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# Elution Efficiency of Airborne Virus Captured by the Zefon Vira-Pore Cassette under Various Sampling and Sample Storage Conditions

A preliminary report prepared by

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## 1. Objective

The overall goal of this work is to determine the elution efficiency of Human Coronavirus OC43 (HCoV OC43), a surrogate for the novel SARS-CoV2 virus, collected on 37mm 1 µm pore-size PTFE-laminated PTFE Zefon® "ZePore" ™ filters after different aerosolization and filter storage times.

The work is in response to the unprecedented crisis and pandemic caused by the novel SARS-CoV2 virus. There is much unknown about the virus, and various means of its collection from the air have to be developed and validated. Zefon Inc. (a Cole-Parmer, Inc. Company) has developed a proprietary filter cassette (Vira-Pore) containing the ZePore filter that can be used to sample the virus from the air to determine its presence. However, the sampling protocol, particularly the elution of the collected virus from the filters, had not previously been studied and had to be validated. Since experiments with the actual SARS-CoV-2 are not feasible for safety reasons, the OC43 human coronavirus (a cause of the "common cold") was selected as a simulant of SARS-CoV-2, which causes COVID-19. Both human coronavirus OC43 and SARS-CoV-2 are positive-sense single-stranded RNA (+ssRNA) beta enveloped coronavirus virus, and thus OC43 is a suitable and close simulant.

## 2. Materials and Methods

### 2.1 Preparation of Human Coronavirus OC43 inoculum

Human Coronavirus OC43 (HCoV OC43) was used as a surrogate for the novel SARS-CoV2. Both human coronavirus OC43 and SARS-CoV-2 are positive-sense single-stranded RNA (+ssRNA) beta enveloped coronavirus virus, and thus OC43 is a very close simulant. There are few published studies with airborne OC43 virus to the best of our knowledge, and its use here adds to the novelty of the study. The OC43 virus was prepared according to published protocols (DeDiego et al., 2007). Briefly, Vero E6 (African green monkey kidney) cells were cultured at 33°C in Eagle's minimum essential medium (MEM; Fisher Scientific Inc., Pittsburg, PA) supplemented with 2% fetal bovine serum (FBS; Fisher

Scientific Inc.). HCoV OC43 was propagated into the Vero E6 cells. Cells were incubated for adsorption at 33°C for one hour. The unbound virus was removed by washing cells three times with ice-cold phosphate-buffered saline solution (PBS; Sigma-Aldrich Co., St. Louis, MO), followed by introducing the fresh medium to the cells. Media from infected cultures was then harvested at various times, and HCoV OC43 titers were determined by plaque assay using Vero E6 cells. The virus was inactivated by heating its suspension in a water bath at 56°C for 30 min. The absence of plaques for the inactivated virus was confirmed by the OC43 plaque assay.

**2.2 Experimental setup for testing in the laboratory**

The test system used for this study was based on the setup described in our previous publications (T. T. Han et al., 2017, 2018) and shown in Figure 1. The test system consists of a flow controller, a particle generator, an air-particle mixing element, a flow straightener, a test chamber, and a particle monitor (T. T. Han et al., 2017). The system is housed in a Class II Biosafety cabinet (NUAIRE Inc., Plymouth, MN). The liquid suspension with the inactivated virus was aerosolized using a three-jet Collison nebulizer with a polycarbonate jar and operated at a flow rate, $Q_A$= 5 L/min (pressure of 19 psia). The use of a polycarbonate jar and low aerosolization pressure minimizes damage to biological particles.

The aerosolized viral particles were combined with a dry airflow, $Q_d$ (5 L/min) (T. Han et al., 2015; Zhen et al., 2013). The flow stream was passed through a 2-mCi Po-210 charge neutralizer (Amstat Industries Inc., Glenview, IL) to reduce aerosolization-imparted particle charges to Boltzmann charge equilibrium. A HEPA-filtered dilution airflow, $Q_D$ (60 L/min), provided by an in-house compressor, was used to dilute the particle stream. A well-mixed flow stream was then pass through a flow straightener (honeycomb). The filter cassette with the filter being tested was positioned six duct diameters downstream of the exit of the flow straightener to provide a uniform cross-sectional particle profile (T. T. Han et al., 2018). The concentration of airborne particles (#/L) was monitored by an SMPS 3080 + CPC 3776 system (both TSI

Inc., Shoreview, MN) and GRIMM MiniWRAS 1371 (GRIMM aerosol technology, Ainring, Germany) that were connected to an isokinetic probe (Apex Instruments Inc., Fuquay-Varina, NC) via a flow splitter.

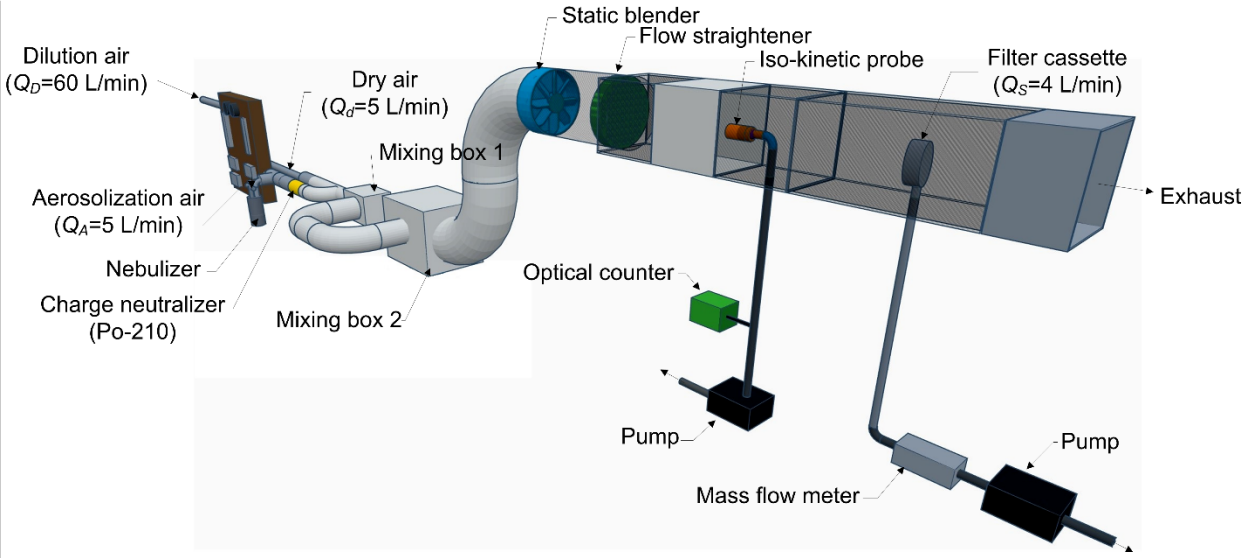


Figure 1. Schematic of the test setup.

### 2.3 Sampling

The airborne virus was sampled using filters and cassettes provided by Zefon Inc. The filters were 37 mm diameter, 1 µm pore-size PTFE-laminated PTFE Zefon® "ZePore" ™ filters with and without support pads in 37 mm three-piece static-dissipative ("conductive") polypropylene cassettes, used in the "open-face" configuration. Static-dissipative ("conductive") cassettes are preferred for fine particle sampling. Two different sampling flow rates were used:

1. 10 L/min, which with a support pad included leads to a pressure drop across the sampler of 17 inches w.g. (4.23 kPa), which allows the use of certain personal pumps, such as the Gilian 12 or SG-10, in addition to vacuum pumps.
2. 3 L/min which with a support pad included leads to a pressure drop across the sampler less than 5.5 inches w.g. (1.37 kPa), which is compatible with many personal sampling pumps.

The flowrates were verified using a mass flowmeter (TSI Inc.). The duration of sampling virus aerosol varied between 10 and 60 min depending on the experiments, as described below.

## 2.4 Test Parameters

### *2.4.1 Testing of elution method of viral particles spiked onto filters*

Filters were placed in 60 mm Petri dishes, and 500 µL of the virus suspension was added onto the filters in small droplets (Figure 2). The filters were then placed in an incubator at 25°C (room temperature) for approximately four hours: until the liquid evaporated. PrimeStore® MTM (Longhorn Vaccines and Diagnostics™, San Antonio, TX, USA) was used as the elution liquid since it has been used to inactivate viruses and act as an RNA stabilization agent (Daum et al., 2011, 2014; Zar et al., 2016). Once the suspension dried out, the viral particles were eluted using two methods:

(1) The filters were placed face down in a 60 mm petri dish (Fisher Scientific Inc.) with 700 µL of molecular transport media (PrimeStore® MTM (Longhorn Vaccines and Diagnostics™ Inc.) and placed on an orbital shaker for 20 mins at 360 rpm (Booth et al., 2005; Myatt et al., 2003, 2004).

(2) The filters were rolled inwards and placed in a 2 mL microcentrifuge tube (Fisher Scientific Inc.) with 1 mL of MTM media (Longhorn Vaccines and Diagnostics™, Inc.) and vortexed three times for 10 seconds each (Coleman et al., 2018; Fabian et al., 2009; Jonges et al., 2015; Tseng & Li, 2005).

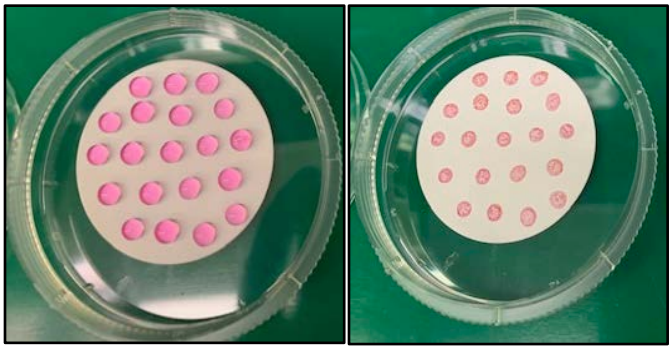

Figure 2: Spiking and drying of filters with OC43 stock virus solution

### *2.4.2 Testing of elution method of airborne viral particles collected onto filters*

The viral particles were aerosolized as described in Figure 1 and then sampled on filters at 10 L/min for 10 mins. The captured virus was eluted in triplicate using the two methods described above. PrimeStore® MTM (Longhorn Vaccines and Diagnostics™, San Antonio, TX, USA) was used as the elution liquid, and samples were then immediately placed on ice for RNA extraction:

(1) The filters were placed face down in a 60 mm petri dish (Fisher Scientific Inc.) with 700 µL of molecular transport media (PrimeStore® MTM, Longhorn Vaccines and Diagnostics™ Inc.) and placed on an orbital shaker for 20 mins at 360 rpm (Booth et al., 2005; Myatt et al., 2003, 2004).

(2) The filters were rolled inwards and placed in a 2 mL microcentrifuge tube (Fisher Scientific Inc.) with 1 mL of MTM media (Longhorn Vaccines and Diagnostics™, Inc.) and vortexed three times for 10 seconds each (Coleman et al., 2018; Fabian et al., 2009; Jonges et al., 2015; Tseng & Li, 2005).

### *2.4.3 Testing of support pads for RNA interference*

Cellulose support pads and porous plastic support pads (item # PFSP37; Zefon Inc.) of 37 mm were tested for any RNA interference with the collected virus samples. Cellulose pads were folded into 5 ml sterile tubes (Fisher Scientific Inc.) with 2 ml of MTM (Longhorn Vaccines and Diagnostics™ Inc.). The tubes

were vortexed thrice for 10 seconds each. When testing porous plastic pads (Zefon Inc.), each side of the pad was rinsed with 2 ml of sterile RNA-free water (Fisher Scientific Inc.) and dried before placing them in the sampling cassette. The rinsed off and vortexed liquids plus controls (e.g., water and MTM) were immediately quantified for their total RNA concentration.

### *2.4.4 Recovery of the virus after airborne sampling*

The recovery of the virus from the filter after airborne sampling stress was evaluated by comparing the RNA yields from the following six experiments:

(1) Sampling of the aerosolized virus for 10 mins at 10 L/min (100L volume) followed by immediate sample processing

(2) Sampling of the aerosolized virus for 60 mins at 10 L/min (600L volume) followed by immediate sample processing

(3) Sampling of the aerosolized virus for 10 mins at 10 L/min (100L volume) followed by exposure of the collection filter to a clean airstream for 1 hour, followed by immediate sample processing

(4) Sampling of the aerosolized virus for 60 mins at 3 L/min (180L volume) followed by immediate sample processing

(5) Sampling of the aerosolized virus for 60 mins at 3 L/min (180L volume) followed by exposure of the filter to a clean airstream for 5 hours, followed by immediate sample processing

(6) Sampling of the aerosolized virus for 60 mins at 3 L/min (180L volume) followed by exposure of the filter to a clean airstream for 15 hours, followed by immediate sample processing

Each experiment was performed at least in triplicate. In addition to filters with the sampled virus, positive (fresh viral inoculum) and negative (elution liquid and MEM) controls were analyzed. The collected viral

particles were eluted into the MTM media (Longhorn Vaccines and Diagnostics™, Inc.) immediately after sampling, and the liquid was quantified for its total RNA concentration.

### *2.4.5 Recovery of the virus after storage*

The recovery of the virus from the filter after storage was evaluated by comparing the yields from the following experiments:

(1) Sampling of the aerosolized virus for 60 mins at 3 L/min (180L volume) and storing the filter at 25°C (room temperature) for two days.

(2) Sampling of the aerosolized virus for 10 mins at 10 L/min (100L volume) and storing the filter at 4°C (refrigerator) for seven days

(3) Sampling of the aerosolized virus for 10 mins at 10 L/min (100L volume) and storing the filter at 25°C (room temperature) for seven days

Each experiment was performed at least in triplicate, and the sample filters, as well as positive (fresh viral inoculum) and negative (elution liquid and MEM media) controls, were analyzed. After the specified storage period, the collected viral particles were eluted into the MTM media (Longhorn Vaccines and Diagnostics™, Inc.), and their total RNA concentration was quantified.

## 2.5 Viral quantification method

The QIAamp Viral RNA Mini Kit (Qiagen Co., Germantown, MD) was used for extraction, and the manufacturer's protocol was optimized to obtain a better RNA yield and quality. This protocol uses 5.6 µg carrier RNA for each sample. The carrier RNA has to be taken into account during sample analysis: the signal produced by the carrier RNA in the blank media is arithmetically subtracted from the signal produced by the samples. The signal produced by the samples is the sum of the signal by the virus in the sample and the carrier RNA. Currently, the kit has a yield of 35-40% compared to the standard trizol-chloroform extraction procedure. However, it consists of no hazardous materials and is faster than the

trizol-chloroform extraction procedure. 60 µL of RNA was extracted from the eluted samples and kept on ice. The extracted RNA samples were quantified using the Nanodrop 1000 (Fisher Scientific Inc.) in ng/µL. The NanoDrop measures nucleic acid concentration by absorbance using a 2 µL RNA sample and assesses extracted RNA's purity using the A260/A280 ratio.

### 2.6 Data presentation: Relative Recovery

The results were expressed as relative recovery ($RR_{mass}$). The $RR_{mass}$ is the ratio of the viral mass concentration eluted from a filter (ng/ml) relative to the mass concentration of viral particles in the initial nebulizer suspension (ng/ml). All samples for this ratio were analyzed using QIAamp Viral RNA Mini Kit and the Nanodrop device. The mass concentration of viral RNA eluted from the filter was normalized to the sampled air volume.

$$RRmass(L^{-1}) = \frac{Mass\ concentration\ of\ viral\ RNA\ eluted\ from\ filter\ (\frac{ng}{ml})}{Mass\ concentration\ of\ viral\ RNA\ in\ the\ initial\ sample\ \left(\frac{ng}{ml}\right) * Sampled\ air\ volume\ (L)} \quad (1)$$

### 2.7 Quantitative RT-PCR

Several samples were analyzed by qRT-PCR to confirm that the virus samples could be amplified after their elution and extraction from the filter. Primers specific for OC43 were used.

### 2.8 Biosafety protocol

Rutgers Environmental, Health and Safety Service (REHS) approved the project's protocol, specifically by Biosafety officers. The PIs worked with REHS to obtain the necessary approvals before the start of the project.

## 3. Results and Discussion

### 3.1 Size distribution of HCoV OC43 virus

We aerosolized the virus from the MEM growth medium and then from the MEM medium alone. By comparing the SMPS spectra of the two measurements (subtracting one from the other), we obtained the electrical mobility size distribution with a peak number concentration at ~50 nm (Figure 3). This is consistent with the 80-120 nm projected physical diameter from electron microscopy, and in some *beta coronaviruses*, including HCoV-OC43, smaller sizes were also observed (Liu et al., 2020).

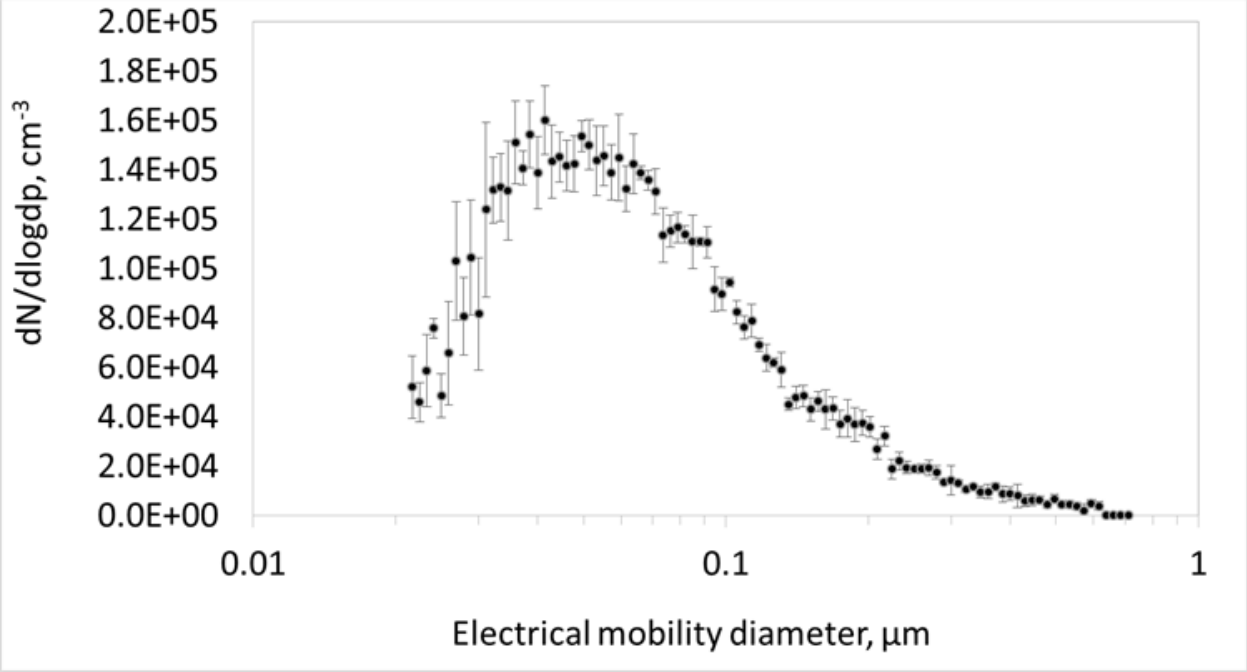


Figure 3: Particle size distribution of HCoV OC43 measured by SMPS.

### 3.2 Test Parameters

#### *3.2.1 Method to elute viral particles spiked onto filters*

Our results showed that RNA concentration eluted by vortexing was ~2x higher than the RNA concentration eluted by the orbital shaker. The average total mass of the virus eluted by vortexing and

shaker was 2.68 x $10^3$ ng (± 1.84 x $10^3$) and 1.71 x $10^3$ ng (± 1.06 x $10^3$), respectively. For comparison, the viral RNA mass in the 500 µL control sample added to the filter was 3.18 x $10^3$ ng. The viral RNA in the 500 µL control sample was not subjected to any stress before RNA extraction.

### *3.2.2 Method to elute airborne viral particles collected onto filters*

Our results showed that vortexing eluted an average RNA concentration of 1.76 x $10^3$ ng/mL (± 1.26 x $10^3$) while the shaker eluted an average RNA concentration of 5.46x $10^2$ ng/mL (± 8.59x $10^1$). The $RR_{mass}$ for the vortexing and shaker were 0.0067 and 0.0034, respectively.

Since in both experiments with the two elution methods higher RNA concentrations and relative recovery ratios were obtained by vortexing a filter with viral particles three times for 10 seconds each, we chose the vortexing method as our elution method for subsequent testing.

### *3.2.3 Testing of support pads for RNA interference*

Based on experiments with 7 cellulose pads and 3 porous plastic pads, the average RNA concentration measured in the liquid used to rinse off cellulose and porous plastic pads did not differ significantly from the control samples ($p > 0.05$). However, in 2 samples rinsed off from cellulose pads, the RNA concentrations were higher than in the control samples.

Furthermore, previous experiments with water and MTM liquid (Longhorn Vaccines and Diagnostics™ Inc.) spiked with carrier RNA showed no difference in the resulting RNA once the samples have been processed. So, either one of the two liquids could be used to rinse off the porous plastic pads.

### *3.2.4 **Recovery of the virus after sampling stress***

The virus from filters was eluted immediately after sampling for 10 mins at 10 L/min (100L volume), 60 mins at 10 L/min (600L volume), 10 mins at 10 L/min (100L volume) followed by exposure to clean airstream for 1 hour, 60 mins at 3 L/min (180L volume), 60 mins at 3 L/min (180L volume) followed by exposure to clean airstream for 5 hours, and 60 mins at 3 L/min (180L volume) followed by exposure to clean airstream for 15 hours. The obtained relative recovery ($RR_{mass}$) results are presented in Figure 4.

For the 10 min samples at 10 L/min (100L volume), the RNA mass concentration was $1.76 \times 10^3$ ng/mL ($\pm 1.26 \times 10^3$), and the $RR_{mass}$ (purple bar in Figure 4) was 0.0067 (± 0.0013). For the 60 min samples at 10 L/min (600L volume), the RNA mass concentration was $4.02 \times 10^3$ ng/mL ($\pm 2.46 \times 10^3$), and the $RR_{mass}$ (blue bar in Figure 4) was 0.0054 (± 0.0033). For the 10 min samples at 10 L/min (100L volume) followed by exposure to a clean airstream for 1 hour, RNA mass concentration was $2.17 \times 10^3$ ng/mL ($\pm 6.97 \times 10^2$), and the $RR_{mass}$ (orange bar in Figure 4) was 0.0050 (± 0.0016). For the 60 mins samples at 3 L/min (180L volume), the RNA mass concentration was $5.54 \times 10^3$ ng/mL ($\pm 2.33 \times 10^3$), and $RR_{mass}$ (black bar in Figure 4) was 0.0055 (± 0.0023). For the 60 mins samples at 3 L/min (180L volume), followed by exposure to clean airstream for 5 hours, the RNA mass concentration was $4.74 \times 10^3$ ng/mL ($\pm 2.08 \times 10^3$), and the $RR_{mass}$ (olive green bar in Figure 4) was 0.0048 (± 0.0020). For the 60 mins samples at 3 L/min (180L volume) followed by exposure to clean airstream for 15 hours, the RNA mass concentration was $3.57 \times 10^3$ ng/mL ($\pm 1.57 \times 10^3$) and $RR_{mass}$ (grey bar in Figure 4) was 0.0042 (± 0.0018).

These results show that the relative recoveries of the aerosolized virus normalized to the sampling volume were similar to each other after sampling and immediate analysis for 100L, 180L, and 600L as well as 100L followed by exposure to clean airstream for 1 hour and 180L volume followed by exposure to clean airstream for 5 hours and 15 hours; the difference was not statistically significant ($p = 0.805$).

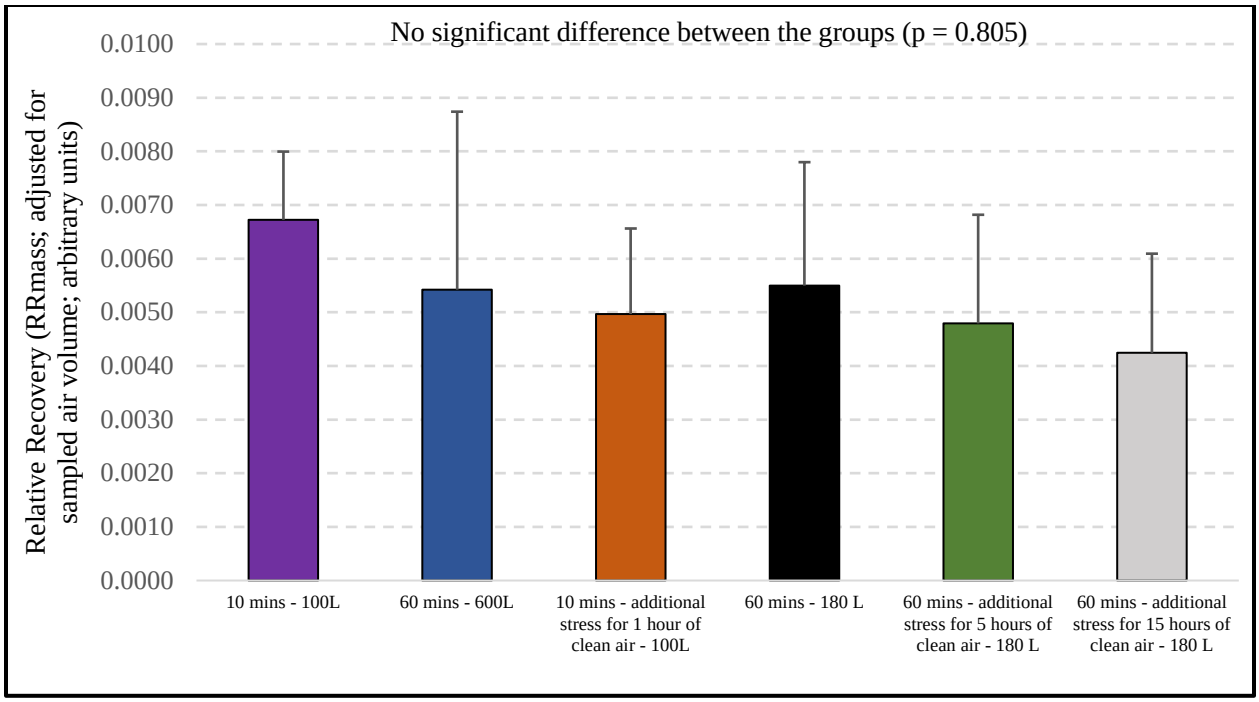


Figure 4: The effects of sampling stress on Relative Recovery ($RR_{mass}$) of airborne OC 43 virus sampled on Zefon Filters (reference: mass concentration). The purple, blue, orange, black, olive green, and grey bars represent sampling for 10 mins at 10 L/min (100L), 60 mins at 10 L/min (600L), 10 mins at 10 L/min (100L), followed by exposure to clean airstream for 1 hour, 60 mins at 3 L/min, 60 mins at 3 L/min (180L) followed by exposure to clean airstream for 5 hours, and 60 mins at 3 L/min (180L) followed by exposure to clean airstream for 15 hours, respectively.

### *3.2.5 Recovery of the virus after storage*

For recovery after 2 days of storage at 25°C, to simulate unrefrigerated transport to the laboratory, with an air sample volume of 180L, the RNA mass concentration was $2.63 \times 10^3$ ng/mL ($\pm 9.91 \times 10^2$), and the $RR_{mass}$ (brown bar in Figure 5) was 0.0037 (± 0.0014). Although after 2-day storage the average RNA mass concentration and the relative recovery are lower compared to no storage (black bar in Figures 4 and 5), the difference is not statistically significant (p = 0.24).

Recovery was also evaluated after 7 days of storage at 4°C and 25°C (room temperature) for a sampled air volume of 100L, to simulate different storage conditions in the laboratory prior to analysis. For the storage at 4°C, the RNA mass concentration was 4.41 x $10^3$ ng/mL (± 2.51 x $10^3$), and for the 7-day storage at 25°C, the RNA mass concentration was 1.34 x $10^3$ ng/mL (± 8.23 x $10^2$). Thus, the average concentration for 7-day storage was about 3.3x lower for room temperature storage condition than for refrigerator storage condition. As shown in Figure 5, the $RR_{mass}$ for the 7-day storage at 4°C (green bar in Figure 5) and the 7-day storage at 25°C (yellow bar in Figure 5) were 0.0055 (± 0.0031) and 0.0017 (± 0.0010), respectively. When comparing the $RR_{mass}$ of three data sets for the same sampled air volume of 100 L but different storage conditions, i.e., 7-day storage at 25°C, 7-day storage at 4°C, and no storage (purple bar in Figures 4 and 5), the statistical difference was borderline significant ($p = 0.053$).

Overall, when all five sampling scenarios in Figure 5 were considered, the statistical difference in $RR_{mass}$ was borderline significant ($p = 0.059$). When pairwise comparisons within this group of five were considered, only sampling for 10 mins at 10 L/min (100L volume) with no storage and 1-week storage at 25°C were significantly different from each other ($p < 0.05$).

One eluted sample from each set of 1 week's storage was analyzed by qPCR. The detected RNA concentration was similar for these two samples: 4.01 x $10^6$ RNA copy/ml (for 4°C storage) and 4.18 x $10^6$ RNA copy per ml (for 25°C storage). For both samples, the $C_T$ value of the qPCR was above 30 – i.e., at the lower end of detectability. This could explain the similarity of the values between the two samples and why, unlike in the sample analysis with carrier RNA kit, the difference between the two samples is not apparent.

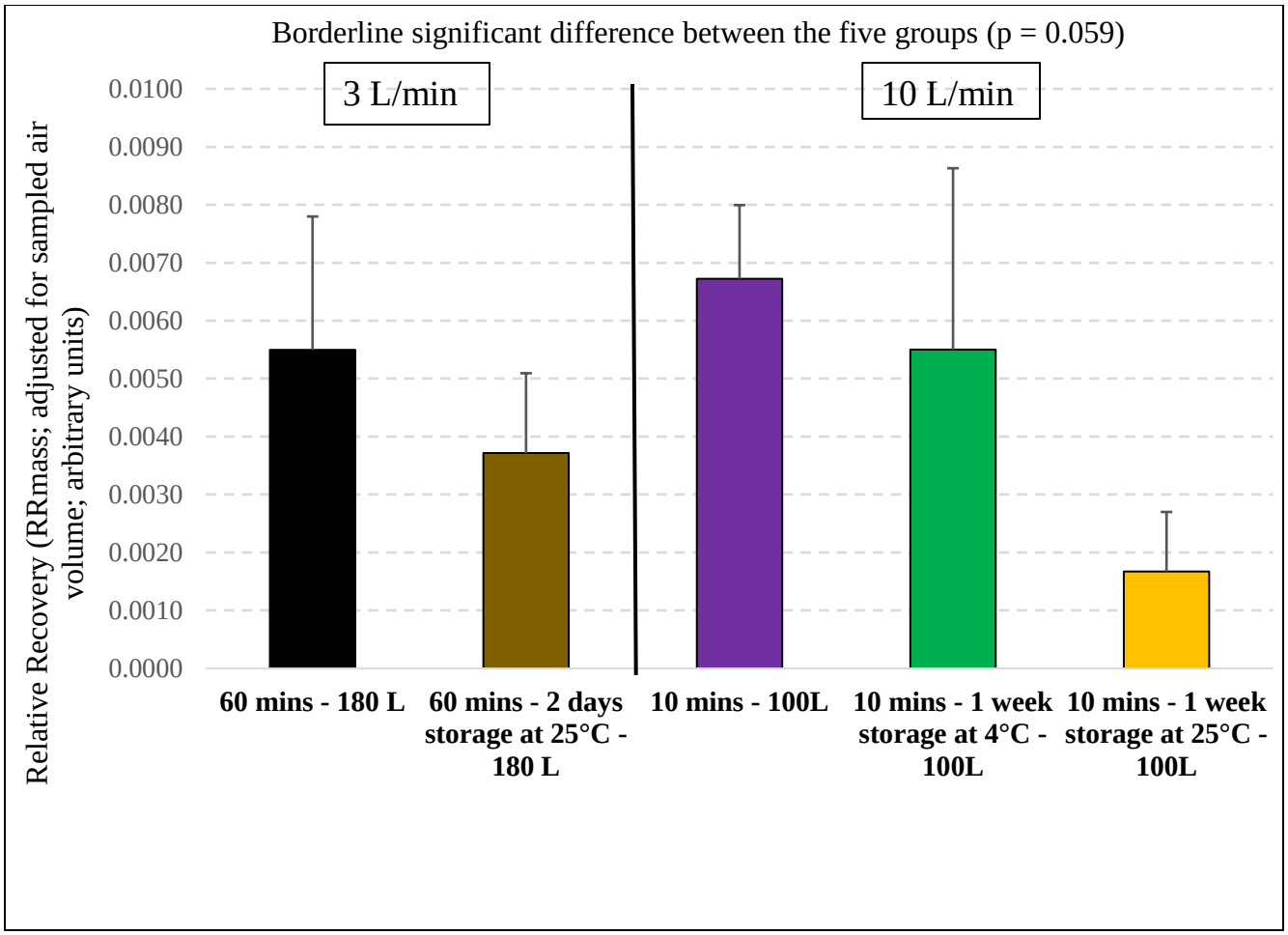


Figure 5: The effects of storage conditions on Relative Recovery ($RR_{mass}$) of airborne OC 43 virus sampled on Zefon Filters (reference: mass concentration). The black and brown bars represent sampling for 60 mins at 3 L/min (180L volume) with no storage and 2-day storage at 25°C, respectively. The purple, green, yellow bars represent sampling for 10 mins at 10 L/min (100L volume) with no storage, 1-week storage at 4°C, and 1-week storage at 25°C, respectively. The five groups were borderline significantly different from each other, with a p-value of 0.059. When pairwise comparisons within this group of five were considered, 10 mins at 10 L/min (100L volume) with no storage and 1-week storage at 25°C were significantly different from each other ($p < 0.05$).

## 4. Conclusions

- We successfully aerosolized Human Coronavirus OC43 (HCoV OC43), a surrogate for the novel SARS-CoV2 virus. There are few published studies with airborne OC43 virus to the best of our knowledge, and its use here adds to the novelty of the study.
- We found that the vortexing method yielded higher RNA concentrations compared to the shaker method for both samples spiked on the filter and airborne samples collected on the filter.
- We recommend using porous plastic support pads to minimize pressure drop during sampling; Cellulose pads are not recommended because of potential contamination and RNA interference.
- Sampling was evaluated at 10 L/min for short-term sampling and at 3 L/min for long-term sampling.
- The relative recovery of the aerosolized virus from filters after the six different sampling scenarios (100L, 180L, and 600L with immediate analysis as well as 100L followed by exposure to clean airstream for 1 hour, and from experiments at 180L volume followed by exposure to clean airstream for 5 hours and 15 hours) were not significantly different from each other ($p = 0.805$).
- When comparing the relative recovery of the virus after storage at 25°C for 2 days and no storage, the average relative recovery of the virus after storage at 25°C for 2 days was ~35% lower compared to immediate processing after sampling; however, due to experimental variability, the difference was not statistically significant ($p=0.24$). Nevertheless, we recommend sample shipping under reduced temperatures.
- When comparing the recovery of the virus after storage for seven days at 4°C and 25°C and immediate processing with no storage, the difference in relative recovery among these three groups was borderline statistically significant ($p = 0.053$). Therefore, we recommend storage in a laboratory refrigerator prior to analysis.

- When pairwise comparisons within the five subgroups of sampling with various storage conditions (Figure 5) were performed, sampling for 10 mins at 10 L/min (100L volume) with no storage and 1-week storage at 25°C was significantly different from each other ($p < 0.05$).
- Based on the results above, we recommend shipping filters within a week of sampling.
- For qualitative and quantitative analysis of the airborne virus, shipping filter cassettes in an insulated Styrofoam box with ice packs is highly recommended.

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