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 3.1 × 10⁻³ m s⁻¹ for 10-μm particles to 3.5 × 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
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 (Lendicky 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 biotreats 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 (Oursini 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 (Lindsay 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
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<sub>10</sub> (particulate matter ≤10 μm) and 0–40% for PM<sub>2.5</sub> (particulate matter ≤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) (Lindsey et al. 2006) is a multistage cyclone operating at a flowrate of 3.5 l min<sup>−1</sup>: 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 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
reeaerosolization 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 (Lindsay 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 × 200 × 250 nm³ (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 nanoparticle 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

| Collection methods | Impactors and options | Filters | Liquid impingers | Water-based condensation | Other devices |
|---------------------|----------------------|---------|-----------------|--------------------------|---------------|
|                      |                      |         |                 |                          | Good for specific types of viruses |
|                      |                      |         |                 |                          | High for sampling viruses not fully evaluated |
|                      |                      |         |                 |                          | Low efficiency for small virus particles |
|                      |                      |         |                 |                          | Bulky |
|                      |                      |         |                 |                          | Complicated to operate |
|                      |                      |         |                 |                          | Efficiency for particle from 20 nm to 10 μm or even larger |
|                      |                      |         |                 |                          | Maintain viability of viruses |
|                      |                      |         |                 |                          | Efficiencies for sampling viruses not fully evaluated |
|                      |                      |         |                 |                          | Low efficiency for submicrometre particles |
|                      |                      |         |                 |                          | Ozone formation deactivate viruses |
|                      |                      |         |                 |                          | Efficiencies for sampling viruses not fully evaluated |
|                      |                      |         |                 |                          | Low efficiency for small virus particles |
|                      |                      |         |                 |                          | Efficiencies for sampling viruses not fully evaluated |
|                      |                      |         |                 |                          | Low efficiency for small virus particles |
|                      |                      |         |                 |                          | Ozone formation deactivate viruses |
|                      |                      |         |                 |                          | Efficiency 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 (Benburg 1971). Biological collection efficiency of these samplers also strongly depends on the sampling conditions, aerosolization 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 (Lindicky 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 (Verreaut 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 Sizer 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 Gehhart 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 $7 \times 10^7$ 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)
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 range of virus aerosols, from 20 nm to >10 μm.

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

| Knowledge gap                                      | Recommended action                                                                 |
|----------------------------------------------------|------------------------------------------------------------------------------------|
| Increasing the collection efficiency of the sampler for a wide range of virus aerosols, from 20 nm to >10 μm |  |
| Decrease re-aerosolization, 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) |  |
| 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 |  |
| Integrate sensitive, rapid and preferably, real-time detection with air sampling devices |  |

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.

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