Extended Abstract

Filter membrane screening to increase sensitivity for passive sampling of SARS-CoV-2 in sewage

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Highlights

- Three membranes showed high capacity to passively accumulate SARS-CoV-2 fragments over 24 hours
- A cellulose nitrate membrane showed high capacity to accumulate SARS-CoV-2 fragments over 24 hours
- A polyvinylidene fluoride filter may have a longer linear uptake of SARS-CoV-2 fragments than other membranes

Introduction

Since 2020, monitoring SARS-CoV-2 in sewage systems is being extensively performed in the State of Victoria for early detection and prevention of COVID-19 outbreaks. Black et al. (2021) observed that, through wastewater surveillance, it is possible to detect people infected with SARS-CoV-2 from 2 to 8 weeks after becoming ill. Even though sewage testing can be effective to detect COVID-19 cases, increasing its sensitivity is needed by exploring and improving sampling techniques and methods. Filter membranes are commonly used to accumulate viral particles (Ahmed et al, 2015; Conceição-Neto et al, 2015). Some of those membranes have been tested for virus recovery in sewage (Ahmed et al, 2015) and a few are currently used to capture SARS-CoV-2 fragments by inserting them in passive samplers (Shang et al, 2021). In this research, we aim to evaluate the uptake and desorption rate of SARS-CoV-2 fragments in seven types of filter membranes in order to evaluate their potential use in passive samplers to capture SARS-CoV-2. Positive outcomes of this research would lead us to re-design and manufacture passive samplers with a longer linear uptake rate of SARS-CoV-2 fragments, while maintaining high sensitivity during the first days of deployment.

Methodology

A total of seven filters were carefully selected for analysis based on their use in other studies to accumulate viral particles, availability, material composition and range of porosity (Table 1). Three 1L plastic bottles were filled with 1L of sewage wastewater (SWW) and spiked with 5 µl of 10^6 copies/µl of gamma radiated fragments of SARS-CoV-2. One filter per type was inserted in each bottle. At day zero, one filter per type was dunked three times in bottle one and rinsed three times by deepening it in RNA-free water. The same procedure was done for filters dunked in bottles two and three. After 24 hours of tumbling the three bottles, filters were removed from the bottles and rinsed three times with RNA-free water. At day zero and after 24 hours, 50 mL of SWW per bottle was filtered. All filters were stored at -80 °C until analysis.

Table 1. Physical properties of the seven filters selected for this study

Filter name	Material	Porosity	Pore size	Diameter
CN	Cellulose Nitrate	Not found	0.45	47 mm
HA	mixed cellulose esters (MCE)	79%	0.45	47 mm
GS	mixed cellulose esters (MCE)	75%	0.22	47 mm
PV	Polyvinylidene Fluoride (PVDF)	70%	0.45	47 mm
GT	Polycarbonate	13.80%	0.22	47 mm
HT	Polycarbonate	10-20%	0.45	47 mm
T4	Polypropylene	15%	0.22	47 mm

Total RNA was extracted from all filter membranes using the NucleoSpin®RNA Stool kit (Macherey-Nagel) and RNA extracts were analysed using the PerkinElmer®SARS-CoV-2 Real-time RT-PCR kit. Bio-Rad Laboratories CFX-96 qPCR instrument (Bio-Rad, USA) was used to run all essays with 45 cycles. Results were analysed and plotted using GraphPad Prism 9.

Results and discussion

We evaluated the capacity of seven filter membranes to accumulate SARS-CoV-2 fragments over 24 h (Figure 1). Cellulose membranes (CN, HA and GS) showed the highest accumulation of the virus. The polyvinylidene fluoride (PV) membrane accumulated six times more SARS-CoV-2 fragments after 24 h, which indicates that PV membrane has a lower capacity to accumulate the virus than CN and HA membranes in a short period of time. Membranes made of polycarbonate showed the lowest capacity to accumulate the virus, indicating that these membranes are not appropriate for using them in passive samplers. The T4 membrane had the highest load after dunking it in SWW and almost immediately reached its maximum capacity to accumulate SARS-CoV-2 fragments.

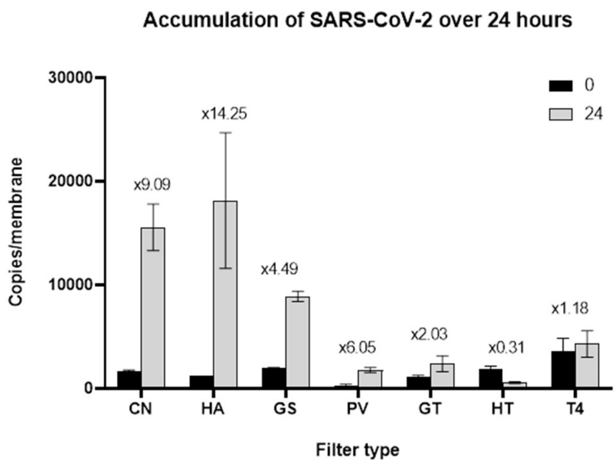

Figure 1. Accumulation of SARS-CoV-2 over 24 hours using different membranes filters. Filter description is given in table 1. Black and grey bars correspond to time 0 and 24h, respectively. Y axis indicate the number of SARS-CoV-2 copies per membrane and X axis the filter type. The value above each grey bar indicates the increase in accumulation of SARS-CoV-2 after 24 hours.

In an ongoing second experiment, we have selected the membranes CN, HA and PV to determine their linear uptake range of SARS-CoV-2 over a period of seven days. We hypothesise that PV filter will show a longer linear uptake than CN and HA membranes, while CN and HA will show a higher capacity to accumulate SARS-CoV-2 in the first days of exposure to the virus. These results will lead us to better estimate the sensitivity of the studied filters to accumulate SARS-CoV-2 at different time points. A third experiment will be performed to determine the desorption rate of SARS-CoV-2 in the CN, HA and PV membranes over a period of seven days, which will allow us to compare the capacity of these membranes to maintain the virus once it is attached to the membrane.

Conclusions and future work

Filter membranes have different potential to capture SARS-CoV-2 fragments. The uptake and desorption rate of the virus need to be carefully considered to determine which and when membranes can be used in passive samplers. Membranes made of cellulose have a high capacity to accumulate SARS-CoV-2 fragments over 24 h, while polyvinylidene fluoride filters could continue accumulating the virus for a longer period than cellulose membranes. In the near future, testing the studied filters in passive samplers will be crucial to determine if by using different types of membranes it is possible to prolong the linear uptake capacity of passive samplers to capture SARS-CoV-2 fragments in sewage systems while maintaining a high performance.

Acknowledgements

We appreciate VIDRL for providing the gamma-irradiated SARS-CoV-2 and acknowledge the funding support of the Department of Health and Human Services.

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