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REVIEW ARTICLE

Collection, particle sizing and detection of airborne viruses

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Abstract

Viruses that affect humans, animals and plants are often dispersed and transmitted through airborne routes of infection. Due to current technological deficiencies, accurate determination of the presence of airborne viruses is challenging. This shortcoming limits our ability to evaluate the actual threat arising from inhalation or other relevant contact with aerosolized viruses. To improve our understanding of the mechanisms of airborne transmission of viruses, air sampling technologies that can detect the presence of aerosolized viruses, effectively collect them and maintain their viability, and determine their distribution in aerosol particles, are needed. The latest developments in sampling and detection methodologies for airborne viruses, their limitations, factors that can affect their performance and current research needs, are discussed in this review. Much more work is needed on the establishment of standard air sampling methods and their performance requirements. Sampling devices that can collect a wide size range of virus-containing aerosols and maintain the viability of the collected viruses are needed. Ideally, the devices would be portable and technology-enabled for on-the-spot detection and rapid identification of the viruses. Broad understanding of the airborne transmission of viruses is of seminal importance for the establishment of better infection control strategies.

Introduction

Airborne particles of biological origin including bacteria, fungi and viruses, are commonly present in the air we breathe. Any respiratory pathogens able to remain viable (infectious) after aerosolization and air transport are a potential cause of respiratory disease, and they are often associated with other substances to form 'complex particles' (Tang 2009). An example of a complex particle would be an influenza virion within a droplet composed of mucus, salts and water. Virus-containing aerosols can be formed through natural occurrences, for example, sneezing by an individual harbouring a respiratory virus infection, or through mechanical means, for example, when air currents around contaminated surfaces disperse the viruses into the air (Verreault *et al.* 2008).

The dimensions of aerosolized virus particles vary widely, ranging from nanometre (e.g. 'naked' virus particles) to micrometre (e.g. viruses associated with non-viable particles) (Gerone *et al.* 1966). Once airborne, small particles containing virus(es) can remain airborne for long periods of time, allowing for their transport to other locations (Fig. 1). They also remain adrift in air (i.e. airborne) for longer periods of time primarily because of their low settling velocity, for example, from $3{\cdot}1 \times 10^{-3}$ m s^{-1} for 10-μm particles to $3{\cdot}5 \times 10^{-5}$ m s^{-1} for 1 μm particles (Hinds 1998). Among nose breathers, larger particles (>5 μm) tend to deposit on the surfaces of the upper respiratory tract, whereas inhalation of small particles into the lower lungs may pose a greater risk for pneumonia/severe infection than what occurs with deposition onto the upper respiratory tract (Vincent 2005; Killingley and Nguyen-Van-Tam 2013). Overall, smaller particles that contain respiratory viruses are potentially more dangerous because they stay airborne longer (and thus the risk for acquiring an infection is

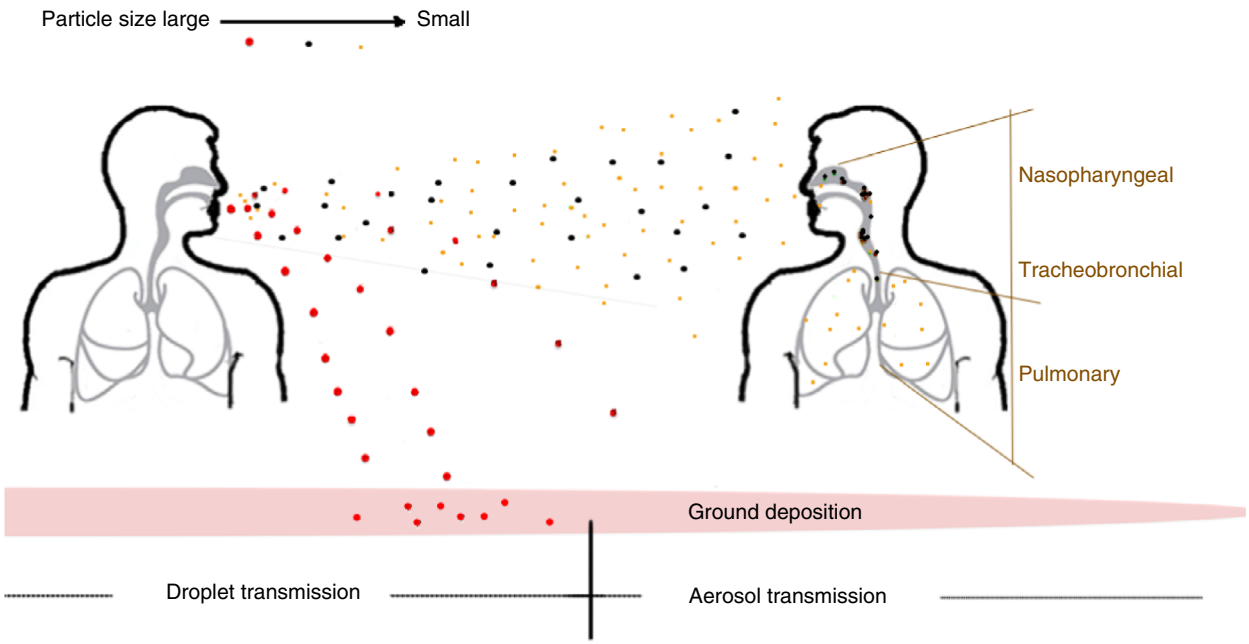

Figure 1 Comparison between droplet transmission (●) and aerosol transmission (•). Large droplets settle close to the source, while smaller aerosol particles stay aloft and can drift long distances. Once inhaled, very small particles can reach deeper to the pulmonary region while larger particles are captured in the nasopharyngeal region in the upper respiratory system. [Colour figure can be viewed at wileyonlinelibrary.com]

prolonged), and they get inhaled into the lower lungs, potentially causing diseases with more severe outcomes.

Adequate understanding about the routes of airborne virus transmission is important for protecting public health, especially as infection control procedures relevant for the spread of respiratory pathogens are founded on this body of knowledge. Based on current dogma, for example, the World Health Organization recommends that when possible, a patient with respiratory illness should be kept at least 1 m away from others to reduce spread of epidemic or pandemic-prone respiratory diseases (WHO, 2014). Transmission of airborne viruses from one subject to another occurs mainly by three routes or a combination thereof: (i) direct or indirect contact of infectious secretions from infected hosts with mucus membranes of susceptible hosts, (ii) contact of virus-containing droplets with surfaces of the upper respiratory tract, and (iii) inhalation of small aerosolized virus-containing particles or droplet nuclei (droplet and aerosol transmissions are compared in Fig. 1). The relative importance of different transmission modes varies from one virus to another, with droplet transmission being traditionally regarded as the main route for respiratory viruses (Gerone *et al.* 1966; Gralton *et al.* 2011). However, due to inherent limitations of conventional impingement/impaction-based bioaerosol samplers, which have low collection efficiencies for nanosized particles containing infectious viruses (Hogan *et al.* 2005; Cao *et al.* 2011), the aerosol transmission mode has not been adequately investigated. Nevertheless, evidence is mounting that aerosols are important for the transmission of airborne viruses (Cowling *et al.* 2013). For example, *Varicella Zoster virus* (VZV) has been proven to be transmitted by the aerosol mode (Gustafson *et al.* 1982; Gardam and Lemieux 2007); VZV DNA was detected in rooms of patients without varicella in a hospital with VZV-infected patients (Sawyer *et al.* 1994), and directional airflow was consistent with VZV transmission (Gustafson *et al.* 1982). The importance of the aerosol transmission mode is still debated for influenza viruses. Although droplet infection is the commonly described mode of transmission for influenza viruses, they have also been detected in aerosols 'far away' (>1 m) from infected patients in a few studies. For example, influenza A H1N1 and H3N2, and B viruses, were collected by a water-based sampler located >2 m from patients in a student infirmary (Pan *et al.* 2017), and airborne influenza A H3N2 was collected by a Sioutas impactor 3·7 m away from sick individuals (Lednicky and Loeb 2013).

Outbreaks due to respiratory viruses spread through aerosol routes can result in pandemics. A recent estimate indicates that up to 646 000 persons in the world die every year of influenza (Iuliano *et al.* 2017). As the world population grows, modern agricultural production systems have greatly expanded, and these large-scale operations provide enzootic opportunities for the spread of

novel pathogens through aerosol routes (Jones *et al.* 2008; Sarkar *et al.* 2012). Increasing threats of bioterrorist attacks performed through aerosol dissemination of dangerous pathogens have also increased the need to develop methods for rapid detection and identification of airborne microbes (Mirski *et al.* 2014). Prompt and accurate detection of airborne pathogens and their identification are key to mitigating these biothreats that may have pandemic potential; they enable minimal exposure of personnel, minimal contamination of surfaces and when possible, allow for the initiation of early treatment, effective decontamination and infection control procedures, and for the selection of protective countermeasures. Methods to better and more reliably measure the concentration, size of particles carrying these viruses and transmission modes of aerosolized virus particles, in relation to their potential risks to humans and animals, are also needed to understand the principles that govern airborne virus transmission from host to host. This knowledge is of foremost importance for infection control. This review presents (i) a review of major limitations of existing sampling technologies for airborne respiratory viruses, (ii) the latest developments regarding samplers for airborne respiratory viruses, (iii) factors affecting their performance, and (iv) virus detection methodologies used in association with air samplers.

Samplers for airborne viruses

The performance of virus aerosol samplers is evaluated by their sampling efficiency. True sampling efficiency is determined in two ways: (i) physical efficiency, which is the ratio of the amount of the collected particles to the amount of particles in the ambient environment, and (ii) biological efficiency, which is a measure of the fraction of biologically active virus that remains viable after collection (Hogan *et al.* 2005; Kulkarni *et al.* 2011). The physical efficiency is usually determined by measuring the particle number concentrations at the sampler's inlet and the exit, with the inlet loss and wall losses ignored (Lin *et al.* 2000). As an aid in data interpretation, tracers have been used for estimating the efficiency through comparison of the collected tracer mass concentration with the total tracer mass concentration at the inlet (Orsini *et al.* 2008). Disadvantages of these tracers are the deactivation of viruses due to the tracer, the interference of tracers in further analyses of the samples (e.g. overlapping fluorescence wavelength) and the difficulty in homogeneously attaching tracers to particles. The biological efficiency is usually determined by comparing the infectious virus count measured by a viability assay, for example, plaque assay (infectious virus titre defined in terms of plaque-forming units or PFU per ml) or median tissue culture infectious dose ($TCID_{50}$) per ml for the collected material, with the total generated infectious virus count calculated from the liquid consumption rate in the aerosol generator (Hogan *et al.* 2005). Alternatively, the virus DNA or RNA genome-equivalent measured by polymerase chain reaction (PCR) is used. Tracers have also been used to evaluate the biological efficiency by comparing the ratios of the virus count to tracers' fluorescence intensity in both the particles entering the sampler and those collected by the sampler (Appert *et al.* 2012; Zuo *et al.* 2013).

The same principles for sampling bacterial and fungal aerosols are typically used for aerosolized viruses (Lindsley *et al.* 2017). These samplers separate the particles from the airstream utilizing various physical mechanisms (Henningson and Ahlberg 1994). The movement of an airborne particle is described in terms of its aerodynamic diameter, which is the diameter of a sphere with unit density having the same settling velocity as the particle (Hinds 1998). Particles with larger aerodynamic diameters have higher inertia (i.e. tendency to maintain their current state) and can be easily separated from the airstream through impaction. Particles with a smaller diameter (<100 nm) can be collected using their higher diffusivity (a measure of the rate at which particles spread), or size-enlarged through condensation to enable impaction. Various aerosol samplers based on these principles have been used to recover airborne viruses (Fig. 2) (Henningson and Ahlberg 1994; Pillai and Ricke 2002; Verreault *et al.* 2008; Xu *et al.* 2011). Studies using these samplers specifically for collection of airborne viruses are discussed in the following sections.

Impactors and cyclones

Impactors like the slit sampler and the Andersen 6-stage sampler, and cyclones, have been used for sampling airborne viruses. They are active samplers that require a vacuum pump to draw in the aerosol, and particles in the incoming airstream get accelerated through small nozzles (in the form of holes or slits). As they are pulled through these devices, particles with high inertia impact onto the surface of collection media (Fig. 2) (Andersen 1958). Then, the collection media are recovered and aliquots thereof used for virus isolation or other analyses (Verreault *et al.* 2008). For example, a high-resolution slit sampler with liquid collection medium was used for the collection of airborne *Severe Acute Respiratory Syndrome* coronavirus (Booth *et al.* 2005); in that work, all virus cultures were negative, although 2 of the 10 samples were RT-PCR (Reverse Transcription-Polymerase Chain Reaction) positive. Reasons for the negative results might be that viruses were present in the air at relatively low levels

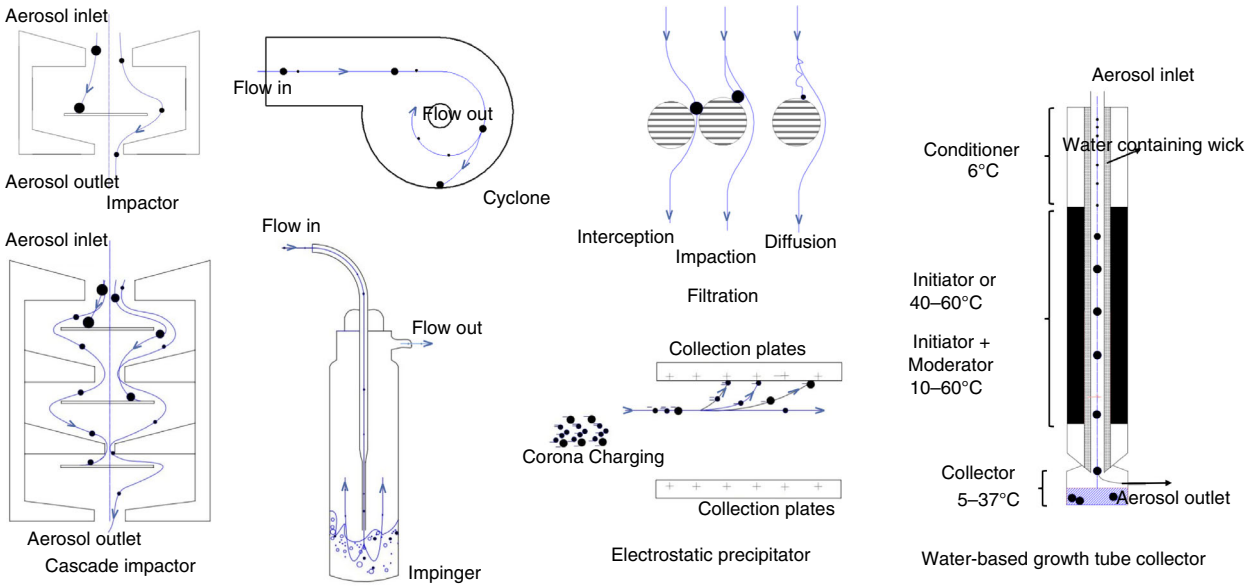

Figure 2 Conceptual schematic diagrams of various air samplers for airborne viruses and their collection mechanisms. Solid circles (•) are aerosol particles and the size of the circle indicates the size of the particle (not in scale). Shown are an impactor, cyclone, filter, impinger, electrostatic precipitator and a water-based growth tube collector. Impactor: particles in the incoming airstream accelerate through small nozzles (in the form of holes or slits), and those with high inertia impact onto the surface of collection media. Cascade Impactor: by successively decreasing nozzle size, particles are classified by their inertia to be collected onto different size stages. Cyclone: centrifugal forces deviate particles from the air flow to impact onto the collection wall. Impinger: Abrupt change in the airstream direction inside the bottle impacts particles into the liquid collection medium. Filtration: Particles are collected onto filter media through interception, inertial impaction, and diffusion. Electrostatic precipitator: Particles are first charged through corona discharge to create electrostatic attraction that draws the charged particles to collection plates (oppositely charged). Water-based growth tube collector: Cold aerosol particles are introduced into a warm growth tube saturated with water vapour. This process encapsulates small particles into larger droplets, thus enabling efficient collection of these enlarged particles through gentle impaction. [Colour figure can be viewed at wileyonlinelibrary.com]

or that these devices were inadequate for sampling aerosolized viruses. Practical limitations for the design of common impactors limit the smallest cut-off size (particle diameter with 50% collection efficiency) to 0·2–0·3 μm. For example, to collect 30-nm particles, a nozzle size of 63 μm (which is extremely challenging to manufacture) running at sonic velocity (which is damaging to viruses) is required.

Cyclones exert centrifugal forces on particles so that they deviate from the air flow and impact onto the collection wall (Fig. 2). They are not designed as high-efficiency (>95%) samplers for size ranges from 10 nm to more than 10 μm; collection efficiency is 30–90% for PM_{10} (particulate matter $\leq$10 μm) and 0–40% for $PM_{2.5}$ (particulate matter $\leq$2·5 μm) for a conventional single-stage cyclone (Cooper and Alley 2010). However, free virus particles can be smaller than 100 nm, and thus would not be collected efficiently. Modification of conventional cyclone samplers has somewhat improved their performance (Kenny *et al.* 2017). One sampler developed by the National Institute for Occupational Safety and Health (NIOSH) (Lindsley *et al.* 2006) is a multistage cyclone operating at a flowrate of 3·5 l min^{-1}: the first stage is a 15-ml tube that collects aerosol particles >4 μm, the second is a 1·5 ml tube that collects particles between 1 and 4 μm, and the third is a polytetrafluoroethylene (PTFE) filter that captures those <1 μm. Using this sampler to collect laboratory-generated influenza A H1N1 virus aerosols inside a settling chamber, Cao *et al.* (2011) found that its collection efficiency for total quantity of collected viruses as measured by RT-PCR was the same as that attained by sampling with an SKC BioSampler (considered the industry standard) for 15 min. With the BioSampler as a reference, however, only 34% of the viruses collected by the NIOSH cyclone remained infectious, probably due to desiccation. Blachere *et al.* (2009) used the NIOSH cyclone to collect airborne influenza virus in a hospital emergency department. They found that influenza A virus (IAV) RNA was detected by RT-PCR in 11 out of 81 samples, and more than half of the aerosol particles that contained the virus were <4 μm, within the respirable particle size range (inhaled particles capable of passing beyond the ciliated airways) (Brown *et al.* 2013).

Liquid cyclonic collectors, wherein liquid medium dislodges and then collects particles trapped against the

cyclone's wall, have also been used for virus aerosol sampling (Corzo *et al.* 2014; Alonso *et al.* 2015). In one study, a liquid cyclonic collector and an Andersen cascade impactor (ACI) were used to collect aerosolized IAV, Porcine reproductive and respiratory syndrome virus and Porcine epidemic diarrhoea virus particles generated by infected pigs (Alonso *et al.* 2015). Aliquots of collection media from both devices were used for RT-PCR and virus isolation in cell cultures. All three viruses were found in that study, and there was no difference in geometric mean particle concentration in samples collected by either the cyclone or the ACI. Despite advancements in cyclone design, cut-off sizes for these devices (mostly >1 μm) cannot meet the sampling requirements for small-sized virus-containing particles. Moreover, physical damage resulting from the actions of cyclones can deactivate viruses, resulting in an underestimate of the infectious viruses collected (Bourgueil *et al.* 1992).

A capability to collect particles containing viruses in different particle size ranges is the major advantage of using the ACI. For particles >1 μm, the ACI or similar devices are the main samplers for size-selective sampling (Xu and Yao 2013; Alonso *et al.* 2015). However, as for slit samplers and cyclones, the ACI relies on after-filters (placed downstream of the impactor) for collecting fine particles. Additionally, its wall loss (particles deposited on the internal wall surface, rather than the designated collection medium) is considerable.

Liquid impinger

Liquid-based impingers are the most commonly used samplers for collecting aerosolized viruses, as the liquid collection medium helps maintain the viability of the virus and often can be used directly with analytical methods such as plaque assay without the need to extract the virus from a surface or filter (Xu *et al.* 2011). However, certain collection media used for bacteria and fungi are inappropriate for viruses. For example, mineral oil, which is non-nutritive and thus does not support the replication of micro-organisms but nevertheless maintains them live (which is important for accurate enumeration of the collected agents), cannot be used for viruses. When used for bacteria and fungi, the mineral oil-collected micro-organism mixture is filtered through a 0·45-μm filter and the micro-organisms retained on the filter are recovered by washing them off into a vessel. For viruses, filtering mineral oil through the smaller pore-size filters needed for viruses (such as 0·22-μm filters) requires excessive pressure that would be damaging to viruses.

Frequently used liquid impingers include all-glass impingers (AGIs) and the BioSampler. AGIs work by forcing the airstream to change direction abruptly inside a bottle. During this sampling process, formation of air bubbles in the liquid collection medium inside the bottle on one hand increases the collection efficiency for small particles through diffusion, while on the other hand causes reaerosolization of collected viruses (Grinshpun *et al.* 1997; Riemenschneider *et al.* 2010). The BioSampler improves on the AGI-30 by (i) minimizing the effects of particle bounce and reaerosolization and (ii) extending its sampling time period while conserving viability of the collected micro-organisms (Willeke *et al.* 1998). It works by depositing particles into the collection media in a swirling motion through three 0·630 mm tangential nozzles at sonic velocity, resulting in a cutoff size of around 300 nm (Lin *et al.* 2000). The BioSampler was used by Anderson *et al.* (2016) to study IAV during summer and fall/winter seasons in five swine farms. They found that shedding of IAV in pigs occurred in both seasons, but the detection of these aerosolized viruses was dependent on factors like climatic conditions or husbandry practices. The BioSampler was also used for the collection of IAV inhaled by a human manikin target (Tang *et al.* 2014) and IAV in live poultry markets (Kang *et al.* 2016). No influenza virus RNA was detected in the former study, possibly due to low virus concentration at distance from the virus source or the relatively short duration of manikin exposure, whereas avian influenza virus nucleic acid was found in aerosol samples in the latter study.

The AGI-4, the AGI-30 (the number refers to the distance from the tip of the orifice to the bottom of the flask in millimetres) and the BioSampler have been used as reference samplers (Henningson and Ahlberg 1994). Despite the advantage that their liquid collection medium can be directly used in molecular analytical technologies like PCR or Enzyme-Linked ImmunoSorbent Assay (ELISA), their use as reference needs to be further evaluated. First, their 0·9-cm inlets are designed to mimic the human upper respiratory system at removing large particles from the airstream (Henningson and Ahlberg 1994; Grinshpun *et al.* 1997). The inlet efficiency, defined as the fraction of particles entering the sampler to that in the ambient air, is above 98% for 1-μm particles, but is substantially reduced to around 80% for 5 μm based on tests at the manufacturer's recommended flow rate of 12·5 l min^{-1} (Grinshpun *et al.* 1994; Seshadri *et al.* 2009). The 20% observed loss for 5-μm particles not getting into the sampler warrants caution in its use for sampling larger virus-containing particles. Second, Han and Mainelis (2012) found that the adhesion of the deposited particles (fluorescent Polystyrene Latex (PSL)) and *Bacillus subtilis* cells and *Cladosporium cladosporioides* spores to the inner wall of the BioSampler was as high as 30% and the reaerosolization rates ranged from 0·2% to 6·9%. For the AGI-30, Riemenschneider *et al.* (2010) found that

reaerosolization of MS2 bacteriophage (single virion particle size ~28 nm) increases as the flow rate increases. Moreover, Hogan *et al.* (2005) found the collection efficiencies of the AGI-30, the BioSampler and frit bubblers for bacteriophages MS2 and T3 (single virion particle size ~45 nm) are below 10% in the size range of 20–100 nm. The 50% cut-off sizes of both the BioSampler and the AGI-30 are 300 nm at 12·5 l min^{-1}. Anwar (2010) found that 8 l min^{-1} for the BioSampler was more effective for the sampling of MS2 aerosols due to collection by diffusion and maintenance of virus infectivity. While collection by diffusion is enhanced at a low flow rate, reduction in flow rate results in lower physical collection efficiency that relies on inertia. Hence, caution should be exercised in further reduction in the flow rate, and research to determine the optimal flow rate for sampling airborne virus is warranted. Finally, the BioSampler's high centrifugal force also inactivates a significant fraction of influenza viruses (Fabian *et al.* 2009; Lednicky *et al.* 2016). Hence, new samplers for airborne viruses that allow for less violent but efficient sampling in the wide size range of virus-containing particles are in great need.

These impingers have also been modified in attempts to increase their collection efficiencies for particles containing infectious agents. A modified piston-style mechanical spirometer, which is used as an accumulation chamber for exhaled or coughed aerosols, combined with the BioSampler, was used for collecting influenza viruses during coughs and exhalation (Lindsley *et al.* 2016). Temperature-controlled or relative humidity (RH)-controlled AGI-30 and filters were used for sampling aerosols containing bacteria (Springorum *et al.* 2011; Walls *et al.* 2017). Relatively better collection efficiencies were achieved by these modified samplers compared with traditional ones, especially under extreme conditions (dry or cold conditions), as extreme sampling environments can lead to the inactivation of the micro-organisms. Some of these modified samplers have not been tested for the collection of airborne virus, but modification of standard bioaerosol samplers for virus collection has been actively pursued (see sec 'Water-based condensation').

Filters

Given that particle sizes of virus-containing aerosols range widely and impactors or impingers are less efficient for particles <500 nm, filters are widely used for sampling airborne viruses. The collection mechanisms of filters include interception, inertial impaction, diffusion and electrostatic attraction (Fig. 2) (Hinds 1998). PTFE (Myatt *et al.* 2004; Jonges *et al.* 2015) and cellulose filters (Sawyer *et al.* 1994) have been used for sampling virus-containing aerosol. Airborne rhinovirus RNA, low pathogenicity avian influenza virus RNA and VZV DNA were detected in these studies. However, filtration processes are likely to dehydrate viruses during sampling (Verreault *et al.* 2008); as well, the extraction of the collected viruses off the filters after sampling results in inactivation of a significant fraction of the collected viruses (Tseng and Li 2005). Fabian *et al.* (2009) reported that Teflon and gelatin filters recovered only 22 and 10%, respectively, of infectious influenza viruses compared with the BioSampler. Li *et al.* (2017) evaluated the performance of a 5 ml BioSampler, gelatin filter and glass fibre filter for the collection of influenza H1N1 virus, and found that deactivation of most of the trapped viruses was a result of extraction of virus off the filters. Similarly, relative extraction efficiencies attained using alumina nanofibre *vs* glass fibre filters were compared for MS2 phage with the BioSampler as a reference sampler by Li *et al.* (2009); the extraction efficiency of the nanofibre filter was less than 10%, while that for the glass fibre filter varied from 32·3% to 162%.

Among the commonly used filters, the gelatin filter is unique in that it can be dissolved into liquid for molecular or virus enumeration in cell cultures without significantly affecting the viability of many viruses. Zhao *et al.* (2014) evaluated four samplers (six-stage ACI, AGI-30, OMNI-3000 and MD8 with gelatin filter) for the collection of aerosolized infectious bursal disease virus (IBDV), and found that gelatin filters had a 100% physical collection efficiency without significant dehydration effects, probably due to the 'stress resistance' of IBDV. The collection efficiencies of gelatin filters for viable hydrophilic viruses were found to be 10 times better than for Nucleopore polycarbonate filters, since many hydrophilic viruses need to be hydrated to remain viable (Tseng and Li 2005). Nevertheless, sampling and extraction problems still prevail with gelatin filters. Fabian *et al.* (2009) retrieved 23% of the total viable IAV using a gelatin filter, whereas 100% could be retrieved from the BioSampler during bench top virus spike recovery experiments. A simple system composed of a portable MD8 air sampler connected by a hose to a gelatin filter was used for the capture of influenza viruses in cough aerosols, although virus deactivation was reported to be a problem (Hatagishi *et al.* 2014). Sampling conditions are very important for successful virus collection on gelatin filters; low RH can lead to desiccation of viruses and high RH leads to dissolution of gelatin filters (Verreault *et al.* 2008). These filters must be used in short periods of time (<15 min sampling time) as they dry out quickly, and at higher temperatures they melt (Fabian *et al.* 2009). Thus, filter-collected viruses are typically more suitable for molecular analyses than for assessments of infectivity, as

desiccation, extraction and postsampling processes significantly inactivate a significant fraction of the infectious viruses (Li *et al.* 1999; Tseng and Li 2005; Burton *et al.* 2005).

Electrostatic precipitators

Another type of sampler is the electrostatic precipitator (ESP), wherein electrostatic attraction is used to collect a wide size range of airborne particles (Jang *et al.* 2008; Kettleson *et al.* 2009; Dybwad *et al.* 2014). The ESP works by creating a corona discharge that places charges on airborne particles, resulting in an electrostatic attraction that draws the charged particles to electrodes (Fig. 2; Hinds 1998). The ESP has a size-dependent collection efficiency; total mass-based collection efficiencies are high (e.g. 99%), but typically low for submicrometre or nanometre particles (Yoo *et al.* 1997; Kettleson *et al.* 2009). Jang *et al.* (2007) developed a flow-swirling–based ESP without a corona discharger, and this device successfully collected *Vaccinia* viruses, which are brick shaped with dimensions around $200 \times 200 \times 250$ nm^3 (Jang *et al.* 2008). Problems with this device are its inability to collect larger particles (>10 μm), which are less likely to swirl as a result of increased inertia compared to viruses in nanometre ranges. Also, fewer *Vaccinia* viruses were collected compared with nanoparticles of similar size as the viruses could aggregate to form large particles rather than remaining as individual particles. Hong *et al.* (2016) applied a personal ESP for sampling submicrometre-sized MS2 and T3 viruses, and found out that the recovery rate for MS2 and T3 were more than 10 times higher than the BioSampler at 12·5 l min^{-1}; its collection efficiency at the flow rate of 1·2 l min^{-1} reached 99·3–99·8% for 0·05–2-μm diameter PSL particles, although the efficiency for virus-containing particles is not available. Compared with inertia-based samplers, the ESP consumes less energy and it is easier to make it portable. However, ozone formation in the ESP limits its use for sampling infectious viruses, as ozone is intrinsically a virus-inactivating agent (Wells *et al.* 1991).

Water-based condensation

Condensation particle counters (CPCs) have been used since 1888 to measure the number concentration of aerosols, when Aitken (1889) first amplified dust particles through water vapour condensation using adiabatic expansion. After 2000, systems based on water vapour condensation have been developed for collection of airborne viruses. Oh *et al.* (2010) designed two bioaerosol amplification units (BAU); their tests with MS2 showed that the mixing type BAU (mBAU) performed better than the cooling type BAU and the number of viable MS2 collected by mBAU increased two to three fold after amplification compared to that without amplification. Orsini *et al.* (2008) combined a condensation growth chamber with a cyclone to collect a rod-shaped plant virus (*Tobamovirus*) and a protein-enveloped insect virus (*Baculovirus*). Using an adiabatic chamber, Yu *et al.* (2018) increased MS2-containing particles to >1 μm by controlling compression pressure and temperature. The exhaled breath condensate (EBC) is another condensation-based device used for sampling influenza A H3N2 virus (Horvath *et al.* 2005; Xu *et al.* 2012). In this device, exhaled breath condenses into tiny liquid droplets on a hydrophobic collection surface (parafilm) due to the low temperature caused by ice and the hydrophobic nature of the surface. As the EBC was specifically designed for sampling exhaled breath, the efficiency for sampling airborne infectious viruses is unknown (Horváth *et al.* 2017). Above all, collection efficiencies of viable viruses by the above-mentioned water-based samplers still have much space for improvement due to particle wall loss or less effective amplification (e.g. unable to create supersaturation to enable condensation).

Although laminar-flow CPCs were developed long ago, they were not practical for studying airborne viruses because they require a slowly diffusing fluid such as butanol as the condensing material and butanol deactivates viruses (Stolzenburg and McMurry 1991). More recently, water-based condensational technologies have undergone changes to overcome the limitations of butanol based CPCs. This includes introduction of cooled aerosols into warm wet-walled growth tubes to minimize wall losses (Oh *et al.* 2010), and maintaining the condensation system at a lower temperature to sustain the viability of viruses being sampled (Orsini *et al.* 2003). An emerging virus aerosol sampler, the water-based laminar-flow condensational growth tube collector (GTC), has been tested for collecting laboratory-generated bacteriophage MS2 and IAV aerosols, and for airborne viruses in a student infirmary. For MS2-containing particles, the collection efficiency of the GTC for infectious MS2 was more than 10 times higher than that of the BioSampler (Pan *et al.* 2016). For laboratory-generated infectious IAV, the GTC's collection efficiency was seven times higher than that of the BioSampler (Lednicky *et al.* 2016). For real-world sampling, the GTC collected more types of airborne viruses and higher quantities per sampling run than the BioSampler (Pan *et al.* 2017). These results indicate the GTC can be used for surveillance of airborne viruses. The GTC mimics what happens in human lungs on a cold day by introducing cold aerosol particles into a warm growth tube saturated with water vapour. This process encapsulates small particles into larger droplets, thus

enabling efficient collection of these enlarged particles through gentle impaction (Fig. 2) (Hering *et al.* 2005; Hering and Stolzenburg 2005). Physical collection efficiencies of this GTC are above 90% for particles as small as 30 nm and as large as 10 μm (Lednicky *et al.* 2016). Current limitations of the GTC samplers are that they are bulky (considerable size and weight) and they require special skills to operate or maintain.

Other devices

Other samplers have also been developed for sampling airborne viruses. Some combine different sampling mechanisms by using inertia-based methods for large particles, and diffusion based methods like filtration, or water condensation, for small particles. Some devices can distinguish between coarse and fine particles, and they usually have higher collection efficiencies in a wider particle size range than attainable with the older devices. As an example, McDevitt *et al.* (2013) built a sampler (G-II) that operates at a high flow rate of 130 l min^{-1} to collect exhaled breath particles, and the collected viruses can be used in infectivity analyses. The G-II incorporates three parts: (i) an impaction substrate to collect particles >5 μm, (ii) a Condensation Growth Unit and (iii) a slit impactor to collect particles >1 μm. Test results obtained using spherical PSL particles revealed more than 85% collection efficiency for particles larger than 50 nm, although collection for influenza virus was equitable with that obtained using a BioSampler. Agranovski *et al.* (2002) designed a personal sampler that passes aerosol particles through a porous medium submerged in a liquid layer. Evaluated for the collection of influenza viruses at a flow rate of 4 l min^{-1} (Pyankov *et al.* 2007), it recovered 65–68% of the virus particles. This device was also used for monitoring airborne measles virus in a natural environment (Agranovski *et al.* 2008). The applicability of these samplers designed to operate using multiple mechanisms is still exploratory, as their overall collection efficiencies, both physically or biologically, have not been fully evaluated, and these combinatorial systems have complicated features. Most have been only tested for one or two types of viruses. In addition, compared with water-based CPCs, collection efficiencies using these devices are lower.

The pros and cons of each type of samplers for airborne viruses are summarized in Table 1. Overall, the collection efficiencies for airborne viruses by samplers such as the water-based CPC or the integration of inertial or diffusion-based samplers have improved steadily, but development is still needed for light-weight and portable samplers that can collect a variety of infectious airborne viruses present in a wide size range of aerosolized

Table 1 Summary of the pros and cons of common samplers for airborne viruses

Collection methods	Impactors and cyclones	Liquid impingers	Filters	Electrostatic precipitators	Water-based condensation	Other devices
Pros	• Collect viruses in different particle sizes • Efficient for large particles	• Maintain viability of viruses • No need to extract viruses from a surface or filter	• Efficient for particles from 20 nm to 10 μm or even larger • Easy to use	• Have size-dependent collection efficiency • Consume less energy and easier to be portable	• Efficient for particles from 8 nm to 10 μm or even larger • Maintain viability of viruses	• Good for specific types of viruses
Cons	• Wall loss • Virus deactivation upon collection • Low efficiency for small virus particles	• Wall loss or inlet loss • Low efficiency for small virus particles	• Inactivation of viruses due to dehydration or extraction from filters	• Low efficiency for submicrometre or nanometre particles • Ozone formation deactivate viruses	• Bulky • Complicated to operate	• Efficiencies for sampling viruses not fully evaluated

particles. Until a standard sampler and procedure for sampling aerosols containing infectious viruses are developed, our understanding of airborne virus transmission remains stilted. It is impossible to accurately reconcile results from different laboratories that use different samplers based on different sampling and analytical methods for the collection of airborne viruses. Moreover, flow rates used for virus-containing aerosol sampling are generally low (less than 12·5 l min^{-1}), and this limits the amount of air that can be sampled. Given the low virus concentration in the air, sampling at high flow rates might facilitate fast detection of airborne viruses, although the associated high flow velocity may damage viruses. Hence, a balance between high volume of sampled air and maintenance of virus viability is needed. Finally, the 'collection efficiencies' reported in many virus aerosol studies are in reality 'relative collection efficiencies'; they are measures of the amount of virus collected by one sampler compared to that collected or calculated by a reference sampler. One reason for this gap in reporting is that a large percentage of the test virus is deactivated during aerosol generation or sampling processes (Zhen *et al.* 2014; Walls *et al.* 2016), and quantification of their inactivation rate proves challenging. The absolute efficiency of a virus sampler is very important for health risk assessments, because even if the tested sampler has high collection efficiency compared with some sampler considered to be state-of-the-art, it might still be possible that both samplers do not collect sufficient quantities of airborne viruses for accurate risk assessments. Thus, future studies should develop reference samplers that have verifiable absolute collection efficiency, although currently there is no clear solution yet.

Factors affecting the sampling efficiency of virus aerosols

While each sampler's efficiency is dependent on the dominating collection mechanism, other factors can also affect their performance, including RH, temperature, light, irradiation, suspension media and sampling media (Benbough 1971). Biological collection efficiency of these samplers also strongly depends on the sampling conditions, aerosolilzation method and the virus type, such as virus morphology, surface charge and the hydrophilic or hydrophobic nature of the viruses (Tseng and Li 2005).

Relative humidity is one commonly studied factor for biological collection efficiency (Cox and Wathes 1995). If aerosolized, non-lipid-enveloped viruses can be unstable below about 70% RH as a result of denaturation of virus surface structures. In contrast, lipid-enveloped viruses such as vaccinia virus, may have reduced stability in air if RH is above 70% (Cox 1987; Tellier 2006). These observations fit well with the general belief that phospholipid–protein complexes in enveloped viruses are usually more likely to denature in the air at medium to high RH, whereas the protein coats of non-enveloped viruses are more readily to denature at low RH (Cox and Wathes 1995). One the other hand, when influenza virus is suspended in a medium that closely mimics respiratory tract fluids, the effect of humidity on survival is greatly diminished (Kormuth *et al.* 2018). Transmission of airborne viruses is also affected by RH; another plausible explanation for the fact that influenza is more likely to occur in winter (low RH) is that settling of airborne viruses due to condensation occurs to a lower extent in winter, and therefore the chances of inhaling airborne influenza viruses is much greater than during summer (high RH) (Lowen *et al.* 2007). Moreover, RH is important in filtration collection, as desiccation has always been a problem for viruses trapped on filters (Tseng and Li 2005; Fabian *et al.* 2009; Lindsley *et al.* 2010). These studies illustrate that sampling or transport, rather than aerosol generation or sample storage, account for the loss of virus viability. Hence, sampling process should provide the optimum RH for the targeted infectious virus.

Temperature is another factor for biological collection efficiency. Once aerosolized, viruses can be inactivated by heat (Norman and Veomett 1960). Thus, attempts have been made to control temperature and RH for improved efficiency. Walls *et al.* (2017) designed a temperature and RH-conditioned filtration process, although it has only been tested for bacteria, wherein their polyurethane nanofibre filter could be used for as long as 5 h of sampling without affecting the viability of *Escherichia coli*. Similarly, Springorum *et al.* (2011) insulated the AGI-30 with a holder to control the temperature of the collection fluid. Their tests showed that tempering strongly affected the volume of the sampling liquid and the number of culturable microbes collected in the sampling liquid and subsequently the total biological collection efficiency. The tempered impingers preserved viability three times better than in the untempered ones. Similar devices should be investigated for the sampling of infectious viruses.

Choices of suspension media for aerosol generation and collection media in the samplers are also important considerations for successful collection of viable airborne viruses. Sterile phosphate-buffered saline (PBS) with 0·5% bovine serum albumin fraction V has been used as both suspension and collection media for influenza viruses, as it helps maintain viability of the viruses (Lednicky *et al.* 2010). The use of serum as an aid for maintaining virus viability is not a new concept, for example, PBS containing 5% inactivated ox serum was used for sampling *Foot-and-mouth disease virus* (Sellers and Parker 1969). Serum and other stabilizers have not always been

included in liquid collection media, for example, PBS with or without calcium and magnesium has been used for the collection of influenza virus (Fabian *et al.* 2009). Appert *et al.* (2012) evaluated the effects of nebulizer fluids on the viability of phage MS2 and human adenovirus serotype-1, and showed that recovery of MS2 aerosolized with tryptic soy broth (TSB) was much higher than with DI water or TSB with 7·6% w/v glycerol. However, most adenoviruses are environmentally stable viruses, and for that virus, there was no statistical difference for virus recovery when the nebulization fluids were Eagle's minimum essential medium (MEM) lacking supplements such as serum, or DI water, or MEM with 7·6% w/v glycerol. Taken together, with regard to virus viability in aerosol studies, more work is needed to better understand the type of suspension media for superior performance during nebulization in laboratory studies, and the most suitable collection media for laboratory and field tests, as well as the optimum conditions for different types of viruses.

Size distribution of airborne infectious viruses

Health risks due to exposure to airborne virus particles partly depend on the particle size distribution of the aerosols containing infectious viruses. The sizes of the virus-containing particles affect their transport, lifetime, their deposition in the human respiratory system, infectious dose and the selection of the right sampling and detection methods (Lednicky *et al.* 2010; Zuo *et al.* 2013; Clauß 2015). If infectious viruses are preferentially associated with particles smaller than 1 μm, wearing respiratory protectors such as an N95 respirator rather than a surgical mask might be strongly advised (Seto 2015). Moreover, virus dispersion models rely heavily on the particle sizes of these virus-containing particles (Sørensen *et al.* 2000).

As virus-containing particles are generally a complex mixture of various components (e.g. salts, proteins), sizes of these virus-containing particles range from the 'naked' virus diameter (20–30 nm) to the sizes of the carrier particles (>20 μm) that include many other components (Verreault *et al.* 2008). Sellers and Parker (1969) recovered airborne viruses excreted by cattle, sheep and pigs with FMDV with a multistage liquid impinger; they found that 65–71% of the viable FMDV assayed by inoculation of mice and calf thyroid tissue culture tubes were over 6 μm in diameter, 19–24% ranged from 3 to 6 μm and 10–11% were under 3 μm. Noti *et al.* (2012) found that influenza viruses can be transmitted across the space of a patient examination room with the NIOSH cyclone sampler: 5% of the infectious influenza analysed by plaque assay was recovered in aerodynamic diameter >4 μm, 75·5% in 1–4 μm and 19·5% in <1 μm. By combining the BioSampler with a piston spirometer, Lindsley *et al.* (2015) showed that the highest concentration of infectious influenza virus was in the smaller particle size fractions (0·3–8 μm). Using a Sioutas cascade impactor, Lednicky and Loeb (2013) found that infectious IAV was concentrated in particles below 250 nm. Studying the infectious size fractions of aerosols containing MS2 bacteriophage and adenovirus with Andersen and MOUDI cascade impactors, Appert *et al.* (2012) found that the infectious adenovirus number concentration depended on aerodynamic particle size with higher concentration of viruses in the 0·56–1·9 μm range. As virus samplers are improved, a better understanding can be attained regarding the size distribution of aerosolized viruses, especially those in fine particles.

Efforts have been attempted to correlate virus infectivity with aerosol particle size. Using gelatin filters, Zuo *et al.* (2013) observed that the infectious virus distribution for MS2 phage aerosols in the size range of 100–500 nm was better represented by particle volume distribution rather than number distribution, although they offered no mechanistic explanation. Walls *et al.* (2016) conducted laboratory studies on sampling size-selected MS2 aerosols (45, 90, 300 nm) with a water-based condensation sampler, and suggested that the number of infectious virions per particle was proportional to the cube of the particle diameter. Meanwhile, Pan *et al.* (2019) found out that the composition of the nebulization suspension also affects the infectious count of viruses carried per particle, not always leading to volume size distribution.

Size distribution of these virus-containing particles also depends on the way they are generated. With microscopic measurement, Duguid (1946) reported that most of the droplets produced by coughing were between 8 and 32 μm while sneezing generated relatively smaller droplets. Using a transmission electron microscope and an impactor, Papineni and Rosenthal (1996) showed that coughing produced the largest droplet concentrations and nose breathing the least. Lindsley *et al.* (2016) suggested that exhalation might generate more airborne infectious material than coughing over time, but both respiratory activities are important for the transmission of airborne influenza virus. Therefore, future studies using samplers capable of sampling fine virus aerosols are needed to better clarify and update distribution of infectious viruses in aerosolized particles.

Measurements of concentration and size distribution of virus-carrying particles are very important for a better understanding of virus transmission modes. Natural virus-containing particles consist of multiple components and are usually irregular in shape; both factors make size

distribution measurement of virus-containing particles challenging. Particle physics-based devices like the Cascade Impactor, the Optical Particle Counter (OPC), the Wide Range Aerosol Spectrometer, the Aerodynamic Particle Sizer (APS) and the Scanning Mobility Particle Sizer (SMPS), are commonly used. They provide physical counts of the particles, but no information about viruses contained in the particles. Problems arise in getting size distribution of particles in a wide size range (20 nm–10 μm), as each device can only cover certain range of particle size, for example, both SMPS and OPC were used in Yu *et al.*'s (2018) study to measure the size distribution of MS2-containing particles from 27 nm to more than 10 μm. Liu *et al.* (2010) introduced a system capable of measuring aerosol size distributions from 10 nm to 10 μm in diameter, which included an SMPS for particle measurement from 0·01 to 0·5 μm and a Laser Particle Spectrometer for measurement in 0·4–10 μm range. Another problem is the definition of particle size. The APS measures aerodynamic particle size (Baron 1986), particle sizing of the OPC is based on single particle's elastic light scattering that follows the Mie theory (Heyder and Gebhart 1979), while the SMPS is based on the particles' electrical mobility (Wang and Flagan 1990). Thus, conversions between different definitions of particle sizes are necessary, and translating particle size distribution measured by one device to another is a technological gap to be filled.

Detection of viruses in collection media

Apart from traditional animal models and virus isolation in cell cultures for infectious viruses, for example, Madin-Darby Canine Kidney Epithelial Cells (MDCK cell line) and ferrets for influenza viruses, nucleic acid-based technologies such as PCR, quantitative PCR and RT-PCR, and biochemical tests such as ELISA are used for the detection of viruses in collection media. Reviews of these methods are available in published literature (Pillai and Ricke 2002; Xu *et al.* 2011). However, it is very important to note that detection of nucleic acid in aerosol does not correlate with virus viability in the aerosol. The ability to detect both nonviable and viable viruses, and determine the fraction thereof that is infectious, is important for risk assessments, as nonviable viruses do not cause infections. Electron microscopy has also been attempted in some studies to identify virus particles, for example, avian infectious laryngotracheitis viruses in the study of Williams *et al.* (1994), but this approach lacks sensitivity, is costly, and has not proven to be practical. For the identification of infectious viruses, virus-containing aerosol particles are first collected with a sampler, and then transported to a virology laboratory for further analyses.

Sensitive and rapid detection of viruses in collection media, preferably in real-time, is an ongoing goal of aerovirology. Promising technologies include loop-mediated isothermal amplification, which has the potential to detect and offer a presumptive identification of a virus in under an hour, as demonstrated for influenza virus (Mori and Notomi 2009), and real-time PCR. However, more work is needed towards the integration of these technologies with air sampling devices. Shen *et al.* (2011) developed a sensor for real-time detection of influenza H3N2 virus by integrating silicon nanowire field effect transistors, microfluidics and electrostatic air sampling. Although they successfully detected influenza viruses, this device has low charging efficiency for nanometre-sized virus particles, that is, they do not easily get charged and thus not captured efficiently. Usachev *et al.* (2012) described several technologies used in conjunction with a personal bioaerosol sampler for real-time detection of viruses. They found that real-time PCR technique allows detection of bacteriophages MS2 and T4, but the whole procedure takes a long time (h). The combination of a personal sampler with surface plasmon resonance (SPR)-based immunosensor allows for rapid detection of MS2; the entire sampling and analysis procedure can be done in 6 min (Usachev *et al.* 2013). Later, Usachev *et al.* (2015) used multiplexed SPR for simultaneous detection of MS2 bacteriophage and IAV. The SPR response units increased with increasing virus concentration, and the sensitivity of this technology was high enough to minimize false alarm. Although the overall response for multiplex SPR slightly decreased compared with singleplex SPR, there was no statistical difference in sensitivity between the two for the target viruses. A limitation with these types of real-time samplers and analyses is that at the current state-of-art, viruses can only be detected when they are present at relatively high concentrations. For example, the detection limit of the SPR was 7×10^7 PFU per ml in the sampling liquids, with a liquid volume of 0·1 ml used in their work, whereas for viruses such as IAV, the quantity exhaled by human beings might be lower than 1000 per 30 min (Milton *et al.* 2013).

Summary and Conclusions

Commonly used samplers for airborne viruses are designed and operated following the same principles used for bioaerosol samplers, including solid impactors, liquid impingers, filters and ESPs. Problems with these traditional samplers include: (a) inefficiency at the collection of fine particles, (b) dehydration of viruses during the collection process, (c) damage of the virus during collection due to impaction forces, resulting in the loss of viability of some or all the collected viruses, (d)

Table 2 Knowledge gaps resulting from inconclusive information that warrants future research

Knowledge gap	Recommended action
Factors affecting sampling efficiency of viable virus aerosol	• Increasing the collection efficiency of the sampler for a wide range of virus aerosols, from 20 nm to > 10 μm • Decrease re-aerosolizaion, bounce, inlet and wall losses in samplers • Carry out a systematic assessment to optimize collection/storage temperatures for each type of virus • Establish standardized procedures and methods for sampling airborne viruses and enable measurement of the detection limit of the virus samplers • Evaluate optimal media for suspension or collection beyond empirical experiences • Conduct a systematic evaluation of the effects of relative humidity on the viability of aerosolized viruses, considering the biochemical and biophysical characteristics of viruses and in the presence of aerosol components (e.g. mucus, salt)
Size distribution of infectious viruses within aerosol particles	• Investigate how the distribution of viruses in aerosol particles is affected by the virus aerosol composition (e.g. saliva, dust) interaction • Study how the aerosol generation method (e.g. coughing, sneezing, breathing, speaking) affects the distribution of viruses
Detection of airborne viruses	• Integrate sensitive, rapid and preferably, real-time detection with air sampling devices

reaerosolization leading to the loss of viruses from the collection media, and (e) losses due to viruses being trapped by the inlet or the samplers' wall. Samplers based on newer technologies, such the water-based condensation or the integration of multiple principles, are in development for the sampling of airborne viruses. Information resulting from the use of these technologies will enhance our knowledge of virus transmission through airborne routes and the biothreats posed by virus aerosols.

At present, the lack of a standard sampler and standardized procedure for sampling virus aerosols has hindered progress towards a better understanding of the occurrence of airborne viruses, the persistence of viruses in the aerosols, movement of aerosol particles in air currents, residence time of aerosolized particles and the biothreats posed by the aerosols. No single device has been demonstrated capable of serving as the gold standard sampler, one that can efficiently sample a wide size range (10 nm to >10 μm) of virus-containing aerosols, and conserve the viability of the collected viruses. In addition, collection efficiencies reported in most virus aerosol studies refer to relative collection efficiency, not absolute collection efficiency, resulting in underestimates of the concentrations of infectious virus particles. Moreover, due to the low concentration of airborne viruses and the inactivation of infectious viruses due to sampling processes, methods to balance the need for high volume sampling air and maintaining virus viability during their collection are needed. Finally, for proper biothreat analyses, further investigations are needed on the size distribution of aerosolized particles that contain infectious viruses, as well as on the factors that affect their concentrations and size distributions. Knowledge gaps resulting from inconclusive information are summarized in Table 2 as a reference for future research studies.

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Conflict of Interest

No conflict of interest declared.

References

Agranovski, I.E., Agranovski, V., Reponen, T., Willeke, K. and Grinshpun, S.A. (2002) Development and evaluation of a new personal sampler for culturable airborne microorganisms. *Atmos Environ* **36**, 889–898.

Agranovski, I.E., Safatov, A.S., Agafonov, A.P., Pyankov, O.V. and Sergeev, A.N. (2008) Monitoring of airborne mumps and measles viruses in a hospital. *CLEAN – Soil Air Water* **36**, 845–849.

Aitken, J. (1889) On the number of dust particles in the atmosphere. *Earth Env Sci T R SO* **35**, 1–19.

Alonso, C., Raynor, P.C., Davies, P.R. and Torremorell, M. (2015) Concentration, size distribution, and infectivity of airborne particles carrying swine viruses. *PLoS ONE* **10**, e0135675.

Andersen, A.A. (1958) New sampler for the collection, sizing, and enumeration of viable airborne particles. *J Bacteriol* **76**, 471–484.

Anderson, B.D., Ma, M., Xia, Y., Wang, T, Shu, B, Lednicky, J.A., Ma, M.J., Lu, J. *et al.* (2016) Bioaerosol sampling in modern agriculture: a novel approach for emerging pathogen surveillance? *J Infect Dis* **214**, 537–545.

Anwar, D. (2010) Virus collection efficiency of biosampler versus impinger with variable time and flow rate. Bachelor's thesis, University of Florida.

Appert, J., Raynor, P.C., Abin, M., Chander, Y., Guarino, H., Goyal, S.M., Zuo, Z., Ge, S. *et al.* (2012) Influence of suspending liquid, impactor type, and substrate on size-selective sampling of MS2 and adenovirus aerosols. *Aerosol Sci Technol* **46**, 249–257.

Baron, P. A. (1986). Calibration and use of the aerodynamic particle sizer (APS 3300). *Aerosol Sci Technol*, **5**, 55–67.

Benbough, J.E. (1971) Some factors affecting the survival of airborne viruses. *J Gen Virol* **10**, 209–220.

Blachere, F.M., Lindsley, W.G., Pearce, T.A., Anderson, S.E., Fisher, M., Khakoo, R., Meade, B.J., Lander, O. *et al.* (2009) Measurement of airborne influenza virus in a hospital emergency department. *Clin Infect Dis* **48**, 438–440.

Booth, T.F., Kournikakis, B., Bastien, N., Ho, J., Kobasa, D., Stadnyk, L., Li, Y., Spence, M. *et al.* (2005) Detection of airborne severe acute respiratory syndrome (SARS) coronavirus and environmental contamination in SARS outbreak units. *J Infect Dis* **191**, 1472–1477.

Bourgueil, E., Hutet, E., Cariolet, R. and Vannier, P. (1992) Air sampling procedure for evaluation of viral excretion level by vaccinated pigs infected with aujeszkys disease (pseudorabies) virus. *Res Vet Sci* **52**, 182–186.

Brown, J.S., Gordon, T., Price, O. and Asgharian, B. (2013) Thoracic and respirable particle definitions for human health risk assessment. *Part Fibre Toxicol* **10**, 12.

Burton, N.C., Adhikari, A., Grinshpun, S.A., Hornung, R. and Reponen, T. (2005) The effect of filter material on bioaerosol collection of *Bacillus subtilis* spores used as a *Bacillus anthracis* simulant. *J Environ Monit* **7**, 475–480.

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. (2011) Development of an improved methodology to detect infectious airborne influenza virus using the NIOSH bioaerosol sampler. *J Environ Monit* **13**, 3321–3328.

Clauß, M. (2015) Particle size distribution of airborne micro-organisms in the environment—a review. *Landbauforschung Appl Ag Forest Res* **65**, 77–100.

Cooper, C. D. and Alley, F. C. (2010). *Air Pollution Control: A Design Approach* (4th edn). Long Grove, IL: Waveland Press.

Corzo, C.A., Allerson, M., Gramer, M., Morrison, R. B. and Torremorell, M. (2014) Detection of airborne influenza A virus in experimentally infected pigs with maternally derived antibodies. *Transbound Emerg Dis* **61**, 28–36.

Cowling, B.J., Ip, D.K.M., Fang, V.J., Suntarattiwong, P., Olsen, S.J., Levy, J., Uyeki, T.M. *et al.* (2013) Aerosol transmission is an important mode of influenza A virus spread. *Nat Commun* **4**, 1935.

Cox, C.S. (1987) *Aerobiological Pathway of Microorganisms*. New York, NY: Wiley.

Cox, C.S. and Wathes, C.M. (1995) *Bioaerosols Handbook*. Boca Raton, FL: Lewis Publishers.

Duguid, J.P. (1946) The size and the duration of air-carriage of respiratory droplets and droplet-nuclei. *J Hyg* **44**, 471–479.

Dybwad, M., Skogan, G. and Blatny, J.M. (2014) Comparative testing and evaluation of nine different air samplers: end-to-end sampling efficiencies as specific performance measurements for bioaerosol applications. *Aerosol Sci Technol* **48**, 282–295.

Fabian, P., McDevitt, J.J., Houseman, E.A. and Milton, D.K. (2009) Airborne influenza virus detection with four aerosol samplers using molecular and infectivity assays: considerations for a new infectious virus aerosol sampler. *Indoor Air* **19**, 433–441.

Gardam, M. and Lemieux, C. (2007) Transmission of influenza A in human beings – reply. *Lancet Infect Dis* **7**, 761–763.

Gerone, P.J., Couch, R.B., Keefer, G.V., Douglas, R.G., Derrenbacher, E.B. and Knight, V. (1966) Assessment of experimental and natural viral aerosols. *Bacteriol Rev* **30**, 576–584.

Gralton, J., Tovey, E., McLaws, M.L. and Rawlinson, W.D. (2011) The role of particle size in aerosolised pathogen transmission: a review. *J Infect* **62**, 1–13.

Grinshpun, S.A., Chang, C.W., Nevalainen, A. and Willeke, K. (1994) Inlet characteristics of bioaerosol samplers. *J Aerosol Sci* **25**, 1503–1522.

Grinshpun, S.A., Willeke, K., Ulevicius, V., Juozaitis, A., Terzieva, S., Donnelly, J., Stelma, G. N. andBrenner, K.P (1997) Effect of impaction, bounce and reaerosolization on the collection efficiency of impingers. *Aerosol Sci Technol* **26**, 326–342.

Gustafson, T.L., Lavely, G.B., Brawner, E.R., Hutcheson, R.H., Jr, Wright, P.F. and Schaffner, W. (1982) An outbreak of airborne nosocomial varicella. *Pediatrics* **70**, 550–556.

Han, T. and Mainelis, G. (2012) Investigation of inherent and latent internal losses in liquid-based bioaerosol samplers. *J Aerosol Sci* **45**, 58–68.

Hatagishi, E., Okamoto, M., Ohmiya, S., Yano, H., Hori, T., Saito, W., Miki, H., Suzuki, Y. *et al.* (2014) Establishment and clinical applications of a portable system for capturing influenza viruses released through coughing. *PLoS ONE* **9**, e0103560.

Henningson, E.W. and Ahlberg, M.S. (1994) Evaluation of microbiological aerosol samplers – a review. *J Aerosol Sci* **25**, 1459–1492.

Hering, S.V. and Stolzenburg, M.R. (2005) A method for particle size amplification by water condensation in a

laminar, thermally diffusive flow. *Aerosol Sci Technol* **39**, 428–436.

Hering, S.V., Stolzenburg, M.R., Quant, F.R., Oberreit, D.R. and Keadu, P.B. (2005) A laminar-flow, water-based condensation particle counter (WCPC). *Aerosol Sci Technol* **39**, 659–672.

Heyder, J. and Gebhart, J. (1979) Optimization of response functions of light-scattering instruments for size evaluation of aerosol particles. *Appl Optics* **18**, 705–711.

Hinds, W.C. (1998) *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles*. New York, NY: Wiley.

Hogan, C.J., Kettleson, E.M., Lee, M.H., Ramaswami, B., Angenent, L.T. and Biswas, P. (2005) Sampling methodologies and dosage assessment techniques for submicrometre and ultrafine virus aerosol particles. *J Appl Microbiol* **99**, 1422–1434.

Hong, S., Bhardwaj, J., Han, C.H. and Jang, J. (2016) Gentle sampling of submicrometer airborne virus particles using a personal electrostatic particle concentrator. *Environ Sci Technol* **50**, 12365–12372.

Horvath, I., Hunt, J., Barnes, P.J., Alving, K., Antczak, A., Baraldi, E., Becher, G., van Beurden, W.J. *et al.* (2005) Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* **26**, 523–548.

Horváth, I., Barnes, P.J., Loukides, S., Sterk, P.J., Högman, M., Olin, A.C., Amann, A., Antus, B. *et al.* (2017) A European respiratory society technical standard: exhaled biomarkers in lung disease. *Eur Respir J* **49**, 1600965.

Iuliano, A.D., Roguski, K.M., Chang, H.H., Muscatello, D. J., Palekar, R., Tempia, S., Cohen, C., Gran, J.M. *et al.* (2017) Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet* **139**, 1285–1300.

Jang, J., Akin, D., Lim, K.S., Broyles, S., Ladisch, M.R. and Bashir, R. (2007) Capture of airborne nanoparticles in swirling flows using non-uniform electrostatic fields for biosensor applications. *Sens Actuators B Chem* **121**, 560–566.

Jang, J.S., Akin, D. and Bashir, R. (2008) Effects of inlet/outlet configurations on the electrostatic capture of airborne nanoparticles and viruses. *Meas Sci Technol* **19**, 065204.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. and Daszak, P. (2008) Global trends in emerging infectious diseases. *Nature* **451**, 990–993.

Jonges, M., van Leuken, J., Wouters, I., Koch, G., Meijer, A. and Koopmans, M. (2015) Wind-mediated spread of low-pathogenic Avian influenza virus into the environment during outbreaks at commercial poultry farms. *PLoS ONE* **10**, e0125401.

Kang, N., Chen, M., Bi, F.Y., Bi, F.Y., Chen, M.M. and Ta, Y. (2016) First positive detection of H9 subtype of Avian influenza virus nucleic acid in aerosol samples from live poultry markets in Guangxi, South of China. *Chin Med J* **129**, 1371–1373.

Kenny, L.C., Thorpe, A. and Stacey, P. (2017) A collection of experimental data for aerosol monitoring cyclones. *Aerosol Sci Technol* **51**, 1190–1200.

Kettleson, E.M., Ramaswami, B., Hogan, C.J., Lee, M.H., Statyukha, G.A., Biswas, P. and Angenent, L.T. (2009) Airborne virus capture and inactivation by an electrostatic particle collector. *Environ Sci Technol* **43**, 5940–5946.

Killingley, B. and Nguyen-Van-Tam, J. (2013) Routes of influenza transmission. *Influenza Other Respir Viruses* **7**, 42–51.

Kormuth, K.A., Lin, K., Prussin, A.J., Vejerano, E.P., Tiwari, A.J., Cox, S.S., Myerburg, M.M., Lakdawala, S.S. *et al.* (2018) Influenza virus infectivity is retained in aerosols and droplets independent of relative humidity. *J Infect Dis* **218**, 739–747.

Kulkarni, P., Baron, P.A. and Willeke, K. (2011) *Aerosol Measurement: Principles, Techniques, and Applications*. Hoboken, NJ: John Wiley & Sons.

Lednicky, J.A. and Loeb, J.C. (2013) Detection and isolation of airborne influenza A H3N2 virus using a Sioutas personal cascade impactor sampler. *Influenza Res Treat* **2013**, 656825.

Lednicky, J.A., Hamilton, S.B., Tuttle, R.S., Sosna, W.A., Daniels, D.E. and Swayne, D.E (2010) Ferrets develop fatal influenza after inhaling small particle aerosols of highly pathogenic avian influenza virus A/Vietnam/1203/2004 (H5N1). *Virol J* **7**, 231.

Lednicky, J., Pan, M.H., Loeb, J., Hsieh, H., Eiguren-Fernandez, A., Hering, S., Fan, H. Z. and Wu, C.-Y. (2016). Highly efficient collection of infectious pandemic influenza H1N1 virus (2009) through laminar-flow water based condensation. *Aerosol Sci Technol*, **50**, I–IV.

Li, C.S., Hao, M.L., Lin, W.H. and Chang, C.W. (1999) Evaluation of microbial samplers for bacterial microorganisms. *Aerosol Sci Technol* **30**, 100–108.

Li, H.W., Wu, C.Y., Tepper, F., Lee, J.-H. and Lee, F. (2009) Removal and retention of viral aerosols by a novel alumina nanofiber filter. *J Aerosol Sci* **40**, 65–71.

Li, J., Leavey, A., Wang, Y., O'Neil, C., Wallace, M. A., Burnham, C.-A. D., Boon, A.C.M. *et al.* (2017) Comparing the performance of 3 bioaerosol samplers for influenza virus. *J Aerosol Sci* **115**, 133–145.

Lin, X.J., Reponen, T., Willeke, K., Wang, Z., Grinshpun, S. A. and Trunov, M. (2000) Survival of airborne microorganisms during swirling aerosol collection. *Aerosol Sci Technol* **32**, 184–196.

Lindsley, W.G., Schmechel, D. and Chen, B.T. (2006) A two-stage cyclone using microcentrifuge tubes for personal bioaerosol sampling. *J Environ Monit* **8**, 1136–1142.

Lindsley, W.G., Blachere, F.M., Thewlis, R.E., Vishnu, A., Davis, K.A., Cao, G., Palmer, J.E., Clark, K.E. *et al.* (2010) Measurements of airborne influenza virus in aerosol particles from human coughs. *PLoS ONE* **5**, e15100.

Lindsley, W.G., Noti, J.D., Blachere, F.M., Thewlis, R.E., Martin, S.B., Othumpangat, S., Noorbakhsh, B.,

Goldsmith, W.T. *et al.* (2015) Viable influenza A virus in airborne particles from human coughs. *J Occup Environ Hyg* **12**, 107–113.

Lindsley, W.G., Blachere, F.M., Beezhold, D.H., Thewlis, R. E., Noorbakhsh, B., Othumpangat, S., Goldsmith, W.T. and McMillen, C.M. (2016) Viable influenza A virus in airborne particles expelled during coughs versus exhalations. *Influenza Other Respir Viruses* **10**, 404–413.

Lindsley, W.G., Green, B.J., Blachere, F.M. and Beezhold, D. (2017) *Sampling and Characterization of Bioaerosols. NIOSH Manual of Analytical Methods* (5th edn). Cincinnati, OH: National Institute for Occupational Safety and Health.

Liu, B.Y.H., Romay, F.J., Dick, W.D., Woo, J. and Chiruta, M. (2010) A Wide-range particle spectrometer for aerosol measurement from 0.010 μm to 10 μm. *Aerosol Air Qual Res* **10**, 125–139.

Lowen, A.C., Mubareka, S., Steel, J. and Palese, P. (2007) Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog* **3**, e151.

McDevitt, J.J., Koutrakis, P., Ferguson, S.T., Wolfson, J.M., Fabian, M.P., Martins, M., Pantelic, J. and Milton, D.K (2013) Development and performance evaluation of an exhaled-breath bioaerosol collector for influenza virus. *Aerosol Sci Technol* **47**, 444–451.

Milton, D.K., Fabian, M.P., Cowling, B.J., Grantham, M.L. and McDevitt, J.J. (2013) Influenza virus aerosols in human exhaled breath: particle size, culturability, and effect of surgical masks. *PLoS Pathog* **9**, e1003205.

Mirski, T., Bartoszcze, M., Bielawska-Drozd, A., Cieślik, P., Michalski, A.J., Niemcewicz, M., Kocik, J. and Chomiczewski, K. (2014) Review of methods used for identification of biothreat agents in environmental protection and human health aspects. *Ann Agric Environ Med* **21**, 224–234.

Mori, Y. and Notomi, T. (2009) Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *J Infect Chemother* **15**, 62–69.

Myatt, T.A., Johnston, S.L., Zuo, Z.F., Wand, M., Kebadze, T., Rudnick, S. and Milton, D.K (2004) Detection of airborne rhinovirus and its relation to outdoor air supply in office environments. *Am J Respir Crit Care Med* **169**, 1187–1190.

Norman, A. and Veomett, R.C. (1960) Heat inactivation of poliovirus ribonucleic acid. *Virology* **12**, 136–139.

Noti, J.D., Lindsley, W.G., Blachere, F.M., Cao, G., Kashon, M.L., Thewlis, R.E., McMillen, C.M., King, W.P. *et al.* (2012) Detection of infectious influenza virus in cough aerosols generated in a simulated patient examination room. *Clin Infect Dis* **54**, 1569–1577.

Oh, S., Anwar, D., Theodore, A., Lee, J.-H., Wu, C.-Y. and Wander, J. (2010) Development and evaluation of a novel bioaerosol amplification unit (BAU) for improved viral aerosol collection. *J Aerosol Sci* **41**, 889–894.

Orsini, D.A., Ma, Y.L., Sullivan, A., Sierau, B., Baumann, K. and Weber, R.J. (2003) Refinements to the particle-into-liquid sampler (PILS) for ground and airborne measurements of water soluble aerosol composition. *Atmos Environ* **37**, 1243–1259.

Orsini, D.A., Rhoads, K., McElhoney, K., Schick, E., Koehler, E. and Hogrefe, O. (2008) A water cyclone to preserve insoluble aerosols in liquid flow – an interface to flow cytometry to detect airborne nucleic acid. *Aerosol Sci Technol* **42**, 343–356.

Pan, M., Eiguren-Fernandez, A., Hsieh, H., Afshar-Mohajer, N., Hering, S.V., Lednicky, J., Fan, Z.H. and Wu, C.Y. (2016) Efficient collection of viable virus aerosol through laminar-flow, water-based condensational particle growth. *J Appl Microbiol* **120**, 805–815.

Pan, M., Bonny, T., Loeb, J., Jiang, X., Lednicky, J.A., Eiguren-Fernandez, A., Hering, S., Fan, Z.H. *et al.* (2017). Collection of viable aerosolized influenza and other respiratory viruses in a student health care center through water-based condensation growth. *mSphere*, **2**, e00251-17.

Pan, M., Leah, C., Lednicky, J. A., Eiguren-Fernandez, A., Hering, S., Fan, Z.H. and Wu, C.-Y. (2019) Determination of the distribution of infectious viruses in aerosol particles using water-based condensational growth technology and a bacteriophage MS2 model. *Aerosol Sci Technol*, **53**, 583–593.

Papineni, R.S. and Rosenthal, F.S. (1996) The size distribution of droplets in the exhaled breath of healthy human subjects. *J Aerosol Med* **10**, 105–116.

Pillai, S.D. and Ricke, S.C. (2002) Bioaerosols from municipal and animal wastes: background and contemporary issues. *Can J Microbiol* **48**, 681–696.

Pyankov, O.V., Agranovski, I.E., Pyankova, O., Mokhonova, E., Mokhonov, V., Safatov, A.S. and Khromykh, A.A. (2007) Using a bioaerosol personal sampler in combination with real-time PCR analysis for rapid detection of airborne viruses. *Environ Microbiol* **9**, 992–1000.

Riemenschneider, L., Woo, M.H., Wu, C.Y., Lundgren, D., Wander, J., Lee, J.H., Li, H.W. and Heimbuch, B. (2010) Characterization of reaerosolization from impingers in an effort to improve airborne virus sampling. *J Appl Microbiol* **108**, 315–324.

Sarkar, A., Aronson, K.J., Patil, S., Hugar, L.B. and vanLoon, G.W. (2012). Emerging health risks associated with modern agriculture practices: a comprehensive study in India. *Environ Res*, **115**, 37–50.

Sawyer, M.H., Chamberlin, C.J., Wu, Y.N., Aintablian, N. and Wallace, M.R. (1994) Detection of varicella-zoster virus-DNA in air samples from hospital rooms. *J Infect Dis* **169**, 91–94.

Sellers, R.F. and Parker, J. (1969) Airborne excretion of foot-and-mouth disease virus. *Epidemiol Infect* **67**, 671–677.

Seshadri, S., Han, T., Krumins, V., Fennell, D.E. and Mainelis, G. (2009) Application of ATP bioluminescence method to

characterize performance of bioaerosol sampling devices. *J Aerosol Sci* **40**, 113–121.
Seto, W.H. (2015) Airborne transmission and precautions: facts and myths. *J Hosp Infect* **89**, 225–228.
Shen, F.X., Tan, M.M., Wang, Z.X., Yao, M., Xu, Z., Wu, Y., Wang, J., Guo, X. *et al.* (2011) Integrating silicon nanowire field effect transistor, microfluidics and air sampling techniques for real-time monitoring biological aerosols. *Environ Sci Technol* **45**, 7473–7480.
Sørensen, J., Mackay, D., Jensen, C. andDonaldson, A.I (2000) An integrated model to predict the atmospheric spread of foot-and-mouth disease virus. *Epidemiol Infect* **124**, 577–590.
Springorum, A.C., Clauss, M. and Hartung, J. (2011) A temperature-controlled AGI-30 impinger for sampling of bioaerosols. *Aerosol Sci Technol* **45**, 1231–1239.
Stolzenburg, M.R. and McMurry, P.H. (1991) An ultrafine aerosol condensation nucleus counter. *Aerosol Sci Technol* **14**, 48–65.
Tang, J.W. (2009) The effect of environmental parameters on the survival of airborne infectious agents. *J Royal Soc Interface* **6**(Suppl. 6), S737.
Tang, J.W., Gao, C.X., Cowling, B.J., Koh, G.C., Chu, D., Heilbronn, C., Lloyd, B., Pantelic, J. *et al.* (2014) Absence of detectable influenza RNA transmitted via aerosol during various human respiratory activities – experiments from Singapore and Hong Kong. *PLoS ONE* **9**, e107338.
Tellier, R. (2006) Review of aerosol transmission of influenza A virus. *Emerg Infect Dis* **12**, 1657–1662.
Tseng, C.C. and Li, C.S. (2005) Collection efficiencies of aerosol samplers for virus-containing aerosols. *J Aerosol Sci* **36**, 593–607.
Usachev, E.V., Pankova, A.V., Rafailova, E.A., Pyankov, O.V. and Agranovski, I.E. (2012) Portable automatic bioaerosol sampling system for rapid on-site detection of targeted airborne microorganisms. *J Environ Monit* **14**, 2739–2745.
Usachev, E.V., Usacheva, O.V. and Agranovski, I.E. (2013) Surface plasmon resonance-based real-time bioaerosol detection. *J Appl Microbiol* **115**, 766–773.
Usachev, E.V., Agranovski, E., Usacheva, O.V. and Agranovski, I.E. (2015) Multiplexed surface plasmon resonance based real time viral aerosol detection. *J Aerosol Sci* **90**, 136–143.
Verreault, D., Moineau, S. and Duchaine, C. (2008) Methods for sampling of airborne viruses. *Microbiol Mol Biol R* **72**, 413–444.
Vincent, J.H. (2005) Health-related aerosol measurement: a review of existing sampling criteria and proposals for new ones. *J Environ Monit* **7**, 1037–1053.
Walls, H.J., Ensor, D.S., Harvey, L.A., Kim, J.H., Chartier, R.T., Hering, S.V., Spielman, S.R. and Lewis, G.S. (2016) Generation and sampling of nanoscale infectious viral aerosols. *Aerosol Sci Technol* **50**, 802–811.
Walls, H.J., Kim, J.H., Yaga, R.W., Harvey, L.A., Haines, L.G., Ensor, D.S., Hering, S.V., Spielman, S.R. *et al.* (2017) Long-term viable bioaerosol sampling using a temperature- and humidity-controlled filtration apparatus, a laboratory investigation using culturable *E. coli*. *Aerosol Sci Technol* **51**, 576–586.
Wang, S.C. and Flagan, R.C. (1990) Scanning electrical mobility spectrometer. *Aerosol Sci Technol* **13**, 230–240.
Wells, K.H., Latino, J., Gavalchin, J. and Poiesz, B.J. (1991) Inactivation of human immunodeficiency virus type 1 by ozone in vitro. *Blood* **78**, 1882–1890.
WHO (2014) *Infection Prevention and Control of Epidemic-and Pandemic-Prone Acute Respiratory Infections in Health Care*. Geneva, Switzerland: World Health Organization.
Willeke, K., Lin, X.J. and Grinshpun, S.A. (1998) Improved aerosol collection by combined impaction and centrifugal motion. *Aerosol Sci Technol* **28**, 439–456.
Williams, R. A., Savage, C. E. and Jones, R. C. (1994). A comparison of direct electron-microscopy, virus isolation and a DNA amplification method for the detection of avian infections laryngotracheitis virus in-field material. *Avian Pathol*, **23**, 709–720.
Xu, Z. and Yao, M. (2013) Monitoring of bioaerosol inhalation risks in different environments using a six-stage Andersen sampler and the PCR-DGGE method. *Environ Monit Assess* **185**, 3993–4003.
Xu, Z.Q., Wu, Y., Shen, F.X., Tan, M. and Yao, M. (2011) Bioaerosol science, technology, and engineering: past, present, and future. *Aerosol Sci Technol* **45**, 1337–1349.
Xu, Z., Shen, F., Li, X., Wu, Y., Chen, Q., Jie, X. and Yao, M. (2012) Molecular and microscopic analysis of bacteria and viruses in exhaled breath collected using a simple impaction and condensing method. *PLoS ONE* **7**, e41137.
Yoo, K.H., Lee, J.S. and Oh, M.D. (1997) Charging and collection of submicron particles in two-stage parallel-plate electrostatic precipitators. *Aerosol Sci Technol* **27**, 308–323.
Yu, H., Afshar-Mohajer, N., Theodore, A.D., Lednicky, J.A., Hugh Fan, Z. and Wu, C.Y. (2018) An efficient virus aerosol sampler enabled by adiabatic expansion. *J Aerosol Sci* **117**, 74–84.
Zhao, Y., Aarnink, A.J.A., Wang, W., Fabri, T., Groot Koerkamp, P.W. and de Jong, M.C (2014) Airborne virus sampling – efficiencies of samplers and their detection limits for infectious bursal disease virus (IBDV). *Ann Agric Environ Med* **21**, 464–471.
Zhen, H., Han, T., Fennell, D.E. and Mainelis, G. (2014) A systematic comparison of four bioaerosol generators: affect on culturability and cell membrane integrity when aerosolizing *Escherichia coli* bacteria. *J Aerosol Sci* **70**, 67–79.
Zuo, Z., Kuehn, T.H., Verma, H., Kumar, S., Goyal, S.M., Appert, J., Raynor, P.C., Ge, S. *et al.* (2013) Association of airborne virus infectivity and survivability with its carrier particle size. *Aerosol Sci Technol* **47**, 373–382.