




Development of an efficient viral aerosol collector for higher sampling flow rate

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Abstract

Viral aerosol infection through cough generates large amounts of viral aerosol and can result in many adverse health effects such as influenza flu and severe acute respiratory syndrome (SARS). To characterize the coughed viral aerosol, the sampler needs to sample at higher flow rate and possess high physical collection efficiency as well as high viral preservation. However, most current inertia-based high flow bioaerosol samplers are not suited for viral aerosol sampling since the viability will be lost doing the sampling process. Current condensation growth methods only have good physical collection efficiency and viral preservation at low flow rate (< 10 LPM). In this study, we developed a viral aerosol sampling system using a cooler and steam-jet aerosol collector (SJAC) for bioaerosol collection for the first time. The system is based on mixing condensation growth method and has high viral preservation at a higher flow rate (12.5 LPM). We control the inlet aerosol flow temperature and the SJAC mixing reservoir temperature to improve the physical collection efficiency and viability preservation of the viral aerosol. Results indicate that the physical collection efficiency is 70–99% for aerosol 30–100 nm when the aerosol flow and mixing reservoir temperature was 19 and 50 °C, respectively. In addition, the system was 7 and 22 times more efficient for viability preservation of MS2 bacteriophage than the commonly used All Glass Impinger 30 (AGI-30) and BioSampler®, respectively. Finally, the system can be applied to sample at a lower concentration (10^5 PFU/m³), and results shows the system was 4.7 times more efficient for viability preservation than using AGI-30 alone. The developed viral collection system will improve our understanding of the characteristics of coughed aerosol and can be used for future evaluation of respiratory protective equipment and environmental sampling.

Keywords Viral aerosol · Steam-jet aerosol collector · Condensation growth · Bacteriophages · Higher flow rate · Viral preservation

Introduction

Viral aerosol spreads infectious diseases such as severe acute respiratory syndrome (SARS) and influenza, which can have

significant adverse health effects (Lee et al. 2003; Lipsitch et al. 2003; Thompson et al. 2003). Considerable global economic losses and public fear are also associated with viral aerosol infection. In 2008, 90 million children were infected by influenza which can result in acute lower respiratory infections, birth defects, and even death. Among the recorded number of deaths, 99% occurred in developing countries (Liang et al. 2017; Osterhaus et al. 2015). Another example is the outbreak of pandemic influenza H1N1 in 2009, resulting in an estimated 201,200 of respiratory deaths and an additional 83,300 cardiovascular deaths worldwide (Dawood et al. 2012). Viral aerosol originated from cough has initial sizes of 1–100 μm, but evaporates to droplet nuclei of 20–300 nm in a short amount of time (Kowalski et al. 1998; Morawska 2006; Tseng and Li 2005). Due to their small size, viral aerosol can penetrate deep into the lungs and are less easily removed by macrophages (Buzea et al. 2007). In addition, viral aerosol can remain airborne for hours to

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days (Verreault et al. 2008), adding the chance of infection. The viability of the aerosol is also affected by the content of salt concentration in saliva and relative humidity during droplet evaporation (Yang et al. 2012). Agencies such as the US and European Centers for Diseases Control (CDC) and World Health Organization (WHO) have expressed urgent need for research in influenza virus transmission (see references within (Bischoff et al. 2013)). Therefore, detecting the viral aerosol concentration and viability is critical in identifying and preventing disease transmission in public health.

Some commonly used instruments for viral aerosol collection are the All Glass Impinger 30 (AGI-30) and SKC BioSampler® (Willeke et al. 1998). AGI-30 and BioSampler® collect aerosol by liquid impingement and tangential impingement, respectively. An ideal viral aerosol sampler should possess both high physical collection efficiency and good viability preservation. However, studies point out that for aerosol 30–100 nm, AGI-30 and BioSampler® have physical collection efficiency less than 10% (Hogan et al. 2005). Using filter and electrostatic precipitators can lead to higher physical collection efficiency for viral aerosol, but can damage the viability of viral aerosol (Kettleston et al. 2009; Roux et al. 2016; Yang et al. 2011). Recently, condensation growth method gained significant attention in viral aerosol collection due to its ability to possess high physical collection efficiency and viability preservation. Condensation growth method helps “grows” the aerosol to a larger size that can be easier for collection or analysis. Another advantage of the condensation growth methods is that the bioaerosol is collected in liquid phase which is beneficial for later biochemical analysis (Alvarez et al. 1995). Several studies have achieved promising results using this principle at lower flow rates (< 10 LPM). When pairing the condensation growth method with the AGI-30 or BioSampler®, the physical collection efficiency and viability preservation of viral aerosol can be significantly enhanced. For instance, when adding condensation growth methods such as the mixing-type bioaerosol amplification unit (mBAU) and growth tube collector (GTC) to the BioSampler®, the collected viable MS2 bacteriophage efficiency increased 2–3 and 10–100 times, respectively (Oh et al. 2010; Pan et al. 2016). However, at higher flow rates (> 10 LPM) as compared to button samplers or deposition devices, applying condensation growth methods for viral aerosol collection were only similar, not significantly better than using AGI-30 impinger and BioSampler® alone (McDevitt et al. 2013; Milton et al. 2013). For example, Gesundheit II (G-II) is inefficient for collecting aerosol smaller than 50 nm. However, many viral aerosols have sizes smaller than 50 nm. Therefore, when using G-II for sampling coughed influenza virus, the system has a high physical collection efficiency, but a low viability preservation (10%) (Hatagishi et al. 2014). Adopting higher sampling flow rate can increase the amount of sampled air and improve the limit of detection (LOD). In addition, a higher flow rate viral aerosol sampler is also needed for sampling exhaled aerosol during tidal breathing. Nevertheless, most of the current

bioaerosol sampler that uses condensation growth methods cannot increase the viable aerosol collection efficiency and viability preservation of the BioSampler® at flow rates higher than 10 LPM.

One option is to use the steam-jet aerosol collector (SJAC) which is also based on condensation growth methods for viral aerosol collection at higher flow rates. SJAC has been widely used for ambient aerosol collection in different campaigns (Rees et al. 2004; Slanina et al. 2001). SJAC has a wide range of sampling flow rate from 10 to 50 LPM, enabling aerosol sampling at higher flow rates (Khlystov et al. 2000). For aerosol larger than 20 nm, SJAC has a physical collection efficiency over 99% for concentration up to $6 \times 10^5 \text{ #/cm}^3$ (Khlystov et al. 1995; van Rens et al. 2007). In this study, we will collect viral aerosol by pairing the SJAC with the AGI-30 and BioSampler® for the first time. In addition, we will test this system at a lower viral aerosol concentration to simulate ambient conditions. The results will help future viral aerosol collection using condensation growth methods at higher flow rate and for ambient environments.

Materials and methods

Steam-jet aerosol collector

The steam-jet aerosol collector (Fig. 1) is made up of two main parts, the mixing reservoir and cyclone. Aerosol flow mixes with the steam vapor flow in the mixing reservoir, in which supersaturation is reached and condensation growth occurs. The enlarged aqueous phase aerosol is collected by the cyclone connected behind the mixing reservoir. In this study, we only use the mixing reservoir of the SJAC which is made of stainless steel. The cyclone is replaced by the AGI-30 or BioSampler® for viral aerosol collection. We mounted a 500-ml three-neck round-bottom flask

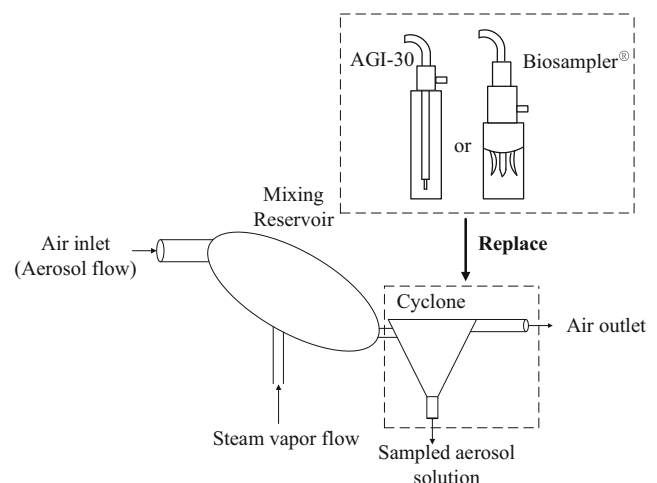


Fig. 1 Schematic figure of the SJAC; the left half is the SJAC mixing reservoir. The right half is the SJAC collection cyclone which is replaced by AGI-30 or BioSampler® in this study

(Pyrex, Tewksbury, MA, USA) which is half filled with ultrapure water (Reference Water Purification System, Milli-Q®, Kenilworth, NJ, USA) to a heating mantle (NewLab Instrument Co., Ltd., New Taipei City, Taiwan). The SJAC is connected to the center of the flask neck. We also inserted a temperature controller (HT-720, Newlab Co Ltd., New Taipei City, Taiwan) and temperature sensor in the side flask to control and monitor the temperature.

Condensation growth in the SJAC mixing reservoir

Condensation growth occurs under supersaturated vapor phase. This occurs when the saturation ratio (S) is larger than 1. Saturation ratio is given as follows:

$$S = \frac{p_i}{p_{sat,T}} \quad (1)$$

where p_i is the vapor partial pressure and $p_{sat,T}$ is the saturation vapor pressure at temperature T . Supersaturation can be reached through mixing condensation (i.e., mixing two flows with different temperatures, relative humidity, and flow rates) (Kousaka et al. 1982; Okuyama et al. 1984). Assume flow one (aerosol flow) has temperature (T_{sl}), relative humidity (H_{sl}), flow rate (Q_{sl}), and specific heat (C_{sl}); flow two (vapor flow) has temperature (T_{sh}), relative humidity (H_{sh}), flow rate (Q_{sh}), and specific heat (C_{sh}). When aerosol flow and vapor flow mix with each other, saturation point i is initially reached. At point i , according to the enthalpy and mass balance equation, the temperature (T_i), relative humidity (H_i), flow rate (Q_i), and specific heat (C_i) become

$$T_i = \frac{C_{sh}T_{sh}Q_{sh} + C_{sl}T_{sl}Q_{sl}}{C_{sh}Q_{sh} + C_{sl}Q_{sl}} \quad (2)$$

$$H_i = \frac{Q_{sh}H_{sh} + Q_{sl}H_{sl}}{Q_{sh} + Q_{sl}} \quad (3)$$

$$Q_i = Q_{sh} + Q_{sl} \quad (4)$$

$$C_i = \frac{C_{sh}Q_{sh} + C_{sl}Q_{sl}}{Q_i} \quad (5)$$

The saturation point i then moves adiabatically towards point f as condensation progresses; during this process, the amount of vapor (ΔH) that condenses on the aerosol is

$$\Delta H = H_i - H_{sf} = \frac{C_i}{\lambda} (T_{sf} - T_i) \quad (6)$$

where λ is the latent heat. The temperature and relative humidity at point f obtained from the saturation vapor curve are T_{sf} and H_{sf} , respectively. According to Eqs. 2–6, at the same vapor flow condition (i.e., same T_{sh} , H_{sh} , Q_{sh} , and C_{sh}) and fixed T_{sf} , lower temperature in the aerosol flow (T_{sl}) will result in a lower T_i . Therefore, ΔH will become larger, indicating more vapors condensing to the aerosol and enhancing aerosol growth.

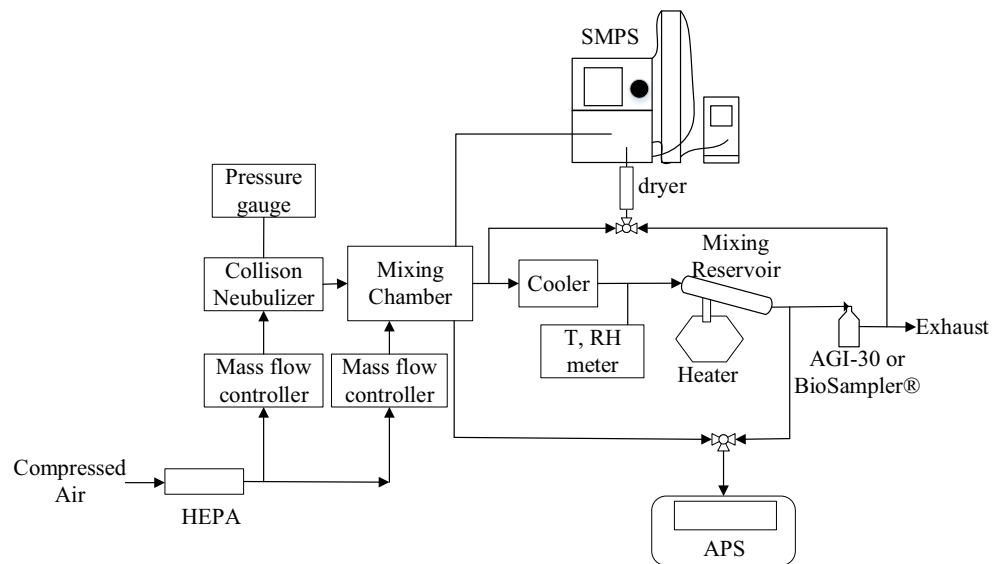
Test virus

We used single-stranded RNA (ssRNA) virus MS2 bacteriophage (cat #70235) obtained from the Bioresource Collection and Research Center Food Industry Research and Development Institute (BCRC, P.O. Box 246, Hsinchu, Taiwan) as the test viral aerosol. MS2 bacteriophage has aerodynamic diameter around 28 nm and can only infect male *Escherichia coli* (BCRC#50354) (Hogan et al. 2004). In addition, the high stock titer of MS2 bacteriophage has the advantage of better virus detection and lower measurement uncertainty (Ge et al. 2014). Therefore, MS2 bacteriophage is widely used as a viral aerosol surrogate (Balazy et al. 2006; Walker and Ko 2007). Plaque assay technique was used to characterize the collected MS2 bacteriophage concentration, and each sample was assayed in duplicates. All the plates were incubated at 37 °C over 12 h using the spread plate method (Cormier and Janes 2014). We used ultrapure water to dilute the supernatant to a titer of 10^9 plaque-forming units (PFUs)/ml for viral aerosol generation. Note that 10^9 PFU/ml is much higher than in real-life environments, but this concentration works better for viral culture when testing the SJAC system. In addition, we also used a lower MS2 concentration (i.e., a titer of 10^4 PFU/ml) to simulate real-life situations.

Experimental setup

The schematic figure of the experimental setup is shown in Fig. 2. We used a six-jet collision nebulizer (model CN25, BGI Inc., Butler, NJ, USA) to generate MS2 bacteriophage at flow rate of 10 LPM, which is controlled by a mass flow controller (model 5850E, Brooks Instrument, Hatfield, PA, USA). A pressure gauge (model: SGZ-D10422N, Dwyer, Michigan City, IN, USA) is attached to the collision nebulizer to monitor the pressure. The generated aerosol flow then mixes with 12 LPM of aerosol-free dry air (by connecting with a HEPA filter (model 12144, Pall Corporation, Port Washington, NY, USA) in a 20-l mixing chamber to remove moisture. A scanning mobility particle sizer (SMPS, DMA model 3081 and CPC model 3787, TSI Inc., USA) was used to monitor the concentration at the mixing chamber. The scan time of the SMPS was set to 2 min (90 s upscan/30 s downscan) for aerosol size range 5.8–228.8 nm. The mixed air flow then enters the cooler (18 cm wide, 17 cm high, and 90 cm in length) which is filled with ice and can cool the aerosol flow from 22 to 19 °C. The ideal condensation growth condition would be at a lower aerosol flow temperature, preferably close to 4 °C (Olson et al. 2004). However, our current setup only allows our aerosol flow temperature to reach 19 °C. At a lower aerosol flow temperature (19 °C), there is a larger temperature difference between the cooling section and the SJAC mixing reservoir, thus facilitating condensation growth on the viral aerosol (Kousaka et al. 1982). We wrapped the copper tube between the cooling section and the SJAC inlet with a thermal insulator made of polyethylene to maintain constant

Fig. 2 A sketch of the experimental setup. Note that for the control case without the SJAC, the SJAC is still attached to the system. The only difference for with and without SJAC is that the heater of the SJAC is turned on and off, respectively



temperature of the aerosol flow. We also monitored the temperature and relative humidity with a sensor (HMT 330 Series, Vaisala, Helsinki, Finland) at the SJAC inlet. The air flow enters the SJAC at flow rate of 12.5 LPM. Note that the residence time of the aerosol flow in the cooling section and SJAC mixing reservoir are 0.14 and 0.19 s, respectively. The AGI-30 or the BioSampler® was connected behind the SJAC to collect the MS2 bacteriophage. Ultrapure water was used as the collection medium. We used a thermometer (PT100, PU-YANG Electronics Co., Taoyuan, Taiwan) to monitor the temperature in the SJAC mixing reservoir.

In the following physical collection efficiency, particle growth, and viability preservation experiments, we examined the MS2 bacteriophage viability preservation at different temperatures of aerosol flow (19 and 22 °C) and SJAC mixing reservoir (37, 45, and 50 °C). Note that throughout each temperature

combination, the SMPS concentration and mode was within 10% of $2.5 \times 10^6 \text{ #/cm}^3$ and 40 nm, respectively.

Evaluation of physical collection efficiency and particle growth

Figure 2 is the experimental setup for measuring physical collection efficiency and particle growth. We measured the physical collection efficiency (*E*) for different particle sizes before and after the collector (i.e., the cooler, SJAC, and AGI-30 or Biosampler®) using the SMPS (DMA model 3081 and CPC model 3010, TSI Inc., USA). At each temperature combination, we performed three sets of measurement. Each set contains one upstream and one downstream scan; each scan is 90 s (60 s upscan/30 s downscan). The physical collection efficiency is given as follows:

$$E(\%) = \left(1 - \frac{\text{Number concentration after collector at different sizes}}{\text{Number concentration before collector at different sizes}} \right) \times 100\% \tag{7}$$

To evaluate the particle growth, an aerodynamic particle sizer (APS, model 3221, TSI Inc., USA) was connected to the two sampling ports located before and after the cooler and SJAC. The two sampling ports were connected to the APS through a three-way valve, allowing us to switch the sample airflow between the two ports. At each temperature combination, we performed three sets of measurement (APS scan upstream and downstream of the SJAC, each scan is 10 s).

Determination of viability preservation

The viability of the collected MS2 bacteriophage in AGI-30 and BioSampler® was determined by using the PFUs. We

calculated the PFUs with and without the SJAC. It should be noted that in both scenarios, the SJAC and cooler were both attached to the system as seen in Fig. 2. The only difference is in the case without SJAC; the heater of the SJAC was turned off. We can then obtain the viral aerosol collection enhancement factor (VCEF) given as follows:

$$VCEF = \frac{PFU_{SJAC-on}}{PFU_{SJAC-off}} \tag{8}$$

PFU_{SJAC-on} indicates that the heater of the SJAC is turned on and PFU_{SJAC-off} means that the heater of the SJAC is turned off. VCEF values were used to evaluate the viability preservation enhancement when using the SJAC. Higher VCEF

values indicate that the cooler and SJAC improve the viable MS2 bacteriophage viability preservation over using AGI-30 or BioSampler® alone. We also investigated how different sampling time affect the VCEF values. All data points were generated with three replicates.

Application of SJAC system to real life

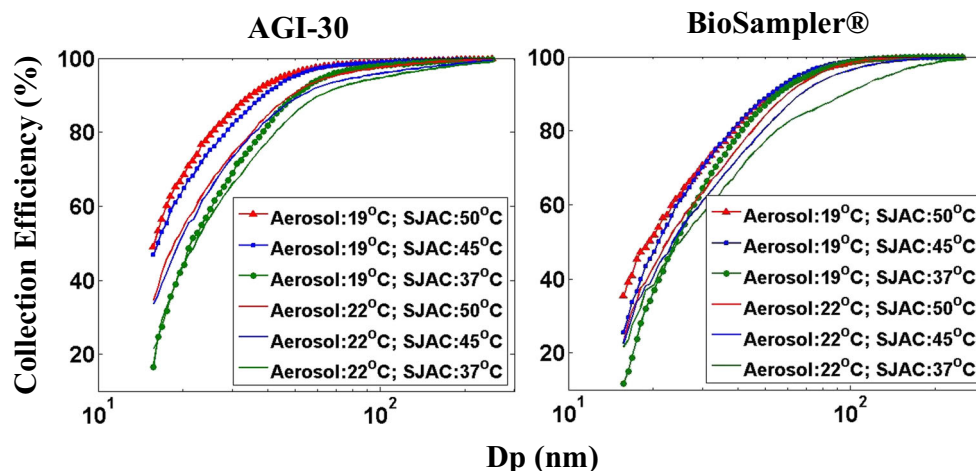
To simulate the real-life viral aerosol concentration, a lower MS2 concentration was used. In ambient concentration, the viral aerosol concentration is 10^5 – 10^7 #/m³ (Prussin et al. 2015; Whon et al. 2012). We first dilute the supernatant to a titer of 10^4 PFU/ml with ultrapure water for viral aerosol generation from the nebulizer. This is equivalent to a concentration of 10^5 PFU/m³ in the mixing chamber. The viability and total virus concentration were determined by using PFUs. We used a longer sampling time (30 min) as recommended by other studies to collect more viral aerosol for later analysis, but not too long as to reduce the physical collection of AGI-30 (Macher et al. 1995; Willeke et al. 1998). The aerosol flow and SJAC mixing reservoir temperatures were set to 19 and 45 °C, respectively.

Results and discussion

SJAC physical collection efficiency and size enlargement

Figure 3 shows the physical collection efficiency of various particle sizes under different temperature combinations of aerosol flow and SJAC mixing reservoir. Overall, for aerosol larger than 30 nm, the physical collection efficiency was over 66 and 57% for AGI-30 and Biosampler®, respectively. The highest physical collection efficiency (70–99% for AGI-30 and Biosampler® when the aerosol was larger than 30 nm) was achieved when the aerosol flow and SJAC mixing

Fig. 3 The physical collection efficiency of AGI-30 (left panel) and BioSampler® (right panel) under different combinations of aerosol flow and SJAC reservoir temperature



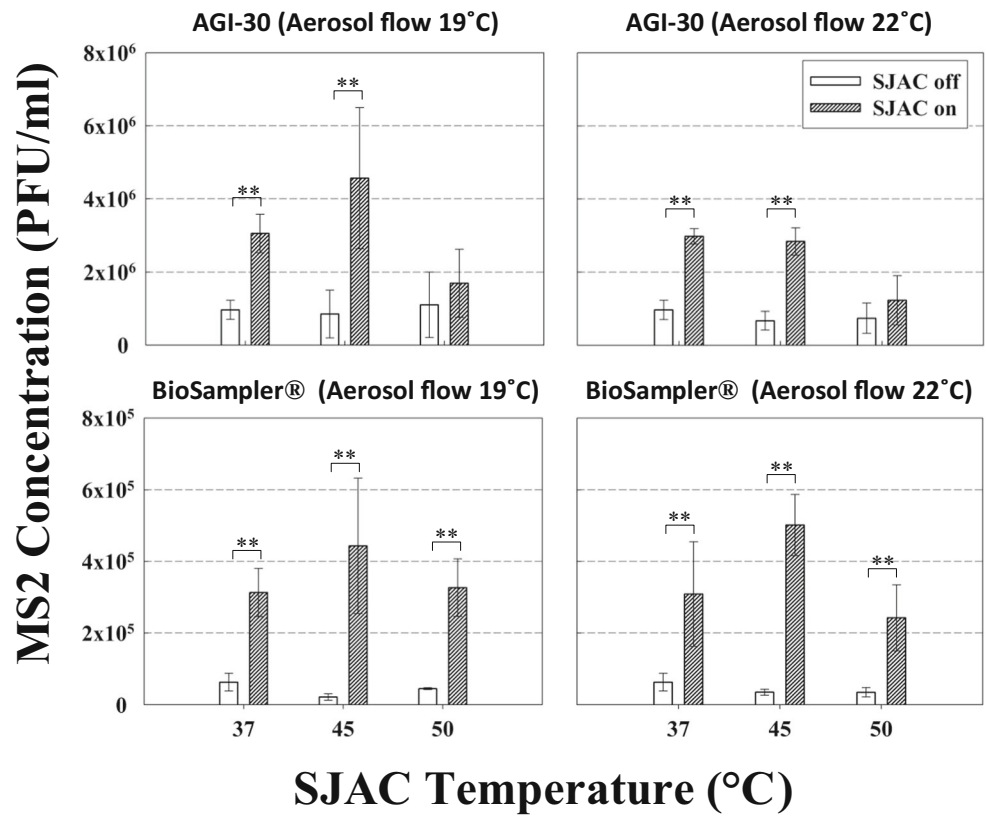
reservoir temperature was 19 and 50 °C, respectively. This is a significant improvement as compared to using the AGI-30 or Biosampler® alone where the physical collection efficiency is less than 10% in the 30–100-nm range (Hogan et al. 2005). Moreover, using AGI-30 as a viral aerosol collector in the SJAC system has a higher physical collection efficiency over Biosampler®. In addition, at the same SJAC mixing reservoir temperature, the physical collection efficiency was higher in both AGI-30 and Biosampler® when the aerosol flow temperature was 19 °C as compared to 22 °C, indicating that more condensation growth in viral aerosol occurred.

Figure 4 shows the aerosol concentration and size distribution of MS2 bacteriophage in front and behind the SJAC under different temperature combinations. Higher SJAC mixing reservoir temperature increased the aerosol number concentration and mode by enhancing aerosol growth. In addition, lower temperature in the aerosol flow showed more condensation growth also resulted in a higher aerosol number concentration. The largest growth was seen when the aerosol flow and SJAC mixing reservoir temperatures were 19 and 50 °C, respectively. During which, the concentration and mode from APS measurements reached 6.3×10^4 #/cm³ and 1.6 μm, respectively. However, the VCEF decrease in this scenario, and this could also be attributed to the high temperature that can damage the viability of MS2 bacteriophage.

Collected viable MS2 bacteriophage in the sampler

Figure 5 shows the collected viable MS2 bacteriophage concentration in the water medium of AGI-30 and BioSampler® over the 15 min of sampling duration. During the 15-min sampling interval, a total of 187.5 l of air was sampled. Assuming indoor viral aerosol concentration to be 10^5 #/m³ and physical collection efficiency of around 66% using SJAC system, a total of 12,375 would be sampled. This amount sampled is higher than the 3375 viral aerosol threshold dose needed to induce infection (Yang et al. 2011). However,

Fig. 5 The concentration of viable MS2 bacteriophage collected with and without connecting the SJAC mixing reservoir to AGI-30 (upper panel) and BioSampler® (lower panel). The aerosol flow temperature was set to 19 and 22 °C in the left and right panel, respectively. The error bar represents one standard deviation and the double stars represent the level of significance ($P < 0.01$)



15 min of sampling time may not be enough for area where the viral aerosol concentration is lower than 10^5 #/m^3 . A longer sampling time may be needed. In general, adding the cooler

and SJAC can significantly increase ($P < 0.01$) the collected viable MS2 bacteriophage concentration in AGI-30 and BioSampler® in most of the temperature combinations. It

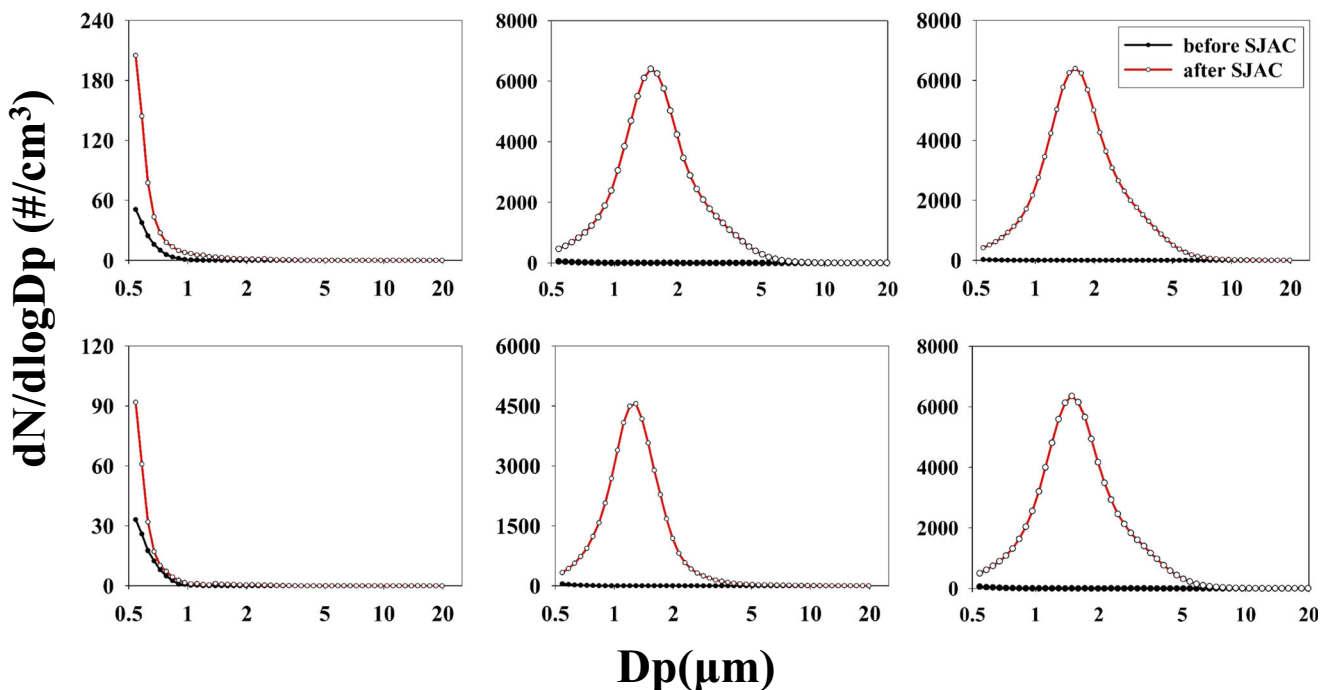


Fig. 4 Viral aerosol size distribution and number concentration before and after the SJAC mixing reservoir at different temperatures in the aerosol flow and SJAC mixing reservoir. The aerosol flow temperature

in the upper and lower panels was 19 and 22 °C, respectively. From left to right, the SJAC mixing reservoir temperature was set to 37, 45, and 50 °C, respectively

Table 1 VCEF values at different operating parameters

Aerosol flow T SJAC T	AGI-30		BioSampler®	
	19°C	22°C	19°C	22°C
37°C	3.31 ± 0.79	3.26 ± 0.81	5.27 ± 1.25	4.77 ± 0.47
45°C	6.69 ± 2.11	4.54 ± 1.02	21.59 ± 6.11	15.04 ± 4.14
50°C	1.94 ± 0.63	1.69 ± 0.18	7.39 ± 2.13	7.32 ± 2.08

*VCEF values were expressed as the mean ± standard deviation.

should be noted that when the SJAC mixing reservoir temperature was 50 °C, no significant increase in the collected viable MS2 bacteriophage concentrations was found in AGI-30. This could be attributed to the higher SJAC mixing reservoir temperature affecting the viability of the MS2 bacteriophage. Researches have indicated that high temperature can result in lost of infectivity of the MS2 bacteriophage (Anders and Chrysikopoulos 2006; Brie et al. 2016; Verreault et al. 2015). Another reason may be attributed to the relatively large error bar at 50 °C from AGI-30. Also, note that the collected viable MS2 bacteriophage concentration in liquid media from AGI-30 was higher than the BioSampler®, and this is similar to other studies that show AGI-30 has a higher viable collection efficiency at higher flow rates (> 9 LPM) (Anwar 2010; Hogan et al. 2005).

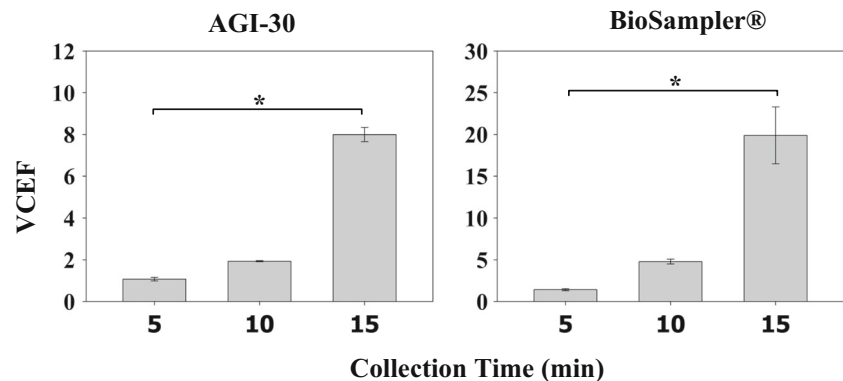
VCEF values

Table 1 shows the VCEF values under different temperature combinations. When the aerosol flow temperature was at 19 °C, larger VCEF values were found as compared to 22 °C. When the SJAC mixing reservoir temperatures were 37–50 °C, the VCEF values were 1.69–6.69 and 4.77–21.59 for AGI-30 and BioSampler®,

respectively. The larger temperature difference between the aerosol flow temperature and SJAC mixing reservoir resulted in a higher viability preservation and viral aerosol condensation growth. This is consistent with the mixing condensation theory mentioned in “Materials and methods” (Kousaka et al. 1982). In addition, the VCEF was highest when the SJAC mixing reservoir temperature was 45 °C instead of 50 °C. At 50 °C, the hot steam vapor from the SJAC may damage the MS2 bacteriophage viability during the condensation growth process.

The VCEF values from this study are mostly higher than the results from the mixing-type bioaerosol amplification unit (mBAU) which has VCEF around 2–3 (Oh et al. 2010), and both studies have a sampling flow rate of 12.5 LPM and use the BioSampler® as the reference sampler. However, the VCEF values herein are mostly lower than the growth tube collector (GTC) (Pan et al. 2016). The GTC has a lower sampling flow rate (7 LPM) compared to this study (12.5 LPM). At higher flow rate, according to Eqs. 2–6, the amount of vapor ΔH condensing on the viral aerosol is smaller, leading to smaller VCEF values. Furthermore, at higher flow rate, the residence time in the SJAC mixing reservoir was also smaller, leading to less time for aerosol condensation growth.

Fig. 6 The viability of MS2 bacteriophage collected using different sampling periods with and without connecting the SJAC mixing reservoir to AGI-30 (left panel) and BioSampler® (right panel). The error bar represents one standard deviation and the stars represent the level of significance ($P < 0.01$)



MS2 bacteriophage viability over different sampling periods

Here, we compared the MS2 bacteriophage viability over different sampling periods (5, 10, and 15 min). Note that 15 min of sampling time is often used for both AGI-30 and BioSampler® (Riemenschneider et al. 2010). The temperature in the aerosol flow and SJAC mixing reservoir was set to 19 and 45 °C, respectively, in this experiment. From Fig. 6, VCEF values increased with increment sampling time for both AGI-30 and BioSampler®. The largest increase was seen between 10 and 15 min. The VCEF values while sampling for 15 min were significantly higher ($P < 0.01$) than sampling for 5 min from the Kruskal-Wallis test. The VCEF result for 15 min sampling duration is similar to another study using the BioSampler®, but with a lower flow rate (Pan et al. 2016).

The AGI-30 and BioSampler® can be used for long sampling duration up to 30 min and 8 h, respectively. As the sampling time increases, the collection efficiencies of the AGI-30 and BioSampler® decrease due to aerosol re-aerosolization (Hogan et al. 2005). At longer sampling periods for viral aerosol, adding the cooler and SJAC to the sampling apparatus could provide better results. This shows the potential of applying the cooler and SJAC to viral aerosol collection. This further indicates the feasibility of using condensation growth method for viral aerosol collection at higher flowrate (> 10 LPM).

SJAC system application in real-life conditions

When sampling at 10^5 PFU/m³ in the mixing chamber for 30 min with AGI-30 sampler, the aerosol flow and SJAC mixing reservoir temperatures were set to 19 and 45 °C, respectively. The collected MS2 concentrations in the AGI-30 were 527 and 113 PFU/ml with and without using the SJAC, respectively. This is equivalent to a VCEF value of 4.7. The system shows the ability to enhance the viability preservation of AGI-30 sampler during low viral aerosol concentration. Therefore, the SJAC system can be applied to sampling real-life conditions where the viral aerosol concentration is lower. However, more field studies is needed to verify the SJAC system.

Conclusions

Significant improvements in viral aerosol physical collection efficiency and viability preservation were seen when adding the cooler and SJAC to the AGI-30 and BioSampler® during aerosol collection. The SJAC system's physical collection efficiency is 57–99% for viral aerosol larger than 30 nm. When the temperature of the aerosol flow was at 19 °C and the SJAC mixing reservoir was 45 °C, the VCEF are 7 and 22 for AGI-30 and BioSampler®, respectively. Furthermore, adding the cooler and SJAC to AGI-30 and BioSampler® can

significantly increase the VCEF values when the sampling time increased from 5 to 15 min. This shows the potential to use this sampling system for viral aerosol sampling for a longer time period. Finally, the SJAC system has VCEF of 4.7 when the MS2 concentration was as low as 10^5 PFU/m³, indicating that the SJAC system can be applied to future ambient environment sampling.

However, the study is limited to flow rates of 12.5 LPM. Future studies on even higher inlet flow rates (preferably over 100 LPM) using condensation methods are also needed for sampling exhaled aerosol during tidal breathing. In addition, application of the SJAC to other coughed viral aerosol collections such as influenza virus is also needed. Furthermore, additional measures such as staining methods for viability or qPCR/ddPCR tools for assessment of total viral particles are also needed to strengthen the study. The work shows the potential of SJAC system in the viral aerosol collection at higher flow rates which can facilitate our understanding towards environmental viral aerosol. This viral collection system can potentially benefit future infectious disease modeling and prevention work.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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