Utility of Three Nebulizers in Investigating the Infectivity of Airborne Viruses

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ABSTRACT

Laboratory-generated bioaerosols are widely used in aerobiology studies of viruses; however, few comparisons of alternative nebulizers exist. We compared aerosol production and virus survival for a Collison nebulizer, vibrating mesh nebulizer (VMN), and hydraulic spray atomizer (HSA). We also measured the dry size distribution of the aerosols produced and calculated the droplet sizes before evaporation and the dry size distribution from normal saline solution. Dry count median diameters of 0.11, 0.22, and 0.30 μm were found for normal saline from the Collison nebulizer, VMN, and HSA, respectively. The volume median diameters were 0.323, 1.70, and 1.30 μm, respectively. The effect of nebulization on the viability of two influenza A viruses (IAVs) (H1N1 and H3N2) and human rhinovirus 16 (HRV-16) was assessed by nebulization into an SKC BioSampler. The HSA had the least impact on surviving fractions (SFs) of H1N1 and H3N2 (89% ± 6% and 94% ± 2%, respectively), followed by the Collison nebulizer (83% ± 1% and 82% ± 2%, respectively). The VMN yielded SFs of 78% ± 2% and 76% ± 2%, respectively. Conversely, for HRV-16, the VMN produced higher SFs (87% ± 8%). Our findings indicate that there were no statistical differences between SFs of the viruses nebulized by these nebulizers. However, VMN produced higher aerosol concentrations within the airborne size range, making it more suitable where high aerosol mass production is required.

IMPORTANCE

Viral respiratory tract infections cause millions of lost days of work and physician visits globally, accounting for significant morbidity and mortality. Respiratory droplets and droplet nuclei from infected hosts are the potential carriers of such viruses within indoor environments. Laboratory-generated bioaerosols are applied in understanding the transmission and infection of viruses, modeling the physiological aspects of bioaerosol generation in a controlled environment. However, little comparative characterization exists for nebulizers used in infectious disease aerobiology, including Collison nebulizer, vibrating mesh nebulizer, and hydraulic spray atomizer. This study characterized the physical features of aerosols generated by laboratory nebulizers and their performance in producing aerosols at a size relevant to airborne transmission used in infectious disease aerobiology. We also determined the impact of nebulization mechanisms of these nebulizers on the viability of human respiratory viruses, including IAV H1N1, IAV H3N2, and HRV-16.

KEYWORDS

airborne viruses, Collison nebulizer, nebulization, spray atomizer, vibrating mesh nebulizer, influenza, rhinovirus

Respiratory viruses are responsible for a large number of deaths and cause substantive indirect costs on the global economic scale (1, 2). Evidence suggests that the airborne mode of transmission plays a significant role in the spread of respiratory viruses (3, 4). Respiratory droplets and droplet nuclei generated from the human host respiratory system are potential carriers of pathogens within indoor environments (5, 6). However, there is no consistent explanation that fully addresses the ability of these viruses to survive in an
airborne state (7). It has been reported that a combination of environmental and biological factors can affect the efficiency of airborne transmission of viruses in indoor environments (8). In modeling airborne transmission, laboratory-generated pathogen-laden aerosols are broadly used to investigate the transmission, infection, and toxicology of respiratory viruses (8–10). Techniques used to generate virus-laden aerosols in the laboratory enable greater control over aerosol characteristics, including droplet concentration, size, and their ability to carry respiratory infectious viruses (11). Animal models have also been used to investigate the infection course and pathogenesis of inhaled microorganisms (12). Here, the route of exposure, aerosol size, and infectious dose can influence the infection development and pathogenic outcomes. In these models, the method of aerosolization is critical, as reduced virus viability due to mechanical preparation would influence study outcomes. Considering this critical factor, the field lacks a comprehensive study that fully characterizes the physical and biological properties of carrier aerosols generated by laboratory-use nebulizers, particularly for the modeling of respiratory virus-laden aerosols in a clinical environment.

The Collison nebulizer, as the gold standard, has dominated bioaerosol generation research since its invention in 1932 (13–15). It applies Bernoulli’s principle, resulting in impaction of the liquid suspension against the interior of a jar to generate small-size aerosols (27). The type of liquid suspension and its viscosity and surface tension are the principal factors that influence the droplet size and concentration of aerosols generated by a Collison nebulizer (17). Within the literature reporting Collison nebulizer-generated aerosols, the various studies have utilized nebulization solutions with a diverse range of viscosity, surface tension, and solute concentration. These variations, as well as differences in the applied compressed air pressure and flow rate, combined with different aerosol measurement techniques, have resulted in a range of size distributions being reported during Collison use (18–20). It has also been hypothesized that impaction, shear forces, and recirculation of an infectious sample within the Collison nebulizer may damage microorganisms, potentially decreasing pathogen viability or infectivity (21, 22). However, there are no available well-designed studies to test these effects on pathogen viability.

The vibrating mesh nebulizer (VMN) has been introduced more recently as a high-performance treatment delivery system for patients with respiratory diseases (23, 24). VMNs employ electroformed plates that vibrate to generate aerosols (25). Two types of VMN, passive and active, have been commercially deployed. An example of a passive VMN is the Omron MicroAir NE-U22, which contains a perforated vibrating plate underlying the fluid reservoir with around 6,000 tapered holes of 3-μm diameter. Alternatively, the Aeroneb Pro nebulizer is an active VMN, utilizing a micropump system which delivers fluid to a vibrating plate containing up to 1,000 dome-shaped apertures. In both systems, aerosols are generated by applying an alternating electric potential to a piezoelectric, triggering the mesh to move back and forth by a few micrometers to aerosolize liquids brought into contact with the mesh surface (17). An alternative to the Collison and VMN nebulization is the hydraulic spray atomizer (HSA). The HSA concentrates the suspension into a stream by forcing it through a very small hole. There is a one-way valve in the nozzle that maintains air from flowing back into the pump and allows for suction within the pump so that liquid can be pulled up the tube. However, these latter two types of nebulizer, VMN and HSA, have rarely been applied in the infectious disease aerobiology research field. In this study, we hypothesized that a VMN or an HSA is superior to the commonly used Collison nebulizer, as they result in less mechanical stress on respiratory viruses and therefore enable a higher viable dose to be delivered for experimental purposes.

This study was therefore designed to determine the influence of nebulization methods of the 1-jet Collison nebulizer, VMN, and HSA on the viability of human respiratory viruses influenza A virus H1N1, IAV H3N2, and human rhinovirus-16 (HRV-16). We also characterized the size and volume of aerosolized fluid droplets ejected by these three nebulizers.

RESULTS AND DISCUSSION

Aerosol output and size analysis. Figure 1 shows a lognormal fit to the aerosol size distribution (ASD) normalized with respect to the maximum sizing channel concentration of
the dried aerosols generated by the Collison nebulizer, VMN, and HSA loaded with 0.5 g liter\(^{-1}\) NaCl solution as measured with a scanning mobility particle sizer (SMPS 3034; TSI Inc., Shoreview, MN, USA). Row data are shown in Fig. S1 in the supplemental material. Initial droplet size of these aerosols before evaporation was calculated using equation 1, and finally, the ASDs were calculated for a solution composed of 9 g liter\(^{-1}\) NaCl. Table 1 summarizes the detailed properties of the aerosols produced. In order to facilitate the investigation of infection risk (in animal exposure studies) and the prediction of physical loss of the carrier aerosols through gravitational settling, both the count median diameter (CMD) and volume median diameter (VMD) of the aerosols generated should be considered. While the CMD can be used to predict the depth of penetration in the airway, the VMD is useful when assessing the viral load of the aerosol. The Collison generated smaller aerosols than the other two nebulizers, with CMD of 0.045 \(\mu\)m for 0.5 g liter\(^{-1}\) NaCl solution. However, the corresponding VMD of this NaCl solution was 0.132 \(\mu\)m. Our detailed characterization predicted that the Collison produced aerosols with CMD and VMD of 0.11 \(\mu\)m and 0.323 \(\mu\)m,

![Graphs showing aerosol size distributions](image-url)
respectively, when derived from a solution of 9 g liter⁻¹ NaCl concentration. This salt concentration is consistent with the salt concentration of standard cell culture media as the potential suspension for viral particles. Based on the particle size distributions generated from a Collison nebulizer, May et al. stated that only 1% of the mass of producing aerosols is larger than 5 μm (27). A study conducted by Ibrahim et al. reported that the CMD of the aerosols produced by the Collison nebulizer is between 33 and 38 nm, which is not large enough to carry an influenza virus particle (>80 nm) (18). The geometric standard deviation (GSD) of the aerosols produced by the Collison was 1.8, indicating that the Collison nebulizer generated a more uniform aerosol size than the other two nebulizers. For Collison nebulizer, similar to our study, several studies reported a GSD at approximately 2 (29, 30). However, a recent study conducted by Bowling et al. reported different GSDs, ranging from 1.54 to 3.67, depending on the animal chamber used (11). The inconsistency between the reported results of physical characteristics of aerosols generated from a Collison nebulizer is a consequence of large variations in the methods used for aerosol production and measurement. Experimental protocols that significantly impact the results include the fluid physiochemical properties (viscosity, surface tension [25], and the type and concentration of solutes [31]) and the measurement techniques used to characterize the aerosol.

VMN generated larger aerosols than the Collison, with CMDs of 0.082 μm and 0.22 μm for 0.5 g liter⁻¹ and 9 g liter⁻¹ NaCl solution and VMDs of 0.65 μm and 1.70 μm, respectively. VMN produced larger aerosols than the Collison nebulizer, suggesting that VMN is suited to investigations of airborne virus-laden aerosols in terms of the aerosol’s physical size. The size diameters of the HRV-16 (32) and IAVs (33) are 30 and 80 to 120 nm, respectively; therefore, any initial aerosols smaller than this would not be able to carry viral particles, although they could carry noninfectious viral fragments.

HSA generated the largest aerosols, with CMDs of 0.10 μm and 0.30 μm, although the VMDs were 0.45 μm and 1.30 μm for 0.5 g liter⁻¹ and 9 g liter⁻¹ NaCl solution, respectively. HSA-atomized aerosols were larger than those generated by the two other nebulizers but were still within the airborne size range. However, HSA nebulization produced a lower concentration of aerosols in the same running time, followed by 1-jet Collison nebulizer. This property could disadvantage studies that require a higher concentration of aerosols or viral doses.

**Viral surviving fractions postaerosolization.** Comparison of SFs for IAVs H3N2 and H1N1, as well as HRV-16 for Collison, VMN, and HSA, were performed by nebulization of viral suspension into an operating SKC BioSampler (part no. 225-9593; SKC), and results are presented in Fig. 2. SFs (arithmetic mean ± standard error) of IAV H1N1 for the Collison, VMN, and HSA were 0.82 ± 0.01, 0.78 ± 0.02, and 0.89 ± 0.03, respectively, while the average SFs for IAV H3N2 were 0.82 ± 0.02, 0.76 ± 0.02, and 0.94 ± 0.02. The corresponding average SFs of HRV-16 after running the Collison, VMN, and HSA were 0.83 ± 0.05, 0.87 ± 0.08, and 0.85 ± 0.03, showing better survival fractions but not statistically significant compared to those of survival of IAV strains. Although HSA is slightly better for IAVs, one-way analysis of variance (ANOVA) demonstrated no statistical differences in SFs of the tested viruses.
between the three mentioned nebulizers. In addition, the large uncertainties in HRV-16 SFs can be attributed to the 50% tissue culture infective dose (TCID₅₀) assay, which has more variability than the PFU assay for IAVs.

We also tested the shear and impact forces delivered by the high-velocity air streams of the 1-jet Collison nebulizer on the viability of viruses suspended during 30-min run times (Fig. 3). There was a gradual decrease in SFs for all three viruses over 30 min. SFs of IAVs H1N1 and H3N2 declined from 0.82 ± 0.01 at 5 min to 0.67 ± 0.02 at 30 min and 0.82 ± 0.02 at 5 min to 0.68 ± 0.01 at 30 min, respectively; these differences between 5 and 30 min operating times were statistically significant (P = 0.003 and P = 0.012, respectively), suggesting that 1-jet Collison may not be an appropriate option for studies that require longer nebulizer operating time or high concentration of viable virus in aerosols. However, the SF of HRV-16 decreased less, from 0.83 ± 0.05 at 5 min to 0.73 ± 0.03 at 30 min, which was not significantly different, suggesting that HRV is less susceptible to mechanical stress of 1-jet Collison compared to IAVs. Our findings showed that the SFs of all three viruses were reduced in samples collected from virus suspensions in the jar of the 1-jet Collison nebulizer after 30 min running time and that the loss was highest for enveloped IAVs compared to the nonenveloped

![FIG 2](image-url) Surviving fraction (calculated based on equation 2) of IAV H1N1, IAV H3N2, and HRV-16 postnebulization. Experiments were conducted in triplicate.

![FIG 3](image-url) IAV H1N1, IAV H3N2, and HRV-16 SF decline following 30 min of 1-jet Collison nebulization. The 5-min SFs are taken from measurements in Fig. 2, and experiments were conducted in triplicate.
HRV-16. Multijet Collisons (6 or 24 jets) can potentially be considered due to a higher aerosol production rate, thus reducing the required run time. However, it might not be easy to control the relative humidity (RH) in the investigated system, as these nebulizers inject water-saturated air at a higher rate than a single-jet Collison. Consistent with our findings, it was previously reported that the method of aerosol production in the Collison jar could damage intact pathogens, affecting the pathogen dose required to identify an infection signal (34). Kim et al. reported a loss of 15% in titer of an enveloped coronavirus (80 to 160 nm) during Collison nebulization over a period of 30 min (35). Conversely, Hermann et al. reported no loss in the viabilities of enveloped porcine reproductive and respiratory syndrome virus (40 to 80 nm) through 55 min of nebulization using a 24-jet Collison (36). The inconsistency between reported results of viable fractions postaerosolization is likely due to large variations in methods used for measuring SFs and differences in the size of viruses tested. Experimental protocols which significantly impact results include aerosol sampling and measuring and the degree of control over temperature and RH. In fact, the Collison nebulizer inherently needs a high volume of virus suspension (10 ml) compared to other nebulizers, which can dilute the titer of initial virus stocks and reduce the aerosolized doses provided. The Collison also generates foam from suspensions with high organic content, which could be a barrier for proper aerosolization of virus-laden droplets (Fig. S2). It should be highlighted that we controlled for all possible experimental aspects in our comparative study, but differences in the nebulizers used are inherent in their design. Naturally, the type, shape, and nebulization process of each of the nebulizers were quite different, and therefore, it is impossible to have a similar experimental setup for Collison and the other two nebulizers. The generated aerosols of VMN and HSA were immediately collected into SKC BioSampler (the aerosols’ residential time was below several seconds); therefore, the aerosols did not experience a dry condition before collection. The different configurations of the SKC BioSampler (Fig. S3) that were used for the Collison nebulizer versus the other nebulizers should have had no impact on our results, as our approach was to measure SFs in a before-and-after nebulization comparison. Finally, we summarized the advantages and disadvantages of investigated nebulizers in Table 2.

In summary, the techniques used to generate laboratory bioaerosols enable greater understanding of the airborne mode in the transmission of respiratory viruses. However, the mechanism of aerosol generation affects the characteristics of the aerosols produced, including their physical and chemical properties, and influences their properties compared to bioaerosols generated from natural sources. This study was conducted to investigate the characteristics of aerosols generated by commonly used nebulizers using SMPS instrumentation. The CMD and VMD of aerosols generated by the Collison placed at 0.11 μm and 0.323 μm, while VMN produced aerosols with CMD and VMD of 0.22 μm and 1.7 μm, respectively, HSA generated larger aerosols with

### Table 2: Advantages and disadvantages of nebulizers for studies in aerobiology field

| Type of nebulizer | Advantage(s)                                                                 | Disadvantage(s)                                                                                       |
|-------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| Collison nebulizer| Minimal aerosol losses in the experimental setup                             | Low aerosol mass injection rate of 3 ml h⁻¹ requiring long-duration injection, poor TCID₅₀ and RT-qPCR signals and poor aging time resolution, injects water saturated air at 8 liters min⁻¹, difficult to control the treatment RH and temp in the experimental setup |
| VMN               | Very high aerosol mass injection rate (2 g min⁻¹) allows short-duration injection, good TCID₅₀ and RT-qPCR signals and good aging time resolution, no air injection, good RH and temp control in the drum, minimal aerosol losses | Huge aerosols losses during experiments, as many large droplets produced which settle before evaporation, impacting airborne assessments |
| HSA               | Very high aerosol mass injection rate allows short-duration injection, good TCID₅₀ and RT-qPCR signals and good aging time resolution, no air injection, good RH and temp control in the experimental setup |                                                                                                       |

*RT-qPCR, reverse transcriptase quantitative PCR.*
CMD and VMD of 0.70 μm and 2.06 μm, respectively. VMN generated higher concentrations of aerosols over the same running time than the other two nebulizers. SFs of IAV H1N1, IAV H3N2, and HRV-16 decreased postnebulization, but this was dependent on virus type (enveloped or nonenveloped) and nebulization mechanisms. HSA elicited the least mechanical stress on the investigated IAV strains; however, the gravitational loss of aerosols was higher due to the generation of large size droplets. It has been recently argued (37) that the airborne size range of <5 μm should be extended to 50 to 100 μm in indoor environments because turbulence and aerosol cloud buoyancy keep larger particles airborne for longer than is predicted by Stokes’ law. Our results indicated that VMN is the best nebulizer for infectious disease aerobiology research due to its production of higher concentrations of aerosols in a usable size range (200 nm to 5,000 nm). This size range is irrelevant to ongoing discussions regarding the role of particle size on infectivity and disease presentation, and it simply refers to the size range which can efficiently carry infectious viruses, as this size is bigger than the size of viruses (IAV and HRV), and they can be suspended in the investigated experimental setup for a prolonged time (38). Besides, VMN has a higher aerosol mass injection rate (2 g min⁻¹), which allows short-duration injection and good aging time resolution. The VMN does not inject air; therefore, it is suitable for studies that require modulation of RH and temperature control within enclosed chambers.

MATERIALS AND METHODS

Aerosol size distribution measurement. The droplet size distribution produced by each of the nebulizers was back calculated after measuring the dry salt aerosol size distribution produced from a saline solution of known concentration. Aerosols (without addition of viruses) were generated by briefly nebulizing a suspension of 0.5 g liter⁻¹ NaCl into a 400-liter rotating stainless steel drum developed based on the TARDIS rotator (39). The drum was first flushed with dry and HEPA-filtered air, and the RH was adjusted to 20%, well below the efflorescence RH of NaCl (40, 41) and the nebulization time limited to ensure generated aerosols dried rapidly and were sampled by the aerosol measurement instrumentation in the dry state (RH ≈ 30%). ASDx of aerosols produced by the 1-jet Collison nebulizer, VMN, and HSA were measured using an SMPS. A dilute sample solution (0.5 g liter⁻¹) was used for measurements to ensure that the dry aerosols were within the size range of the SMPS, which is between 9 to 1,000 nm, depending on operating conditions. We then calculated the initial droplet size of each particle measured with the SMPS using equation 1. Subsequently, we were able to calculate the dry sizes of aerosols expected from a solution with salt concentration of 9 g liter⁻¹, similar to a physiological saline solution, and this size distribution was validated through direct measurement of the portion of that dry size distribution accessible to the SMPS.

\[
D_{dry} = D_{wet} \times \left( \frac{C_{solution}}{P_{solution}} \right)^{1/3},
\]

where \(D_{dry}\) is the final dry diameter of aerosol, \(D_{wet}\) is the initial diameter of the solution droplet, \(C_{solution}\) is the concentration of solution (kg m⁻³), and \(P_{solution}\) is the density of solution (kg m⁻³).

Virus propagation. HRV-16 was grown in Ohio HeLa cells in virus production serum-free medium (VP-SFM) (Life Technologies, USA) and propagated as described previously (42) (Text S1 in the supplemental material). IAV H1N1 and H3N2 were propagated in the allantoic fluid of 10-day-old embryonated chicken eggs (26) (Text S2 in the supplemental material).

Virus suspensions. IAV H1N1, IAV H3N2, and HRV-16 virus batches were added to a suspension composed of phosphate-buffered saline (PBS) and fetal bovine serum (FBS). The salt and total protein contents were 10 and 8 g liter⁻¹, respectively, which is approximately comparable to the ratio of salts and proteins found in human respiratory fluid (43). This solution provided an environment with neutral pH that allowed the viruses to remain viable while attempting to physiologically model the actual composition of human respiratory fluid.

Experimental setup for measuring viruses’ viabilities. Figure 4 illustrates the experimental setup for testing the mechanical stress of Collison nebulizer (top), VMN, and HSA (bottom) on the viability of viruses. HEPA-filtered compressed air with a flow rate of 12 liters min⁻¹ and pressure of 26 to 30 lb in⁻² was blown into the jar of the 1-jet Collison nebulizer to produce carrier aerosols. Virus-laden aerosols were collected into an SKC BioSampler directly after nebulization in 5 ml PBS. The photographs of experimental setups also are shown in Fig. S3. The SKC BioSampler is an advanced impinger-type air sampler that collects airborne aerosols using a whirling flow of liquid (PBS) and pump flow rate of 12.5 liters min⁻¹. The SKC swirling flow action is created by drawing air through three 0.630-mm tangential sonic nozzles (44). A HEPA filter was connected to provide required excess air for sampling. Our VMN (Fig. S4) was assembled inside a biosafety cabinet. It consisted of the nebulizer electroform plate (model no. HW-16-112E-EO), 3-ml reservoir, and electrical wires, which were connected to a high-frequency circuit inside the biosafety cabinet. The virus suspension was injected into the nebulizer reservoir via a Luer lock syringe. As the final step, the virus suspension was aerosolized into an SKC BioSampler and simultaneously collected into 5 ml PBS by running the SKC pump. The same experimental setup was applied for testing
HSA, and the virus suspension was nebulized using a 10-ml Luer lock syringe with a feed rate of 0.33 ml s\(^{-1}\). The room temperature during the experiments was 23 ± 2°C.

**TCID\(_{50}\)** to determine HRV-16 titer. The change in titer of HRV-16 before and after nebulization was quantified using a standard TCID\(_{50}\) at which 50% of HeLa cells cultured in monolayer demonstrated a cytopathic effect (45). Cells were exposed to replicate 10-fold serial dilutions of the fluid collected for HRV-16 in the BioSampler for 4 days at 34°C. CPE was then identified for each well in the TCID\(_{50}\) assay using 1% crystal violet solution mixed with 60% ethanol and 40% methanol.

**Plaque assay to determine IAV H1N1 and IAV H3N2 titters.** IAV H1N1 and H3N2 infectivity titers were measured by plaque formation on confluent monolayers of Madin-Darby canine kidney (MDCK) cells cultured in RF10 medium (26). Once cells in 6-well plates were confluent, medium was replaced with 135 μl RPMI medium containing 10-fold serial dilutions of recovered sample. Plates were incubated (37°C, 5% CO\(_2\)) for 45 min and, every 15 min, gently rocked to distribute the virus evenly and prevent drying of the cell monolayer. Cells were then overlaid with 3 ml per well of L15 medium mixed with 0.1% trypsin and 1.8% (wt/vol) agarose. Once set, plates were incubated (37°C, 5% CO\(_2\)) for 3 days. Plaque formation was visualized by staining the monolayer with 1% crystal violet solution, and the number of plaques was counted as a measure of virus infectivity.

**Surviving fraction and statistical analysis.** Surviving fractions (SFs) of IAVs (H1N1 and H3N2) and HRV-16 were calculated using equation 2. RNA was used as a natural tracer of dilution based on the expectation that RNA was present in the same ratio to total virus in the nebulizer before aerosolization and after sample collection (46). Viral RNA was extracted from nebulizers and SKC BioSampler samples by QIAamp viral RNA minikit (Qiagen, USA) and its concentration quantitated using Qubit high-sensitivity RNA kit (Thermo Fisher Scientific, USA).

**FIG 4** Experimental setup for measuring the effects of nebulization on the viability of respiratory viruses. (Top) Collison nebulizer. (Bottom) VMN and HSA.
\[ SF = D \times \left( \frac{LVC_1}{OLVC_1} \right) \]

where \( D = \left( \frac{OLVC_1}{OLVC_2} \right) \) is the dilution factor and \( LVC_1 \) is the live virus concentration (PFU or TCID\(_{50}\)) in the extracted aerosol sample, \( OLVC_1 \) is the original live virus concentration (PFU or TCID\(_{50}\)) in the nebulizer, \( RNA_1 \) is the total RNA mass concentration in the extracted aerosol sample, and \( RNA_2 \) is the original total RNA mass concentration in the nebulizer.

The concentrations of extracted RNA (ng ml\(^{-1}\)), PFU ml\(^{-1}\) (IAV H1N1 and IAV H3N2), and TCID\(_{50}\) ml\(^{-1}\) (HRV-16) for each experiment operated by 1-jet Collison, VMN, and hSA are presented in Table S1. Table S2 also shows the concentrations of extracted RNA (ng ml\(^{-1}\)), PFU ml\(^{-1}\) (IAV H1N1 and H3N2), and TCID\(_{50}\) ml\(^{-1}\) (HRV-16) for each experiment operated by 1-jet Collison after 10, 20, and 30 min incubation. All experiments were conducted in biological triplicate. A one-way analysis of variance (ANOVA) was performed to compare SFs of viruses between nebulizers chosen due to the normal distribution of data, approved by the Shapiro-Wilk test. A P value of less than 0.05 was considered statistically significant.

**Biosafety.** Experimentation was conducted according to the health risk assessment and approval by the QUT biosafety committee (approval no. 1800000969).

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.3 MB.

**ACKNOWLEDGMENTS**

The research described here was sponsored by Australian Research Council (grant number DP170102733).

We thank Kirsty R. Short for allowing us to propagate influenza A viruses in her laboratory.

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