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VIRA-PORE Viral Sampling Cassette

A specialty air sampling cassette for the collection of viral RNA

The Vira-Pore viral sampling cassette featuring the ZePore™ filter has been specifically designed and validated for the collection of airborne RNA from a coronavirus and, by analogy, it could be used for sampling RNA from other viruses.

VIRA-PORE Viral Sampling Cassette Technical Summary

Goal

To determine parameters for the sampling of airborne Human Coronavirus OC43 (HCoV OC43), a surrogate for the novel SARS-CoV2 virus, using 37mm 1 µm pore-size PTFE-laminated PTFE Zefon® "ZePore"™ filters.

Both human coronavirus OC43 and SARS-CoV-2 are positive-sense single-stranded RNA (+ssRNA) beta enveloped coronavirus, and thus OC43 is a suitable and close simulant of SARS-CoV-2.

Previous studies of virus sampling on filters has not often included basic validation data, such as determining the best technique to elute the virus from a filter, the stability of the virus on the filter during sampling or during transportation and storage.

Methods

Extraction of eluted RNA followed by quantification with NanoDrop 1000 (Fisher Scientific Inc.) provided results in ng/µL, normalized to the sampling time. (RT-PCR is used for field samples, and several samples were analyzed by qRT-PCR using primers specific to OC43 to confirm that the virus samples could be amplified after their elution and extraction from the filter). All experiments were performed at least in triplicate.

Results are expressed as relative recovery (RR_{mass}). The RR_{mass} is the ratio of the viral mass concentration eluted from a filter (ng/ml) normalized to the sampled air volume relative to mass concentration in the initial nebulizer suspension (ng/ml).

Filters were used in 37 mm three-piece conductive polypropylene cassettes, in the "open-face" configuration. Conductive cassettes are preferred for fine particle sampling to prevent electrostatic attraction to the cassette walls.

Viral particles were eluted using two different methods with both suspension-spiked filters and air samples for 10 mins at 10 L/m (see below). The elution method with the best recovery in both cases was to roll the filters inwards and place them in a 2 mL microcentrifuge tube with 1 mL of MTM media (Longhorn Vaccines and Diagnostics™, Inc.) and vortex them three times for 10 seconds each.

Virus suspension was aerosolized using a Collison nebulizer in a level II biosafety cabinet. This procedure yielded an average airborne particle concentration of ~1×10^8 #/L.

Flow was set initially at 10 L/min to maximize the amount of virus collected in a short time. The pressure drop with filter and support pad is 17 inches of water gauge (4.23 kPa), which is compatible with vacuum pumps such as the Zefon High Volume Rotary Vane pump

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and certain personal sampling pumps. Samples were collected for 10 minutes, 60 minutes, and 10 minutes followed by 60 minutes of clean air to evaluate the recovery and stability of collected virus during the sampling process.

Flow was also set to 3 L/min to maximize sampling time. At this rate the pressure drop is less than 5.5 i.w.g. (1.37 kPa), which is compatible with most personal sampling pumps, including the Zefon Escort ELF. Samples were collected for 60 minutes, 60 minutes followed by 5 hours of clean air, and 60 minutes followed by 15 hours of clean air to evaluate the recovery and stability of collected virus during the sampling process.

Collected samples were evaluated for stability during transport and storage as follows:

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

Results

Sampling

Colors refer to bars in Figure 1. For the 10 min samples at 10 L/min (100L volume), the RR_{mass} (purple bar) was 0.0067. For the 60 min samples at 10 L/min (600L volume), the RR_{mass} (blue bar) was 0.0054. For the 10 min samples at 10 L/min (100L volume) followed by exposure to a clean airstream for 1 hour, the RR_{mass} (orange bar) was 0.0050. For the 60 mins samples at 3 L/min (180L volume), the RR_{mass} (black bar) was 0.0055. For the 60 mins samples at 3 L/min (180L volume) followed by exposure to clean airstream for 5 hours, the RR_{mass} (olive green bar) was 0.0048. For the 60 mins samples at 3 L/min (180L volume) followed by exposure to clean airstream for 15 hours, the RR_{mass} (grey bar) was 0.0042. There was no statistically significant difference between these results ($p = 0.805$).

Storage

For recovery after 2 days of storage at 25°C, to simulate unrefrigerated transport to the laboratory, with a volume of 180L, the RR_{mass} (brown bar in Figure 2) was 0.0037. Although after 2-day storage the average RNA mass concentration and the relative recovery are lower compared to no storage the difference is not statistically significant ($p = 0.24$).

Recovery was evaluated after 7 days of storage at 4°C and 25°C (room temperature), to simulate different storage conditions in the laboratory prior to analysis, for a sampled volume of 100L. For the storage at 4°C, the RNA mass concentration was 4.41×10^3 ng/mL, and for the 7-day storage at 25°C, the RNA mass concentration was 1.34×10^3 ng/mL. 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 2, the RR_{mass} for the 7-day storage at 4°C (green bar) and the 7-day storage at 25°C (yellow bar) was 0.0055 and 0.0017, respectively. When all five scenarios in Figure 2 are considered, the statistical difference was borderline significant ($p = 0.059$). When comparing the 7-day storage at 25°C, 7-day storage at 4°C, and no storage for the same sampled volume of 100 L the difference was borderline statistically significant ($p = 0.053$). When pairwise comparisons within this group of five were considered, sampling for 10 mins at 10 L/min (100L volume) with no storage and 1-week storage at 25°C were the only pairs significantly different from each other ($p < 0.05$).

Two eluted samples from storage experiments were further analyzed by qRT-PCR in order to show that the total RNA analysis method was not producing misleading results. For these two samples, the detected RNA concentration was similar: 4.01×10^6 RNA copy/ml (for 4°C storage) and 4.18×10^6 RNA copy/ml (for 25°C storage). For both samples, the CT value of the qRT-PCR was above 30 – at the lower end of detectability. This could explain the similarity of the values between the two samples, and why, unlike with the total RNA analysis, the difference between the two samples is not apparent.

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Figure 1

The effects of sampling time and storage conditions on Relative Recovery (RR_{mass}) of airborne OC43 virus sampled on ZePore Filters (reference: mass concentration)

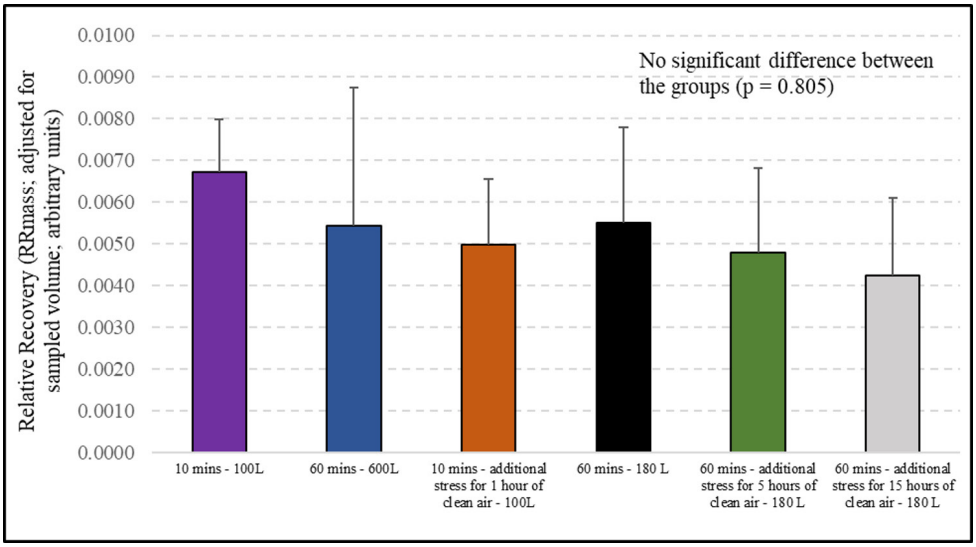
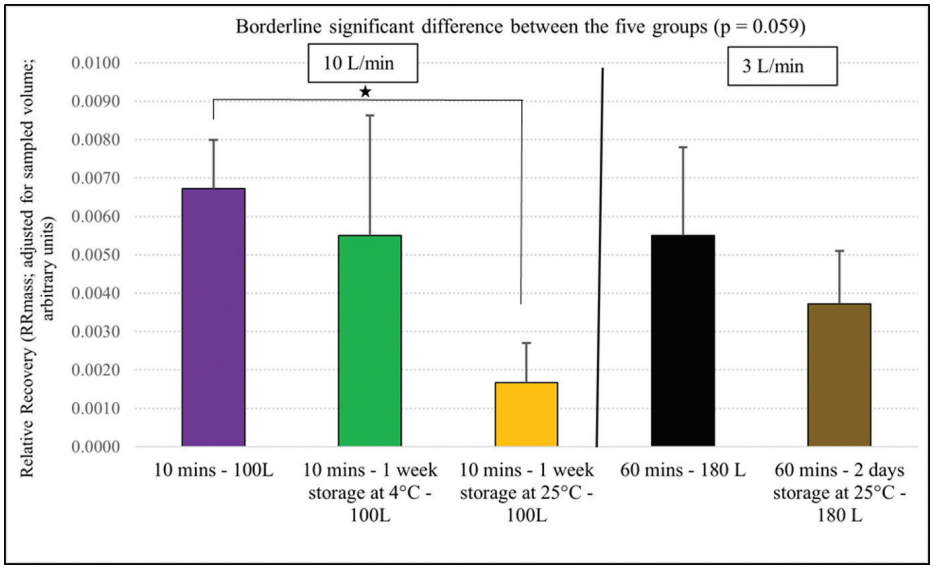


Figure 2

The effects of storage time and temperature on Relative Recovery (RR_{mass}) of airborne OC43 virus sampled on ZePore Filters (reference: mass concentration)



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Conclusions

- Human Coronavirus OC43 (HCoV OC43), a surrogate for the novel SARS-CoV2 virus was successfully aerosolized, collected, and recovered for analysis from the ZePore filters used in the VIRA-PORE cassettes.
- Vortexing elution from the filter yields higher RNA concentrations compared to a shaker method.
- The recovery of the virus from the filter after airborne sampling stress was evaluated by sampling aerosolized virus 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 of the filter to a clean airstream for 1 hour at 10 L/min
  - 60 mins at 3 L/min (180L volume)
  - 60 mins at 3 L/min (180L volume) followed by exposure of the filter to a clean airstream for 5 hours at 3 L/min
  - 60 mins at 3 L/min (180L volume) followed by exposure of the filter to a clean airstream for 15 hours at 3 L/min.
- The recovery of the aerosolized virus from filters after these different sampling scenarios was not statistically significantly different ($p = 0.805$).
- 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 shipping under reduced temperature.
- When comparing the recovery of the virus after storage for seven days at 4°C and 25°C, the difference in the recovery of the virus after storage at 25°C compared to immediate analysis was statistically significantly lower ($p < 0.05$). Therefore, we recommend storage in a laboratory refrigerator prior to analysis.
- These experiments were carried out with a procedure for analyzing total RNA; RNA was also detected in comparison analyses from some of the samples using a qRT-PCR protocol

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