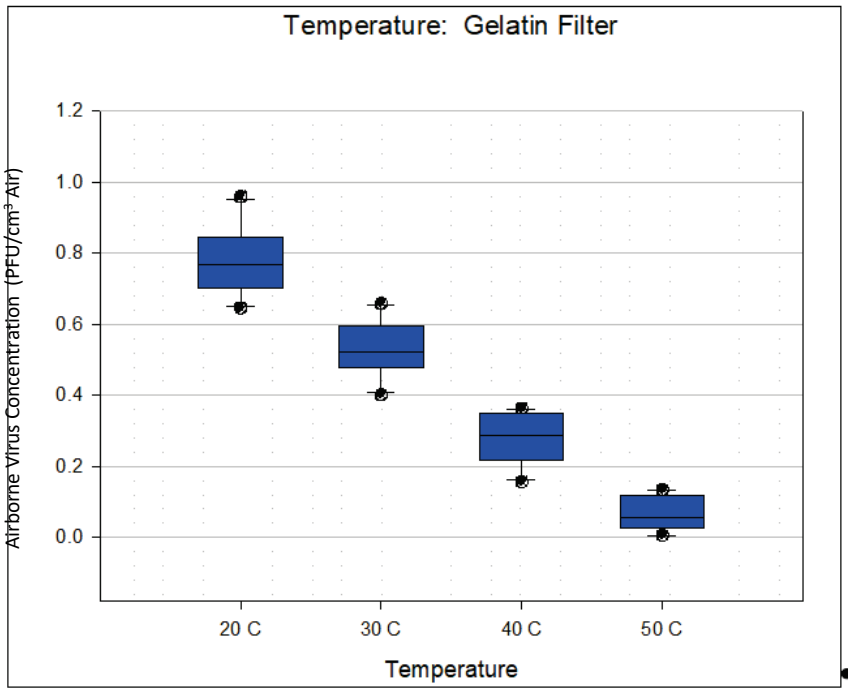

Figure 14. Airborne virus concentration by chamber temperature for the gelatin filter.

The effect of humidity on virus concentration was more noticeable than the effect of temperature. For both the BioSampler system and the gelatin sampling methods, the airborne virus concentration increased significantly as humidity was doubled (Figures 15 and 16). For the BioSampler system, the average plaque-forming unit concentrations were 0.336 PFU/cm^3 at 30% RH and 0.877 PFU/cm^3 at 65% RH. For the gelatin filter, the average plaque-forming unit concentrations were 0.232 PFU/cm^3 at 30% RH and 0.760 PFU/cm^3 at 65% RH.

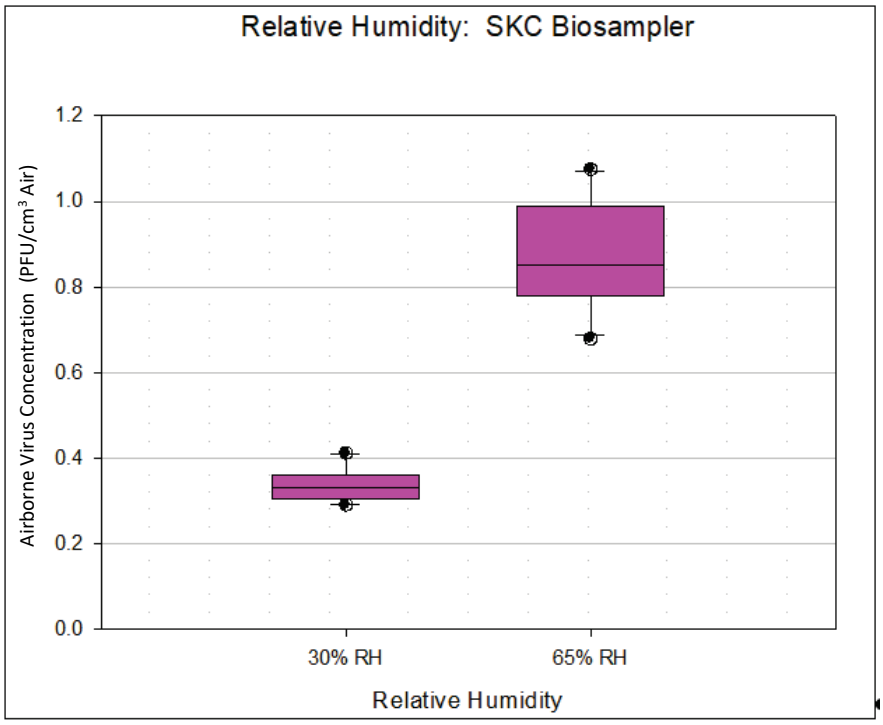

Figure 15. Airborne virus concentration by chamber humidity for the BioSampler system.

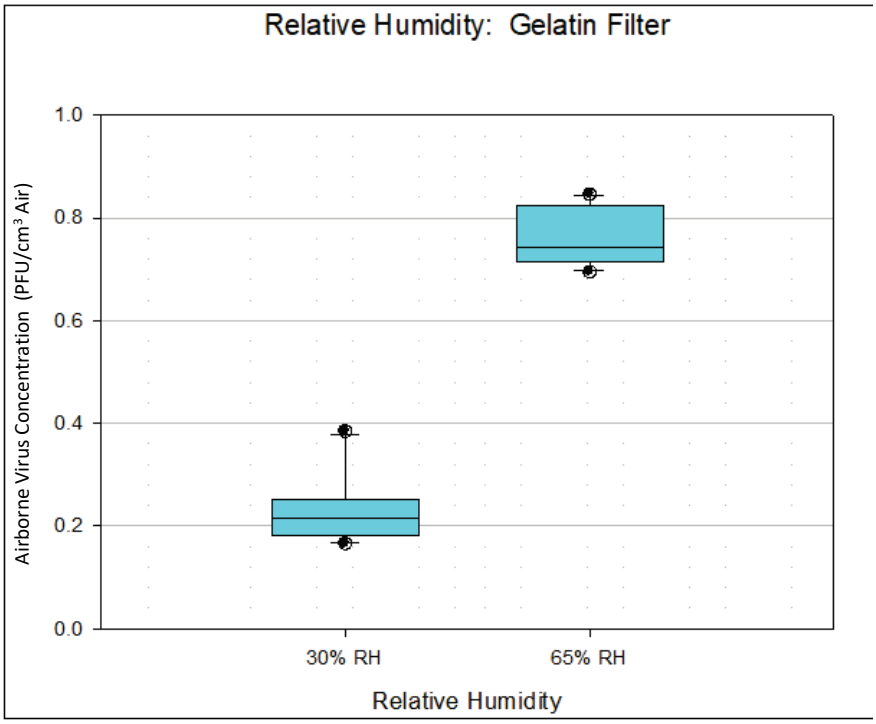

Figure 16. Airborne virus concentration by chamber humidity for the gelatin filter.

The choice of collection media can greatly affect MS2 viability. As compared with PBS (commonly used as the collection medium), tryptone and skim milk increased MS2 viability, whereas albumin and glycerol decreased it. These results pertain to both the BioSampler system and the gelatin filter, as shown in Figures 17 and 18. Also, there was a slight increase in MS2 viability as the tryptone concentration was increased. This effect was more noticeable with use of the gelatin filter. For the BioSampler system, use of skim milk (3.3 g/L) resulted in the highest viability (8.78E-01 PFU/cm^3), and use of glycerol (0.1%) resulted in the lowest viability (1.59E-01 PFU/cm^3). The highest concentration of tryptone (15 g/L) resulted in the second-highest MS2 viability (6.56E-01 PFU/cm^3). Lower concentrations of tryptone, 7.5 and 1.5 g/L, resulted in just slightly less MS2 viability, at 5.59E-01 and 5.73E-01 PFU/cm^3, respectively. The results for the gelatin filter were strikingly similar: use of skim milk (3.3 g/L) resulted in the highest MS2 viability (7.74E-01 PFU/cm^3) and use of glycerol (0.1%) resulted in the lowest viability (1.82E-02 PFU/cm^3). The highest concentration of tryptone (15 g/L) resulted in the second-highest viability (5.74E-01 PFU/cm^3). Lower concentrations of tryptone, 7.5 and 1.5 g/L, resulted in just slightly less MS2 viability at 4.49E-01 and 3.09E-01 PFU/cm^3, respectively. These results are summarized in Table 5.

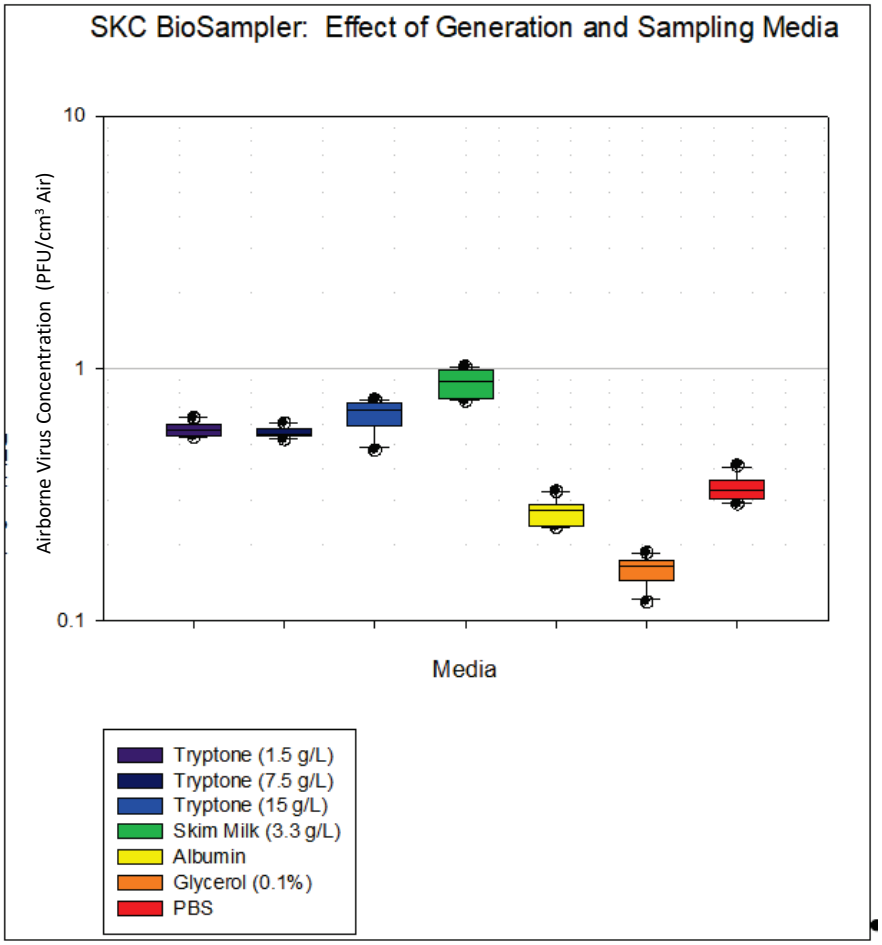

Figure 17. Airborne virus concentration by sample media for the BioSampler system.

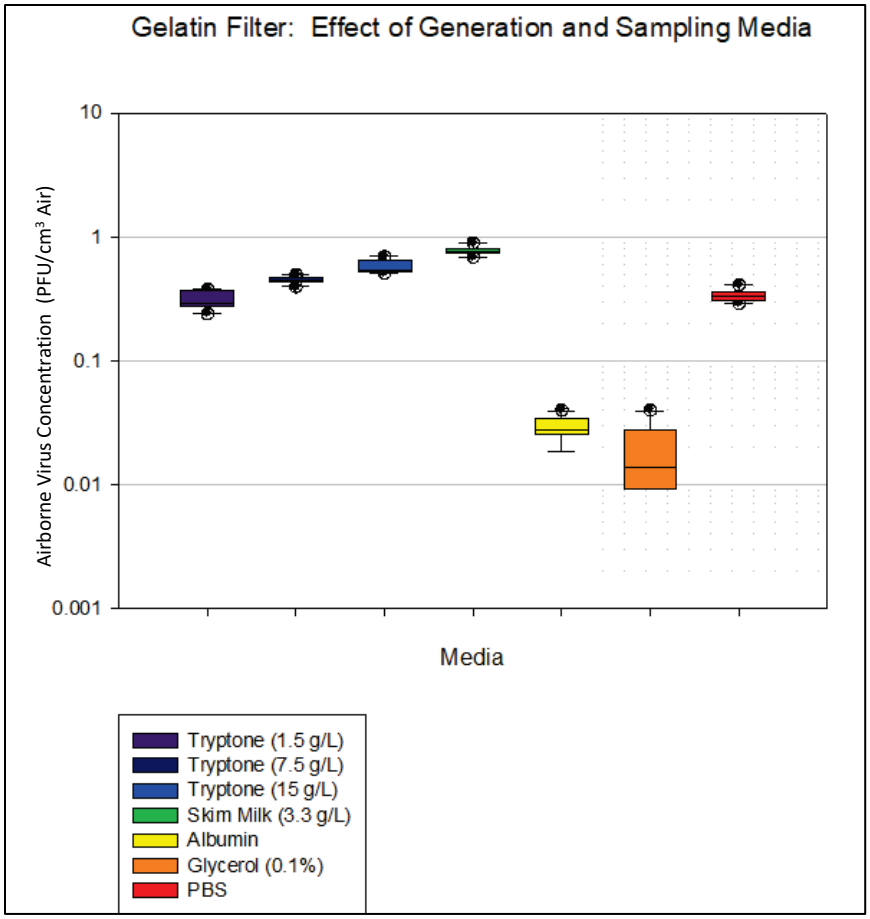

Figure 18. Airborne virus concentration by sample media for the gelatin filter.

Table 5. Viability of MS2 in Samples Collected by BioSampler System and Gelatin Filters with Various Collection Media

Collection Medium		BioSampler System (PFU/cm^3)	Gelatin Filter (PFU/cm^3)
Skim milk		0.878	0.774
Glycerol		0.195	0.0182
Tryptone	15 g/L	0.656	0.574
	7.5 g/L	0.559	0.449
	1.5 g/L	0.573	0.309

5. DISCUSSION AND CONCLUSIONS

When bioaerosol size, shape, and movement are understood, bioaerosol exposure assessment techniques can be used for risk assessment and management purposes. Particular attention must be paid to viral aerosols because they have been less studied than bacterial aerosols. Concern continues to grow over airborne dissemination of viral particles such as coronavirus, influenza virus, and bioterrorism agents. This study examined viral aerosol exposure assessment tools.

To fully analyze aerosol sampling, generation methodology must first be understood. It is evident from the results that generation techniques affect the resultant aerosols: with respect to virus concentration in air, particle concentration, particle GM, and particle GSD, each generation method statistically varied from the other. In previous studies, Collison nebulizers were almost exclusively used for laboratory-scale evaluation and animal respiratory challenges for biological aerosols (Ijaz et al., 1994; Lin et al., 2000; Agranovski et al., 2002; Bray et al., 2002; Mainelis et al., 2002; Tseng and Li, 2005).

The results of this work showed that, regardless of the generation mechanism, viral aerosols have a GM of less than 1.2 μm. Ijaz and colleagues (1987) demonstrated that 87% of aerosolized viruses have a particle size smaller than 2.1 μm. The main difference between the two studies was the generation technique. Most aerosol studies rely on Collison nebulizers, which can vary with respect to the number of jets and the pressure used to generate the aerosol. The Collison nebulizer was the most consistent generation method when GSD was considered. The Willeke circulating bubbler had the second-lowest GSD; however, it is a more complex generation method. Because of its predictable output, ease of use, and consistent results, the Collison nebulizer should be considered the optimal generation method. Thus, it was used during the second phase of the study, when changes associated with humidity, temperature, and media were examined.

Airborne biological particles are most commonly sampled using liquid impingers, which rely on inertial collection mechanisms (Terzieva et al., 1996; Tseng and Li, 2005). Liquid impingers offer a distinct advantage in biological particle collection, given that most analytical methods require samples contained in liquid media (Terzieva et al., 1996; Lin et al., 2000). The disadvantages associated with most commercial impingers derive from loss of sampling liquid through evaporation and reaerosolization of collected particles. Particle collection efficiency and viability can be greatly reduced when using impingement (Lin et al., 1997, 1999, 2000). In a study by Hogan and colleagues (2005), the AGI-30 impinger exhibited consistent collection for T3 bacteriophages; however, MS2 phages within the AGI-30 impinger appeared to decrease in viability after a sampling period of 30 min. Thus, the AGI-30 impinger would not be an effective MS2 sampler for sampling periods longer than 30 min. In this study, the BioSampler system was used to prevent loss of sampling liquid during operation and damage to bacterial cells during collection. The BioSampler system, along with other commercial impingers, has been characterized with respect to collection of airborne bacteria and spores (Lin et al., 1999, 2000). Our study results indicate that the BioSampler system can be an effective sampler for viruses.

The BioSampler system proved to be better suited than filters for sampling airborne viruses. In this study with the Collison nebulizer, the BioSampler system resulted in greater viability by 25.7-fold as compared to the glass fiber filter, 11-fold as compared to the PCM filter, and 9.03-fold as compared to the gelatin filter. Previous studies showed that for infective viruses, the collection efficiency of impingers is greater than that of filters and that for viruses, RH, sampling stress, and sample extraction strongly influence the collection efficiency (Harstad, 1965; Hatch and Warren, 1969; Dubovi and Akers, 1970; Trouwborst, et al., 1972; Ijaz et al., 1987). The decrease in viability that resulted with use of the PCM and glass fiber filters was most likely caused by sample-extraction methodology and desiccation. Often it appeared that the samples were not fully extracted from the PCM filters, which was likely due to the filters' electrostatic properties. As for the glass fiber filters, the virus particles may have become captured by the fibers. Taking into account that the gelatin filters dissolve, sample extraction was less of an issue, but desiccation must be considered. Burton and colleagues (2005) and Tseng and Li (2005) showed similar results regarding the gelatin filter. The tested gelatin filter also had fairly high physical collection efficiency; however, it may not be suitable for long-term sampling because of the potential for desiccation.

Tseng and Li (2005) used four test viruses (φX174, MS2, T7, and φ6) and employed a Collision three-jet nebulizer to aerosolize the bacteriophage suspensions. The AGI-30 impinger and gelatin filter were found to be better than the Nuclepore filter for collecting virus aerosols in terms of preserving higher virus infectivity (Tseng and Li, 2005). The unsuitability of the Nuclepore filter possibly resulted from sampling stress during collection and extraction as well as dehydration during sampling. Their results also illustrated that an impinger and a gelatin filter provide an effective sampling method.

Koller and Rotter (1974) found that gelatin filters had a collection efficiency of >99.95% for particles 0.5–3.0 µm in size. Jaschhof (1992) used a gelatin filter to collect laboratory-generated T1 phage and influenza type A virus particles. In the Jaschhof study, a retention rate of 99.76% was measured for the T1 aerosol, and the workers were able to culture influenza type A virus that had been collected during air monitoring in the room of a patient with influenza type A. Myatt and colleagues (2003) used 2.0 µm pore size PTFE filters with cassette samplers to collect airborne rhinovirus. Burton and colleagues (2007) found PTFE filters to be efficient for collecting submicrometer and nanoscale aerosol particles, including bacteria and viruses. In addition, the 0.3 µm PTFE filter used with the 37 mm three-piece cassette exhibited the highest physical collection efficiency for *B. atrophaeus* and MS2 particles. The other PTFE filters also showed very good physical collection efficiencies across the size range of 10–900 nm with relatively low pressure decrease. PTFE filters exhibited good recovery of aerosolized bacteria when used in button samplers (Burton et al., 2005). Given the results from these studies, filter collection methods can be effectively employed not only for bacteria and spores but also for viruses. However, issues pertaining to viability during collection remain. Furthermore, the BioSampler system was more effective at maintaining viability as compared with filters.

This work also showed that with respect to sampling efficiency, the Andersen single-stage impactor was similar to the glass fiber filter. The collection of the BioSampler system was 22-fold greater than that of the impactor. These results could be skewed; sample overloading is a common problem with the impactor, where more than one particle lands on one

location of the agar plate. Impactor sample times are selected such that only one particle is deposited in one location of the agar plate. However, Tseng and Li (2005) found the impactor to be more effective than a Nucleopore filter. Similar to Benbough's (1971) test results, our study indicated that temperature, RH, and suspending medium influenced the infectivity of airborne viruses. As a result, these factors need to be considered when viral aerosols are generated or sampled. When the BioSampler system was used, temperature had minimal effects. From 20 to 40 °C, there was little change. However, at 50 °C, there was a noticeable decrease in viability. When the gelatin filters were used, the trend varied differently: there was significantly decreased viability as the temperature was increased. Tseng and Li (2005) also noted that temperature affected viability.

Nonlipid viruses are stable at high RH (>65%), whereas lipid viruses are stable at low RH (<35%) (Benbough, 1971). In addition, a virus loses its infectivity in the presence of a NaCl- or peptone-containing medium, whereas phenylalanine protects a virus from aerosol inactivation at various RHs (Dubovi and Akers, 1970; Benbough, 1971; Trouwborst and de Jong, 1973). This study examined the difference between low (30%) and high (65%) RH on viability. For both the BioSampler system and the gelatin filter, there was a 4-fold increase in viability when the RH was high compared to when RH was low. This was expected, as MS2 is a nonlipid virus. Tseng and Li (2005) demonstrated that both the morphology of the virus particles and the presence or absence of a lipid envelope significantly affected the collection efficiency of the four evaluated bioaerosol samplers and also affected the infectivity of the collected virus sample. Their study revealed that the collection efficiencies of these samplers for airborne viruses strongly depended upon virus morphology, hydrophilic nature, and RH. This work similarly showed that humidity and morphology are significantly related to viability.

From the results, it is clear that the type of suspending and sampling media greatly influenced viability. Similarly, Tseng and Li (2005) indicated that collection medium was one of the most significant factors for collected virus samples. This implies that adjusting the composition of the collection medium could minimize the loss of virus infectivity. PBS is a common medium for viral analysis. It is also quite effective for preserving viability. DI water is often used as a collection medium; however, its osmotic properties make it too harsh for MS2. In this work, results from preliminary experiments ruled out the use of DI water. In addition to PBS, other types of media can be used effectively. In this work, tryptone and skim milk were more effective than PBS in preserving viability. Albumin and glycerol actually decreased viability. Although tryptone and skim milk are effective, they produce larger particle sizes. The choice of generation media can also play a significant role in particle size. PBS tends to form smaller particles (0.92 μm), whereas tryptone, albumin, and skim milk formed much larger particles (1.55 μm). Particle sizes increased from 0.92 to 1.55 μm. As another consideration, some media cannot be used with molecular techniques such as PCR or ELISA.

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REFERENCES

Agranovski, I.E.; Agranovski, V.; Reponen, T.; Willeke, K.; Grinshpun, S.A. Development and Evaluation of a New Personal Sampler for Culturable Airborne Microorganisms. *Atmos. Environ.* **2002,** *36*, 889–898.

Aller, J.Y.; Kuznetsova, M.R.; Jahns, C.J.; Kemp, P.F. The Seas Surface Microlayer as a Source of Viral and Bacterial Enrichment in Marine Aerosols. *J. Aerosol. Sci.* **2005,** *36*, 801–812.

Benbough, J.E. Some Factors Affecting the Survival of Airborne Viruses. *J. Gen. Virol.* **1971,** *10*, 209–220.

Bray, M.; Martinez, M.; Kefauver, D.; West, M.; Roy, C. Treatment of Aerosolized Cowpox Virus Infection in Mice with Aerosolized Cidofovir. *Antiviral Res.* **2002,** *54*, 129–142.

Brooks, J.P.; Tanner, B.D.; Gerba, C.P.; Haas, C.N.; Pepper, I.L. Estimation of Bioaerosol Risk of Infection to Residents Adjacent to a Land Applied Biosolids Site Using an Empirically Derived Transport Model. *J. Appl. Microbiol.* **2005,** *98*, 397–405.

Burton, N.C.; Adhikari, A.; Grinshpun, S.A. The Effect of Filter Material on Bioaerosol Collection of *Bacillus subtilis* Spores used as a *Bacillus anthracis* Simulant. *J. Environ. Monit.* **2005,** *7*, 475–480.

Burton, N.C.; Grinshpun, S.A.; Reponen, T. Physical Collection Efficiency of Filter Materials for Bacteria and Viruses. *Ann. Occup. Hyg.* **2007,** *51* (2), 143–151.

Chan, K.H.; Malik Peiris, J.S.; Lam, S.Y.; Poon, L.L. M.; Yuen, K.Y.; Seto, W.H. The Effects of Temperature and Relative Humidity on the Viability of the SARS Coronavirus. *Adv. Virol.* **2011,** 7, 734690; DOI: 10.1155/2011/734690

Cassee, F.R.; Muijser, H.; Duistermaat, E.; Freijer, J.J.; Geerse, K.B.; Marijnissen, J.C.M.; Arts, J.H.E. Particle Size-Dependent Total Mass Deposition in Lungs Determines Inhalation Toxicity of Cadmium Chloride Aerosols in Rats—Application of a Multiple Path Dosimetry Model. *Arch. Toxicol.* **2002,** *76*, 277–286.

Daigle, C.C.; Chalupa, D.C.; Gibb, F.R.; Morrow, P.E.; Oberdorster, G.; Utell, M.J.; Frampton, M.W. Ultrafine Particle Deposition in Humans during Rest and Exercise. *Inhal. Toxicol.* **2003,** *15*, 539–552.

Douwes, J.; Thorne, P.; Pearce, N.; Heederik, D. Bioaerosol Health Effects and Exposure Assessment: Progress and Prospects. *Ann. Occup. Hyg.* **2003,** *47* (3), 187–200.

Dubovi, E.J.; Akers, T.G. Airborne Stability of Tailless Bacterial Viruses S-13 and MS-2. *Appl. Microbiol.* **1970,** *19*, 624–628.

EPA. *Method 1602: Male-Specific (F^+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure*; 821-R-01-029; U.S. Environmental Protection Agency: Washington, DC, 2001.

Esmen, N.A.; Johnson, D.L.; Agron, G.M. The Variability of Delivered Dose of Aerosols with the Same Respirable Concentration but Different Size Distributions. *Ann. Occup. Hyg.* **2002,** 46, 401–407.

Gorny, R.L.; Krysinska-Traczyk, E. Quantitative and Qualitative Structure of Fungal Bioaerosol in Human Dwellings of Katowice Province, Poland. In *Proceedings of Indoor Air 99 Conference,* London, U.K., 1999; Raw, G., Aizlewood, C., Warren, P., Eds.; CRC Ltd: Boca Raton, FL, 1999; vol. 1, pp 873–878.

Grinshpun, S.A.; Willeke, K.; Ulevicius, V.; Juozaitis, A.; Terzieva, S.; Donnelly, J. Effect of Impaction, Bounce and Reaerosolization on the Collection Efficiency of Impingers. *Aerosol Sci. Technol.* **1997,** *26*, 326–342.

Harstad, J.B. Sampling Submicron T1 Bacteriophage Aerosols. *Appl. Microbiol.* **1965,** *13*, 899–908.

Hatch, M.T.; Warren, J.C. Enhanced Recovery of Airborne T3 Coliphage and Pasteurella Pestis Bacteriophage by Means of a Presampling Humidification Technique. *Appl. Microbiol.* **1969,** *17*, 685–689.

Hinds, W.C. *Aerosol Technology Properties, Behaviorism and Measurement of Airborne Particles*, 2nd ed.; John Wiley and Sons: New York, 1999.

Hirst, G.K.; Pons, M.W. Mechanism of Influenza Recombination. II. Virus Aggregation and its Effect on Plaque Formation by So-Called Noninfective Virus. *Virology* **1973,** *56*, 620–631.

Hogan, C.J.; Kettleson, E.M.; Lee, M.-H.; Ramaswami, B.; Angenent, L.T.; Biswas, P. Sampling Methodologies and Dosage Assessment Techniques for Submicrometre and Ultrafine Virus Aerosol Particles. *J. Appl. Microbiol.* **2005,** *99* (6), 1422–1434.

Hull, R.; Hills, G.J.; Plaskitt, A. The In Vivo Behaviour of Twenty-Four Strains of Alfalfa Mosaic Virus. *Virology* **1970,** *42*, 753–772.

Ijaz, M.K.; Karim, Y.G.; Sattar, S.A.; Johnson-Lussenburg, C.M. Development of Methods to Study the Survival of Airborne Viruses. *J. Virol. Methods* **1987,** *18*, 87–106.

Ijaz, M.K.; Sattar, S.A.; Alkarmi, T.; Dar, F.K.; Bhatti, A.R.; Elhag, K.M. Studies on the Survival of Aerosolized Bovine Rotavirus (UK) and a Murine Rotavirus. *Comp. Immunol. Microbiol. Infect. Dis.* **1994,** *17*, 91–98.

Jaschhof, H. Sampling Virus Aerosols Using the Gelatin Membrane Filter—Collection Using a Membrane Filter at a High Sampling Rate. *BioTec* **1992,** 6 (Engl. trans.).

Koller, W.; Rotter, M. Further Investigations on the Suitability of Gelatine Filters for the Collection of Airborne Bacteria. *Zentralbl. Bakteriol Orig. B* (Engl. trans.) **1974,** *159,* 546–559.

Kowalski, W.J.; Bahnfleth, W.P.; Whittam, T.S. Filtration of Airborne Microorganisms: Modeling and Prediction. *ASHRAE Trans. Res.* **1999,** 4–17.

Lin, W.H.; Li, C.S. Collection Efficiency and Culturability of Impingement into a Liquid for Bioaerosols of Fungal Spores and Yeast Cells. *Aerosol Sci. Technol.* **1999,** *30*, 109–118.

Lin, W.H.; Li, C.S. The Effect of Sampling Time and Flow Rates on the Bioefficiency of Three Fungal Spore Sampling Methods. *Aerosol Sci. Technol.* **1998,** *28*, 511–522.

Lin, X.J.; Reponen, T.A.; Willeke, K.; Grinshpun, S.A.; Foarde, K.K.; Ensor, D.S. Long-Term Sampling of Airborne Bacteria and Fungi into a Non-Evaporating Liquid. *Atmos. Environ.* **1999,** *33*, 4291–4298.

Lin, X.J.; Reponen, T.; Willeke, K.; Wang, Z.; Grinshpun, S.A.; Trunov, M. Survival of Airborne Microorganisms during Swirling Aerosol Collection. *Aerosol Sci. Tech.* **2000,** *32*, 184–196.

Lin, X.J.; Willeke, K.; Ulevicius, V.; Grinshpun, S.A. Effect of Sampling Time on the Collection Efficiency of All-Glass Impingers. *Am. Ind. Hyg. Assoc. J.* **1997,** 58, 480–488.

Macher, J.M.; Willeke, K. Performance Criteria for Bioaerosol Samplers. *J. Aerosol Sci.* **1992,** *23,* (Suppl. 1), S647–S650.

Madigan, M.T.; Martinko, J.M.; Parker, J. *Biology of Microorganisms*; Prentice Hall: Upper Saddle River, NJ, 1997.

Mainelis, G.; Willeke, K.; Adhikari, A.; Reponen, T.; Grinshpun, S.A. Design and Collection Efficiency of a New Electrostatic Precipitator for Bioaerosol Collection. *Aerosol Sci. Tech*. **2002,** *36*, 1073–1085.

Mandell, G.L.; Bennett, J.E.; Dolin, R.D. *Principles and Practices of Infectious Diseases,* 6th ed.; Churchhill Livingstone: New York, 2005.

Morris, D.H.; Yinda, K.C.; Gamble, A.; Rossine, F.W.; Huang, Q.; Bushmaker, T.; Fischer, R.J.; Matson, M.J.; Doremalen, N.V.; Vikesland, P.J.; Marr, L.C.; Munster, V.J.; Lloyd-Smith, J.O. Mechanistic Theory Predicts the Effects of Temperature and Humidity on Inactivation of SARS-CoV-2 and Other Enveloped Viruses. *eLife* **2021,** *10,* e65902; DOI: doi.org/10.7554/eLife.65902

Morawska, L. Droplet Fate in Indoor Environments, or Can We Prevent the Spread of Infection? *Indoor Air* **2006,** *16* (5), 335–347.

Myatt, T.A.; Johnston, S.L.; Rudnick, S. Airborne Rhinovirus Detection and Effect of Ultraviolet Irradiation on Detection by a Semi-Nested RT–PCR Assay. *BMC Public Health* **2003**, *3*, article 5.

Nevalainen, A.; Willeke, K.; Liebhaber, F.; Pastuszka, J.; Burge, H.; Henningson, E. Bioaerosol Sampling. In *Aerosol Measurement Principles, Techniques, and Applications*; Willeke, K., Baron, P.A., Eds.; Van Nostrand Reinhold, Oxford University Press: New York, 1993, 471–492.

Oberdörster, G.; Oberdörster, E.; Oberdörster, J. Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. *Environ. Health Perspect.* **2005,** *113*, 823–839.

Otten, J.A.; Burge, H.A. Viruses. In *Bioaerosols Assessment and Controls*; Macher, J., Ed.; American Conference of Governmental Industrial Hygienists: Cincinnati, OH, 1999; vol. 21, pp 1–6.

Reponen, T.; Willeke, K.; Grinshpun, S.A. Biological Particle Sampling. In *Aerosol Measurement, Principles, Techniques, and Applications*; Baron, P.A., Willeke, K., Eds.;Wiley-Interscience: New York, 2001, pp 751–779.

Sattar, S.A.; Ijaz, M.K. Airborne Viruses. In *The Manual of Environmental Microbiology*, 2nd ed.; Hurst, C.J., Crawford, R.L., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D., Eds.; ASM Press: Washington, DC, 2002; pp 871–883.

Terzieva, S.; Donnelly, J.; Ulevicius, V.; Grinshpun, S.A.; Willeke, K.; Stelma, G.N.; Brenner, K.P. Comparison of Methods for Detection and Enumeration of Airborne Microorganisms Collected by Liquid Impingement. *Appl. Environ. Microbiol.* **1996,** *62*, 2264–2272.

Trouwborst, T.; de Jong, J.C. Interaction of Some Factors in the Mechanism of Inactivation of Bacteriophage MS2 in Aerosols. *Appl. Microbiol.* **1973,** *26*, 252–257.

Trouwborst, T.; de Jong, J.C.; Winkler, K.C. Mechanism of Inactivation in Aerosols of Bacteriophage T1. *J. Gen. Virol.* **1972,** *15*, 235–242.

Trouwborst, T.; Kuyper, S. Inactivation of Bacteriophage T3 in Aerosols: Effect of Prehumidification on Survival after Spraying from Solutions of Salt, Peptone, and Saliva. *Appl. Microbiol.* **1974,** *27*, 834–837.

Trouwborst, T.; Kuyper, S.; Teppema, J.S. Structural Damage of Bacteriophage T1 by Surface Inactivation. *J. Gen. Virol.* **1974,** *25*, 75–81.

Tseng, C.-C.; Li, C.-S. Collection Efficiencies of Aerosol Samplers for Virus-Containing Aerosols. *J. Aerosol Sci.* **2005**, *36*, 593–607.

Ulevicius, V.; Willeke, K.; Grinshpun, S.A.; Donnelly, J.; Lin, X.; Mainelis, G. Aerosolization of Particles from a Bubbling Liquid: Characteristics and Generator Development. *Aerosol Sci. Tech*. **1997,** *26*, 175–190.

Utrup, L.J.; Frey A.H. Fate of Bioterrorism-Relevant Viruses and Bacteria, Including Spores, Aerosolized into an Indoor Air Environment. *Exp. Biol. Med.* **2004,** *229* (4), 345–350.

Willeke, K.; Lin, X.J.; Grinshpun, S.A. Improved Aerosol Collection by Combined Impaction and Centrifugal Motion. *Aerosol Sci. Tech.* **1998,** 28, 439–456.

Yang, W.; Marr, L.C. Mechanisms by Which Ambient Humidity May Affect Viruses in Aerosols. *Appl. Environ. Microbiol*. **2012,** *78* (19), 6781–6788.

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ACRONYMS AND ABBREVIATIONS

ANOVA	analysis of variance
BSL	biosafety level
DI	deionized
ELISA	enzyme-linked immunoassay
ELPI	electrical low-pressure impactor
GM	geometric mean
GSD	geometric standard deviation
HEPA	high-efficiency particulate air
PBS	phosphate-buffered saline
PCM	polycarbonate membrane filter
PCR	polymerase chain reaction
PCTE	polycarbonate track etch
PFU	plaque-forming unit
PTFE	polytetrafluoroethylene
RH	relative humidity
RSV	respiratory syncytial virus
SARS	severe acute respiratory syndrome
TSA	tryptic soy agar
TSB	tryptic soy broth
UV-APS	ultraviolet aerodynamic particle sizer

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