Applying passive samplers to SARS-CoV-2 wastewater monitoring: sampling kinetics and sensitivity for infection rates

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Highlights

- Membranes performed time-integrative sampling of viruses in wastewater over 48 h
- Swabs and tampons showed a rapid initial uptake of viruses and reached equilibrium after 8 h
- Membranes could be sensitive to 2 infection cases among 10,000 people in a sewershed

Introduction

Wastewater surveillance for SARS-CoV-2 has been rapidly developed worldwide to complement clinical testing and inform decision making during the COVID-19 pandemic (Ahmed et al., 2020a). Sample collection at inlet of wastewater treatment plants (WWTPs) can reveal disease prevalence in the entire catchment, but it is unable to locate clusters or hotspots. Therefore, sampling in sewage networks is proposed to enable monitoring of wastewater at the building and subcatchment level, such as hospitals, campuses, and different areas within a city (Gibas et al., 2021; Saguti et al., 2021; Spurbeck et al., 2021; Weidhaas et al., 2021). Passive samplers are low-cost and simple tools that could be suitable for deployment in sewersheds where autosamplers cannot be operated. However, neither sampling process nor sensitivity to infection cases is well understood for passive samplers, which are critical factors in the design of sampling strategy. This abstract presents two studies that aimed to improve the application of passive samplers in upstream wastewater surveillance, including 1) in-situ calibration of passive sampling materials (membranes, swabs, and tampons) for the uptake of viruses, and 2) a virus tracking study to determine the sensitivity of passive samplers to infection rates.

Methodology

Design of passive samplers

Two types of passive samplers were used in our studies, including the Torpedo-style sampler (Schang et al., 2021) that contained swabs (Qtips) and electronegative membranes (pore size 0.45 μm, diameter 47 mm), and the tampon-style sampler with an organic cotton tampon (12 mm by 50 mm) enclosed in a hair roller. Each sampling unit was tied to a long cord to ensure submersion in wastewater.

Experimental protocol of calibration study

In order to assess the accumulation of viral gene fragments in passive samplers over time, we conducted an *in-situ* calibration study at a WWTP in Brisbane, Australia. Torpedos and tampon-style samplers were deployed in wastewater influent and retrieved sequentially at multiple time points over 48 h. Wastewater samples were collected in parallel using an autosampler. All samples were transported to a refrigerator on the day of collection. Due to the low prevalence of SARS-CoV-2 in Brisbane during the studying period, we selected three viruses for investigation, including an indicator virus, i.e., pepper mild mottle virus (PMMoV) with high and stable concentrations in wastewater, and two pathogenic viruses, i.e., human adenovirus 40/41 (HAdV 40/41) and enterovirus with less prevalence.

Experimental protocol of virus tracking study

To determine the sensitivity of membranes for SARS-CoV-2 and the related infection rates in wastewater, we deployed a number of passive samplers along sewer networks downstream of a quarantine hospital. According to GIS map, we identified 8 key conjunction points downstream of the hospital and subsequently installed sampling points at 8 manholes receiving wastewater from different areas of the city. Number of inhabitants in each service area was ~4,000 - 119,000. The quarantine hospital was the single known source of SARS-CoV-2 in the wastewater catchment as no community transmission was reported during the study period in this catchment. Three sampling campaigns were conducted and each sampling period lasted 24 h. The number of hospitalized patients infected with SARS-CoV-2 was 13 - 25 during the three campaigns. Samples were transported to laboratory on the day of collection and stored under 4ºC.

Laboratories analyses

All collected samples were pre-processed, extracted and analyzed using RT-qPCR. Different sample pre-processing and RNA extraction methods were applied to liquid samples, membranes, swabs, and tampons (Ahmed et al., 2020b; Schang et al., 2021). The passive samples and wastewater samples collected from the calibration study were used for the analyses of PMMoV, enterovirus, and adenovirus. The membrane samples collected from the virus tracking study were analysed for SARS-CoV-2. Murine hepatitis virus was used as a molecular process control.

Results and discussion

In the calibration study, accumulation of viral gene fragments on passive samplers was observed for up to 48 h (Figure 1). Numbers of viral gene copies (N_s) continuously increased on membranes over time (t) without achieving equilibrium in 48 h. The uptake pattern of membranes can be fitted using the linear regression ($N_s = C_w R_s t$; C_w: viral concentration in wastewater). The estimated sampling rates (R_s) of membrane varied from 0.3 to 33 mL/h for different viruses. We consider the sampling rate for PMMoV (1.0 mL/h) can be applicable to other viruses with high and stable concentrations in wastewater. Different from the uptake pattern of membranes, a rapid increase of viral fragments was found in swabs and tampons following by the equilibrium in approximately 8 h, which can be fitted using the first-order kinetics. During the 48-h sampling period, the numbers or viral RNA accumulated in swabs and tampons were higher than those detected from membranes, which could be attributed to the porewater with viral fragments retained in the inner part of cotton materials.

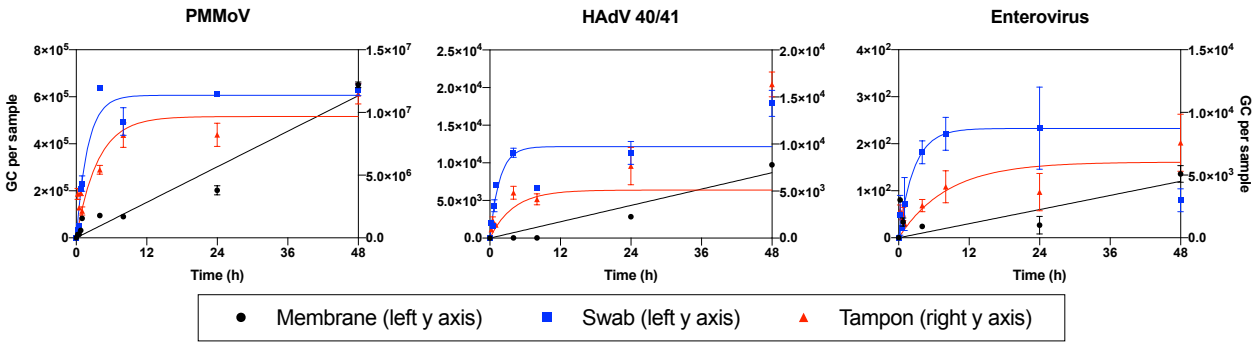

Figure 1. Numbers of gene copies (GC) of PMMoV, HAdV 40/41. and enterovirus in membranes, swabs, and tampons over 48 h during in-situ calibration study, fitted with linear and nonlinear curves.

With the understanding of the time-integrative sampling process of membranes, we further investigated its sensitivity to infection cases in a sewershed. During the virus tracking study, positive signals of SARS-CoV-2 was detected from 15 out of 18 passive samples with gene copies ranging from 5.4 to 1588.9 per sample. The three samples collected at the furthest three manholes from the hospital had negative results. The accumulation of viral fragments on membranes decreased along sewer networks due to the increasing dilution. The lowest amount of SARS-CoV-2 RNA detected from the membranes with 24-h deployment in wastewater was 5.4 gene copies. By assuming the hospitalized COVID-19 patients had the uniform virus shedding levels and defecation rates on the day of sampling, the lowest infection rate that allowed for a positive detection from membranes was 1.9/10,000. We found a positive relationship (R^2=0.55) between

the number of SARS-CoV-2 RNA over 24-h passive sampling and infection rates in the range of 1.9 to 18.5/10,000 (Figure 2). Viral signals were undetectable when the infection rate reduced to 0.7 - 1.6/10,000.

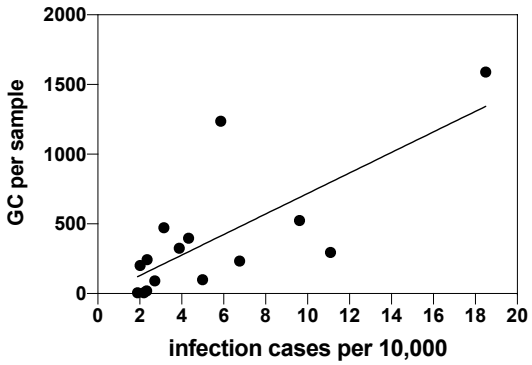

Figure 2. Correlation between numbers of SARS-CoV-2 gene copies (GC) accumulated on membranes through 24-h passive sampling and infection rates during the virus tracking study. Data is fitted with linear regression.

Conclusions and future work

Our studies demonstrated that passive sampling is a simple and practical approach to monitoring the spread of viruses in sewersheds at different scales. The selection of suitable sampling techniques and strategic sampling points at sewer networks is critical to wastewater surveillance in upstream catchments, which should consider the representative sampling of wastewater, maximized coverage of a certain manhole, and huge dilution in sewer pipes, etc. The utilization of membrane-loaded passive sampler can allow for time-integrative sampling over 48 h with the lowest detection limit of 5.4 gene copies per sample and roughly 2 infection cases among 10,000 people. Swabs and tampons have a greater capacity to capture viruses within the effective sampling period of approximately 8 h, which could be suitable for a short-term scanning of wastewater. To further develop upstream sampling for baseline monitoring and source tracking, more studies are needed to understand the impact of different factors on sampling processes (e.g., virus characteristics, viral concentration dynamics, and environmental variables) and more field studies are required to validate the performance of sampling techniques under dynamic sewer conditions.

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