ENVIRONMENTAL EXPRESS®

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 virus, and thus OC43 is a suitable and close simulant.

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.) providing results in ng/µL, normalized to the sampling time. (RT-PCR is used for field samples). All experiments were performed in triplicate, except where noted.

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) 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.

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, which is compatible with certain personal sampling pumps such as the Gilian 12 or GSA SG10 or SG10-2.

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. Sampling was conducted and the virus from filters was eluted immediately after sampling for 10 mins, 60 mins, and 10 mins followed by exposure to clean airstream for 60 mins.

Results

For the 10 min samples, the RNA mass concentration and RR_{mass} (purple bar in Figure 1) were 2.0×10^6 ng/mL (± 1.06×10^6) and 0.55 (± 0.18), respectively. For the 60 min samples, the average RNA mass concentration was 5.98×10^6 ng/mL (± 1.79×10^6), and the RR_{mass} (blue bar in Figure 1) after adjusting for the sampling time was 0.32 (± 0.10). For the 10 mins samples followed by exposure to a clean

airstream for 60 mins, RNA mass concentration and RR_{mass} (orange bar in Figure 1) were 2.17 x 10^6 ng/mL (± 6.97 x 10^5) and 0.50 (± 0.16), respectively. These results show that the recovery of the aerosolized virus after sampling for 10 and 60 mins and 10 mins followed by exposure to clean airstream for 60 mins was similar; the difference was not statistically significant ($p = 0.31$).

In a separate experiment, air samples were stored at 4oC and 25oC for 7 days each. At 4°C, RNA mass concentration was 4.41 x 10^6 ng/mL (± 2.51 x 10^6), and for 25°C storage, 1.34 x 10^6 ng/mL (± 8.23 x 10^5). Thus, the average concentration was about 3.3x lower for room storage condition than for refrigerator storage condition. Due to experimental variability, the difference was not significantly different ($p = 0.11$). As shown in Figure 1, the RR_{mass} for 4°C storage (green bar) and 25°C storage (yellow bar) was 0.55 (± 0.31) and 0.17 (± 0.10), respectively.

One eluted sample from each set of the above experiments was 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 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 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. Until further work is carried out, it is recommended that samples be kept refrigerated before analysis.

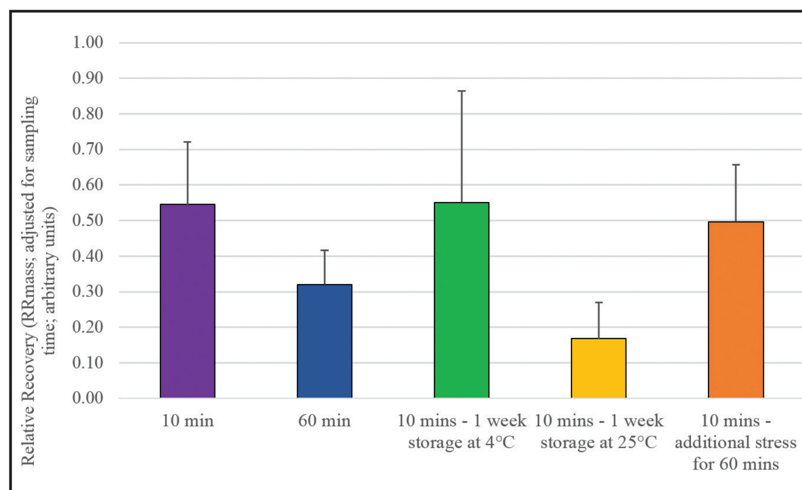

Figure 1

The effects of sampling time and storage conditions on Relative Recovery (RR_{mass}) of airborne OC 43 virus sampled on Zefon Filters at 10 Lpm (reference: mass concentration)

Conclusions

- Human Coronavirus OC43 (HCoV OC43), a surrogate for the novel SARS-CoV2 virus was successfully aerosolized, collected, and recovered for analysis.
- Vortexing elution yields higher RNA concentrations compared to a shaker method.
- The recovery of the aerosolized virus from filters after the three different sampling scenarios (10 mins, 60 mins, and 10 mins followed by exposure to 60 mins of clean airstream) were not significantly different from each other.
- When comparing the recovery of the virus after storage at 4°C and 25°C, the recovery of the virus after storage at 4°C was ~3x higher than after storage at 25°C; however, due to experimental variability, the difference was not statistically significant.

Notice: The VIRA-PORE viral sampling cassette has been tested for collection of RNA from a surrogate virus, but has not been tested for collection of RNA from the COVID-19 virus. Please see the Technical Summary explaning test methodology and test results. The customer is solely responsible for compliance with (i) the Instructions for Use of the product and (ii) all applicable governmental laws, rules, regulations and industry standards related to the use and safe handling of the product and the packaging, labeling and shipment of the product after collection of airborne RNA. Environmental Express recommends that you seek guidance from a qualified environmental microbiology laboratory with expertise in viruses before performing any air sampling. Seek direction, safe handling and transporting procedures as SARS-CoV-2 is highly contagious pathogen with significant health concerns if exposed.