SARS-CoV 2 adsorption rates to passive samplers for wastewater surveillance

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Highlights

- Passive samplers have the ability to adsorb and retain SARS-CoV-2.
- Electronegatively charge membranes showed the highest adsorption rate.
- Linear relationships were found between the concentration of virus on the passive and the concentration in the sewer.

Introduction

SARS-CoV-2, the virus responsible for the current COVID-19 pandemic, is detectable in respiratory secretions as well as the faeces of infected humans. Viral fragments have been found in the stool of both asymptomatic and symptomatic persons infected with SARS-CoV-2 for 6 weeks or more from the time of infection with high intra and inter-individual variability spanning the early infectious and later non-infectious periods (Wu et al., 2020: Gupta et al., 2020). As such, these findings indicate that human wastewater monitoring has the potential to act as a sensitive surveillance system as well as an early warning tool, as was previously shown for poliovirus (Lodder et al., 2012), and help provide targeted health actions.

Passive samplers are a cheap, safe and easy way of monitoring wastewater for SARS-CoV-2 (Schang et al., in press). They offer the possibility of a continuous exposure to the wastewater reducing the errors seen with discrete sampling for example, can be directly introduces into the sewage network, do not require a power supply or the need for confined space entry (Schang et al., in press) and have been significantly used in the field of water chemistry in freshwater (Birch et al, 2013; Almeida et al., 2016). However, questions remain in the literature, like: what is the maximum capacity of these passive samplers?, is the adsorption process reversible?, and hence what deployment durations are optimal for passive samplers?

As such, the aim of this research was to assess the potential of commonly available and cheap materials for passive sampling of SARS-CoV-2 in wastewater. Specifically, our objectives were to (1) assess the relationship between the concentration observed on the materials and the actual concentration of SARS-CoV-2 in wastewater and (2) explore the association kinetics of SARS-CoV-2 to passive materials to give guidance on optimal deployment durations.

Methodology

Experimental set-up

Two batch tests were conducted to assess the performances of three commonly available and cheap materials for passive sampling of viruses in wastewater: 75 mm by 75 mm medical gauze swabs, typical electronegative filter membranes (Cellulose Nitrate, 11406-47-ACN, Sartorius, Germany) and cotton buds (Swisspers, China). Each batch test exposed these materials for different durations to real wastewater spiked with gamma-irradiated SARS-CoV-2. Tests were always run with three biological replicates.

The first batch test included three runs where we compared the performance of different sampling materials (Table 1). The first batch tests showed that electronegative membranes had the highest association rate and hence were further investigated in the second batch test (Table 2). In this second batch test, we exposed the materials for an extended period of time (up to 9 days) and tested whether (1) the material had high desorption rates and (2) whether adsorption rates changed significantly over time (Table 2).

Table 1. First batch tests conducted to explore the adsorption of SARS-CoV-2 onto three passive sampling materials (membranes, gauzes and cotton buds).

Starting date of run	SARS-CoV-2 concentration [copies /mL]	Exposure duration [min]
3rd October 2020	0; 3900; 5300; 13,000	10; 60, 360; 1,440
27th October 2020	0; 80,000	10; 360;
16th November 2020	0; 390; 400; 4,900	10; 60, 360

Table 2. Second batch tests conducted to assess adsorption, first and end flush testing of electronegative membranes

Starting date	Tested exposure durations [days]	Tested desorption rates	Tested effect of time on adsorption rate
2nd May 21	1, 2, 3, 4, 5, 6, 7	✓	✓
18th May 21	1, 2, 3, 4, 5, 6, 7, 8	✓	✓
17th June 21	1, 2, 3, 4, 5, 7, 8, 9	✓	✓

Sample processing, extraction, and qPCR

Samples were analysed for SARS-CoV-2 using similar methods to that described in Schang et al. (2021). In summary, 50mL wastewater samples (spiked and un-spiked) were immediately filtered through 47mm diameter negatively charged membranes and directly extracted. Upon removal, passive sampling materials were immediately washed to remove excess wastewater which may have filled the pores. The washing of each material consisted of dipping them into three new and separate 25mL sterile containers containing sterile RNase free water. After washing, electronegative membranes and cotton buds were used directly for extraction. Gauzes were processed as per Schang et al. (2021). Extraction was conducted using either the Qiagen RNeasy PowerMicrobiome kit (Qiagen, Germany) or the Mackerey-Nagel NucleoSpin RNA Stool kits (Mackery-Nagel, Germany). A method extraction blank was processed with each batch of extraction.

The RNA extracts were then tested in duplicate using the SARS-CoV-2 Real-time RT-PCR Assay (PerkinElmer, USA; hereafter referred to as the PE assay) to detect both the nucleocapsid N and the ORF-1ab genes of the SARS-CoV-2 virus. For more details on this process, see Schang et al. (in press). Standard curves were performed in duplicate using five dilutions of the Twist synthetic SARS-CoV-2 RNA control 1 (GenBank ID: MT007544.1, Cat No: 102019), resulting in very high coefficients of determination (R^2>0.99) and consistent intercepts (mean for N gene: 43.6; ORF-1ab gene: 42.5) and slopes (mean for N gene: -3.47; ORF-1ab gene: -3.38) resulting in acceptable qPCR efficiencies (E=94% and 98%, respectively). All assays were run on a Bio-Rad Laboratories CFX-96 qPCR machine (Bio-Rad, USA).

Results and discussion

Batch test 1. The results showed that the membranes had the highest adsorption capacity from start to finish whilst the cotton buds had the lowest rate (lowest linear coefficient). The gauze relationship had the strongest R^2 (Figure 1) but reached the lowest number of copies per g for a same exposure period as the other two materials.

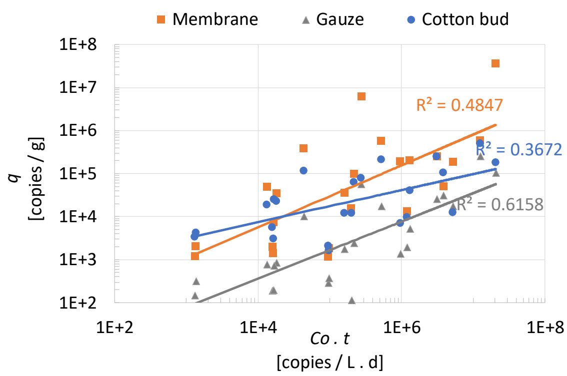

Figure 1. Number of adsorbed virus (per gram of material; No./g) vs. duration of deployment [d] x concentration of water matrix (No./L) for each of the three materials tested in the batch studies.

Batch test 2. The results showed that the membranes were able to continuously attract viral fragments onto their surface for up to 7 days. However, the longer the membranes were exposed to wastewater, the higher the inhibition observed during the qPCR step. After an initial 10 second exposure to SARS-CoV-2 spiked wastewater, membranes demonstrated an ability to retain SARS-CoV-2 for up to seven days in unspiked wastewater, suggesting that the membranes had slow desorption rates. Vice-versa, membranes that were exposed to SARS-CoV-2-free wastewater for seven days were still able to adsorb SARS-CoV-2 when they were once again immersed into SARS-CoV-2 spiked wastewater, however their adsorption rates were halved in comparison to new membranes.

Conclusions and future work

This research showed that out of three easily accessible materials, electronegative membrane have the potential to adsorb SARS-CoV-2 in wastewater for a period of up to 7 days and that a linear relationship could be established between the concentration observed on the passive samplers and the concentration in the wastewater. This study suggested that the electronegative membrane had slow desorption rates as well as an extended but diminished adsorption capacity with time; i.e. the membranes have the ability to retain SARS-CoV-2 for up to 7 days in unspiked wastewater after being exposed to the virus for a short period of time but also to adsorb the virus after 7 days of exposure in unspiked wastewater but with a halved adsorption capacity.

The current work used an inactivated strain of SARS-CoV-2 and therefore it was not possible to assess how the membrane would behave in an environment where natural die-off would occur. Future work will focus on testing wastewater samples with actual strains of SARS-CoV-2 in a very low concentration setting rather than using a laboratory strain.

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