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Passive sampling, a practical method for wastewater-based surveillance of SARS-CoV-2

ARTICLE INFO

Keywords
SARS-CoV-2
Passive samplers
Wastewater surveillance
COVID-19
RT-qPCR
wastewater-based epidemiology (WBE)

ABSTRACT

In search of practical and affordable tools for wastewater-based surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), three independent field experiments were conducted using three passive sampler sorbents (electronegative membrane, cotton bud, and gauze) in Guelph, Ontario, Canada. Total daily cases during this study ranged from 2 to 17/100,000 people and 43/54 traditionally collected wastewater samples were positive for SARS-CoV-2 with mean detectable concentrations ranging from 8.4 to 1780 copies/ml. Viral levels on the passive samplers were assessed after 4, 8, 24, 48, 72, and 96 hrs of deployment in the wastewater and 43/54 membrane, 42/54 gauze, and 27/54 cotton bud samples were positive. A linear accumulation rate of SARS-CoV-2 on the membranes was observed up to 48 hours, suggesting the passive sampler could adequately reflect wastewater levels for up to two days of deployment. Due the variability in accumulation observed for the cotton buds and gauzes, and the pre-processing steps required for the gauzes, we recommend membrane filters as a simple cost-effective option for wastewater-based surveillance of SARS-CoV-2.

1. Introduction

To track the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), researchers around the globe are using wastewater-based epidemiology (WBE) to understand the temporal and spatial spread of the virus (COVIDPoops19 Dashboard, 2021). SARS-CoV-2 infects the absorptive enterocytes of the human gastrointestinal tract (Zou et al., 2020) and large amounts of the virus are shed into municipal wastewater through feces (Parasa et al., 2020; Wang et al., 2020a; Zheng et al., 2020). Accordingly, studies have quantified the virus RNA in raw wastewater (Peccia et al., 2020) and sludge (Balboa et al., 2021) where its genetic material may persist unaffected for days (Wurtzer et al., 2021). Interestingly, the virus has been detected in wastewater days before clinical cases were reported (Medema et al., 2020), and include asymptomatic (Lee et al., 2020) and pre-symptomatic populations. Therefore, monitoring of SARS-CoV-2 RNA is gaining attention as a promising tool in tracking the spread at the community level and serve as an early warning tool for outbreaks and viral caseloads (Tiwari et al., 2021).

The efficacy of WBE of SARS-CoV-2 depends on the ability to accurately characterize the RNA concentration in wastewater which fluctuates continuously depending on time of day (e.g., shedding time), dilution (e.g., precipitation, dilution by industrial waste), and travel time in sewer networks. Hence, continuous sampling is ideal for capturing the variability in wastewater composition. Traditionally, autosamplers are used to collect multiple liquid samples over a specific time interval which are then composited to a wider time range (e.g., 24 hrs). However, autosamplers are not always feasible at every sampling site (e.g., insecure sites, no power, deep sewer mains and within buildings). Moreover, use of autosamplers is costly and processing liquid samples involves time-consuming sample processing techniques prior to nucleic acid extraction (Kitajima et al., 2020). Therefore, development of practical and inexpensive sampling tools that can be used at any site and can reduce processing time to provide timely data, are critical in streamlining WBE surveillance.

Passive sampling, whereby a material is directly deployed in wastewater to sorb the virus over time, may provide an inexpensive and practical alternative to autosampling (Valenzuela et al., 2020). Liu et al. (2020) used a Moore swab (i.e., pieces of gauzes) tied to a fishing line for surveillance of SARS-CoV-2. Although they deployed the swabs for 24–72 hrs, accumulation of SARS-CoV-2 over time, which is critical in establishing ideal deployment times, was not shown. At larger scales (i.e., lot, suburb, and city), Schang et al. (2020) evaluated three passive sampler materials (i.e., sorbents) including electronegative membranes, gauzes, and cotton buds (Q-tips) for detection of SARS-CoV-2 in wastewater. They installed these materials in a torpedo-like perforated device that continuously exposes the materials to flowing wastewater. They reported greater sensitivity of these samplers over traditional sampling particularly when SARS-CoV-2 concentrations were <1 copy/mL. Moreover, Hayes et al. (2021) evaluated four different materials: cotton gauze, cheesecloth, cellulose sponges, and electronegative membrane filters, reporting higher performance (for accumulating SARS-CoV-2) for cheesecloth and membrane filters. However, the optimal deployment times (in relation to available SARS-CoV-2 concentration in the wastewater) were not assessed.

Field studies have yet to demonstrate the ability for passive samplers to linearly accumulate SARS-CoV-2 from wastewater over set durations. However, this is precisely the evidence required to demonstrate that passive samplers can adequately represent time-averaged wastewater concentrations (Gorecki and Namiešnik, 2002). Non-linear uptake may either suggest that the passive sampler has reached its capacity during its exposure period, the adsorption rates are too low to reliably measure SARS-CoV-2 on the sampler, or that adsorption and desorption rates are similar (Gorecki and Namiešnik, 2002).

Consequently, to fill this gap, the goal of the present study was to evaluate whether the three passive sampler materials developed by
Schang et al. (2020) follow a linear process in taking up SARS-CoV-2 (i.e., follow first-order kinetics). We performed three replicate experiments at a wastewater pilot facility where we deployed the three materials (electronegative membranes, gauzes, and cotton buds) and retrieved them at 4, 8, 24, 48, 72 and 96 hrs to assess their ability to accumulate SARS-CoV-2 in raw wastewater.

2. Materials and methods

2.1. Sampling site and the passive sampling tools

We conducted a controlled experiment at a pilot-scale wastewater facility (Text S1) in the city of Guelph, ON, using an apparatus to simulate conditions in a sewer main (Text S1, Fig. S1). The apparatus was fed a continuous flow (7.8L/min) of raw wastewater. Six pairs of torpedo-style samplers were deployed horizontally in the PVC pipe apparatus and remained submerged in the wastewater stream. They were tied to a piece of stiff tubing and placed in the PVC pipe (Fig. S1). Each pair of torpedoes contained either three cotton buds and three membranes or three gauzes and three cotton buds. Details on the design of the samplers are described in Schang et al. (2020). In this study, we processed all of the membranes and the gauzes in triplicate for each time point. An autosampler (Sigma 900 MAX) was connected at the inflow to the PVC pipe, collecting 50mL of sample every 30 minutes, thus enabling simultaneous collection of time-composited wastewater samples. These samples were used to assess the relative accumulation of SARS-CoV-2 on the passive samplers over time.

2.2. Sample collection and storage

Three independent 96 hrs trials were conducted in February 2021, where in each trial 6 pairs of torpedo passive samplers were deployed. The pairs were randomly removed from the PVC pipe at 4, 8, 24, 48, 72, and 96 hrs of deployment. At each time point, torpedoes were immediately disassembled on site and transferred to clean sterile Ziploc bags and stored in –80°C freezer until RNA extraction. We processed all of the membranes, gauzes and cotton buds in triplicate for each time point (3 biological replicates per passive material per time point). In parallel, time-composited wastewater samples were collected from the autosampler. Three 50mL composited samples were collected at 4, 8, and 24 hrs, respectively. For time points 48, 72 and 96 hrs, the previous 24-hrs of sample was composited and three 50mL samples were collected. Each composite was then filtered immediately using 0.45 μm electronegative membrane on site. Membranes were then stored at –80°C until RNA extraction. Viral concentrations during the longer deployment times (i.e., 48, 72, and 96 hrs) were calculated by applying time-weighted mean concentration principle. Filtration blanks of tap water were performed on-site at the end of experiment 1 and 3 to assess for contamination during the filtration process of liquid samples.

2.3. RNA extraction and RT-qPCR

Electronegative membranes (from filtered composite samples collected by the autosampler and torpedoes) and cotton buds were directly used for RNA extraction, whereas gauze samplers were first eluted and then filtered on membranes prior to extraction. Elution was performed per Schang et al. (2020) with slight modifications (see Text S2). Extraction recovery efficiency (%) was assessed by spiking samples with 8.5 log copies bacteriophage Phi6 (a surrogate for SARS-CoV-2 stability) (Aquino de Carvalho et al., 2017; Fedorenko et al., 2020), recoveries were calculated as (copies recovered/copies spiked) × 100 (Fig. S5). RNA extraction was performed using RNeasy PowerMicrobiome Kit (Qiagen) with some modifications (Text S2).

Prior to running TaqMan probe-based reverse transcription quantitative real-time PCR (RT-qPCR), tests for potential inhibition were performed for 81/216 samples (37.5%) (details in Text S3). To further evaluate inhibition, dilutions were performed on 200/216 samples. If the sample still showed evidence of inhibition through dilution (i.e., concentrations increased during dilution), the lower dilutions were used in the calculation of copies per well. The CDC emergency use authorization kits (2019-nCov CDC EUA Kit) which contain N1 and N2 primer-probe sets (IDT, Kanata, Canada) and Reliance One-Step Multiplex RT-qPCR Supermix (BioRad, Hercules, CA) were used to perform one-step RT-qPCR reaction. Details on the calibration curves and limit of detection can be found in Text S4. To support potential accumulation of SARS-CoV-2 on the passive samplers with deployment time, we also quantified the pepper mild mottle virus (PMMoV), an abundant human fecal marker (Rosario et al., 2009). Probes, primers and thermocycling conditions and standard curve efficiencies, R (Zou et al., 2020), slope, and Y-intercepts for both the SARS-CoV-2 and PMMoV can be found in Table S2 and Text S4. All reactions were performed on a CFX96 thermal cycler (BioRad Laboratories), and triplicate reactions per biological replicate were used for SARS-CoV-2 and duplicate for PMMoV. No-template negative controls were performed for all analysis steps: liquid wastewater sample filtration, nucleic acid extraction, reverse transcription, and PCR. All negative controls were negative. Positive PCR controls were run with each sample batch. Data were analyzed using the CFX Maestro software Version 5.0.021.0616.

3. Data analysis

Despite our attempt to quantify both N1 and N2 gene fragments of SARS-CoV-2, the N1 fragment was more sensitive (i.e., consistently detectable) in most of the samples when compared to the N2 gene fragment. Therefore, we present the SARS-CoV-2 results based on the N1 gene fragment. There were some non-detects (NDs) in the technical replicates of the RT-qPCR assays, thus, to avoid overestimation by taking NDs as blanks, NDs were replaced by half the LOD value (4.15, see text S4) when at least one replicate was positive. Using recovery adjusted copies, we calculated accumulation ratios over time for SARS-CoV-2 and PMMoV, calculated as Cp/Cw, where Cw = concentration of target in the composite wastewater sample for that time frame and Cp = concentration of target in the passive sampler for that same time frame. The recovery data are presented in Fig S5. All data reported for SARS-CoV-2 and PMMoV were adjusted for recovery and reported as geometric means.

4. Results and discussion

4.1. Performance of passive samplers

The study was conducted when daily SARS-CoV-2 incidence rates (i.e., new daily confirmed cases in Guelph were lower (2–17/100,000 people) than the rates seen in January and late in April 2021 (Fig. S3a). Considering a 25-day duration of positive signals on feces (Wang et al., 2020b), the total estimated active COVID-19 cases during the study ranged from 211 to 484 per 100,000 people (Fig. S3b). At these case-loads, both the composite and passive samplers, particularly membranes and gauzes, were able to sorb detectable amounts of SARS-CoV-2 RNA within 24 hrs of deployment. Wu et al. (2021), who conducted a large campaign of wastewater surveillance in the US, indicated that detection of SARS-CoV-2 is most likely when daily incidence rates are greater than 13/100,000 people. However, it is important to note that SARS-CoV-2 titers in wastewater may not accurately reflect the incidence rates (Wu et al., 2020) as fecal shedding by asymptomatic and pre/post-symptomatic individuals may also be significant (Wang et al., 2020a; Cevik et al., 2021).

In all three trials, where 216 passive and liquid wastewater samples were processed, total RNA content on the passive samplers increased with deployment time (S4b, c, d). While membranes and cotton buds accumulated RNA gradually, gauzes had quicker saturation where the level of RNA at 4 hrs were equivalent to the RNA contents of membranes.
and cotton buds after 48 hrs (Fig. S4b, c, d).

Extraction recoveries varied between the materials (2–24%) (Fig. S5; Text S5).

Positivity rates for SARS-CoV-2 were comparable among composite, membrane, and gauze samples, which had 43/54, 43/54, and 42/54 positives, respectively (Table S1). Consistent with the results of Schang et al. (2020), the lowest positivity rate was observed for the cotton buds where 27/54 samples were negative, although in our study positivity increased with time (i.e., 2/9 at 4 hrs to 7/9 at 96 hrs). The cotton buds were significantly smaller than the other materials which may have contributed to this observation. Like the cotton buds, there was an increase in positivity with time for the membrane and gauze samplers. Interestingly, after 24 hrs of deployment (a common timeframe for collecting wastewater samples using autosamplers), membranes and gauzes had 8/9 samples positive for SARS-CoV-2 RNA, while only 6/9 of the composite samples were positive. Schang et al. (2020) reported similar results where they observed greater sensitivity of the passive samplers than liquid composite samples.

During this study, up to 1780, 825, and 524 gene copies/mL of SARS-CoV-2 were detected in composite wastewater samples collected during the first, second and third trial, respectively (Table S3). After testing for normality, ANOVA and Tukey’s multiple comparison test indicated that changes in the geometric means of SARS-CoV-2 gene copies (gc) in the wastewater were not significant between the three experimental trials (p = 0.45). When detected, mean copies recovered on the membrane, cotton bud and gauze passives samplers ranged from 270 to 1640 gc/membrane, 379 to 2230 gc/cotton bud and 609 to 3600 gc/gauze, respectively (Table S3).

The detection capabilities of the passive materials were also assessed by measuring the quantities of PMMoV (Table S4). Concentrations of PMMoV in the wastewater ranged from 1.58E+05 to 4.06E+07 gc/mL. Mean copies recovered on the membrane, cotton bud and gauze passives ranged from 9.50E+04 to 1.31E+08 gc/membrane, 3.66E+05 to 3.81E+09 gc/cotton bud and 1.13E+06 to 5.36E+09 gc/gauze, respectively. Interestingly, by 8 hours, PMMoV copies in gauzes reached capacity that membranes and cotton could accumulate after 96 hrs of deployment, (Table S4).

4.2. Accumulation ratios of passive samplers

To compare the relative accumulation of SARS-CoV-2 and PMMoV over time on the different passive samplers, we calculated accumulation ratios whereby we divided the concentration on the passive sampler by the time weighted concentration from the wastewater for the same time-period (Figs 1 and 2). In the three trials, the accumulation of SARS-CoV-2 on the membrane samplers was linear up to 48 hrs and then was variable between 48 and 96 hours (Fig. 1). Accumulation ratios were low and variable for cotton buds, except at 96 hrs where the accumulation ratio spiked to 23 (Fig. 1). The accumulation ratios for gauze samplers were variable and did not follow a trend.

Similar trends were observed for accumulation ratios of PMMoV on the membrane and cotton bud passive samplers, where membranes saw a linear increase to 48 hours and cotton buds were variable (Fig. 2). In contrast to the SARS-CoV-2 results, gauze samplers showed a similar trend to the membranes for PMMoV with a linear increase to 48 hours. For all materials, accumulation ratios for both viruses showed increases and decreases after 48 hrs of deployment, which could be related to the accumulation of inhibitors, RNA washout and decay. The results suggest that at low concentrations, the accumulation of inhibitors on the passives could hinder the quantification of SARS-CoV-2 beyond 48 hrs of deployment (Fig. 1). Based on the accumulation ratios, and the positivity rates, the membranes outperformed the gauze and cotton buds as a passive sampling device for SARS-CoV-2. A lack of linear increase of SARS-CoV-2 on the gauze samplers over time could be indicative of the rapid accumulation of inhibitors which hindered detection of the low copy numbers (Rafiee et al., 2021). This trend was not evident for PMMoV that was much more abundant in the wastestream. Although spiking a synthetic RNA in PCR-grade water, and RNA templates, followed by assessing the CQ values of RT-qPCR showed inhibition in only 4 of the 81 samples assessed, there was clearly inhibition as higher dilutions (i.e., 3, 2.5, 2, and 1 μl of template RNA) often resulted in detections or higher quantities of SARS-CoV-2 RNA in many of the samples. During dilution, 29/54 composite, 24/54 membrane, 15/54 cotton bud, 33/54 gauze samples showed increases in the SARS-CoV-2, indicating the presence of inhibition. On the other hand, when diluted, several samples had no detections. We hypothesize that through dilution the RNA may no longer be detectable or at the dilutions performed, samples were still too inhibited. The use of other methods, such as digital droplet PCR (Racki et al., 2014), may improve quantification of low copy number samples when high amounts of inhibitors are
4.3. Passive samplers as a practical and economical method for wastewater surveillance

The current gold standard for WBE is processing of liquid composite samples often over a 24-hr period. Passive samplers provide a viable and possibly superior alternative as they are continuously exposed to the wastewater stream and, as a result, may capture variabilities in the wastewater missed by composite methods.

From a methodological standpoint, processing of composite and gauze samples involve at least one additional step prior to extraction. Both require filtration on electronegative membranes after preparation of the time-composited wastewater samples and gauze-elution, respectively. The membranes and gauzes performed similarly in terms of yielding positive detections at any time point. However, the additional processing steps (i.e., elution and filtration) required for the gauze are time consuming and might require the use of process controls. Cotton buds, which showed mediocre detection of SARS-CoV-2 RNA after 24 hrs of deployment, did not require additional sample processing or manipulation but technical challenges during nucleic acid extractions were greater for cotton buds than the membrane filters (Text S6).

In conclusion, considering the ease of processing, their high positivity rates, and linear uptake of SARS-CoV-2 RNA (particularly between 4 and 48 hrs of deployment), we recommend electronegative membranes as an effective, practical, and economical passive sampling option for streamlining wastewater-based surveillance of COVID-19.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge the following sources of funding for this research, the National Science and Engineering Council of Canada (Grant # 401655), and the Canada Research Chairs Program (Grant # 950-232787).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112058.

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