VALIDATION OF A PASSIVE SAMPLING METHOD FOR VIRUS DETECTION IN WASTEWATER

Andrea Beltran Rubinat

TREBALL FINAL DE GRAU BIOQUÍMICA I BIOLOGIA MOLECULAR

Academic tutor: Lluís Arola Ferrer (lluis.arola@urv.cat)

In collaboration with: Laboratori de Virus Contaminants d'Aigua i Aliments,
Facultat de Biologia de la Universitat de Barcelona

Supervisors: Sílvia Bofill Mas (sbofill@ub.edu)
Marta Rusiñol Arantegui (martarusinol@gmail.com)
Departament de Genètica, Microbiologia i Biotecnologia

Setembre 2022

Center details

This study was carried out at the Laboratory of Viruses Contaminants of Water and Food of the Faculty of Biology of the University of Barcelona (UB). The laboratory belongs to the Department of Genetics, Microbiology and Statistics and is led by Rosina Gironés and Sílvia Bofill-Mas. This work has been supervised by Sílvia Bofill-Mas, Marta Rusiñol, and Cristina Mejías, researchers of this group.

Acknowledgments

I would like to express my gratitude to the entire team of the Laboratory of Viruses Contaminants of Water and Food for this project. To Sílvia Bofill-Mas, for giving me the opportunity to be part of it for a few months. Thank you all for giving me your time, teaching me, and taking care of me whenever I needed to. I wish to extend my special thanks to Cristina. Thank you for being so supportive and such a hard worker, I really appreciate you.

Finally, I would like to thank my academic tutor Lluís Arola Ferrer for his patience and advice.

INDEX

ABSTRACT AND KEYWORDS

Wastewater-based epidemiology is a promising early warning tool to anticipate potential outbreaks, such as COVID-19. Although most studies focus on wastewater from wastewater treatment plants (WWTPs), studies focusing on small catchments can also provide important information for targeted public health actions.

Passive sampling is a practical and affordable alternative to traditional sampling methods to trace the circulation of pathogens in wastewater streams, especially at smaller scales. Previous studies have shown that the use of a torpedo device (3D-printed sampler with multiple entry points) with electronegative membranes gives the best performance.

To validate the use of passive samplers in urban wastewater, torpedo devices containing electronegative membranes were applied to the analysis of SARS-CoV-2 and 6 other human viruses in wastewater samples from a WWTP and from an aged care facility. Passive samples were compared to 24-h time proportional composite samples.

The results suggest that at least two membranes from each passive sampler should be analyzed to provide more accurate results. Passive sampling obtained equivalent sensitivity to composite sampling at the building level, but lower sensitivity was shown in WWTP. In addition, it has been seen that passive sampling can give semi-quantitative results. In general, passive samplers showed to be a promising and useful tool to replace data obtained with automatic samplers in small facilities.

Keywords: SARS-CoV-2, Wastewater-based epidemiology (WBE), Passive sampling, Torpedo, Electronegative membranes, RT-qPCR

ABBREVIATIONS

- **CS:** Composite Sampling
- **EV:** Enterovirus
- **GC:** Genomic Copies
- **HAdV:** Human Adenovirus
- **JCPyV**: Human Polyomavirus 2
- **NoV:** Norovirus
- **PCR:** Polymerase Chain Reaction
- **PS:** Passive Sampling
- **RoV:** Rotavirus
- **RT-qPCR:** Quantitative real-time PCR
- **WBE:** Wastewater-based epidemiology
- **WWTP:** Wastewater treatment plant

1. INTRODUCTION

Viruses can be shed by humans via multiple bodily secretions. Some viruses can infect the enterocytes of the human gastrointestinal tract and be shed in the feces, leading to the presence of viral pathogens or their fragments in wastewater. Recent evidence shows that SARS-CoV-2, despite being a respiratory virus, was also detected in a considerable proportion of fecal samples from COVID-19 cases (Li et al., 2022).

Wastewater-based epidemiology (WBE) is a useful tool to trace the circulation of pathogens of concern in wastewater streams (Figure 1). Wastewater surveillance is advantageous because it can estimate disease burden within a community without having to collect individual clinical specimens. In addition, this surveillance approach is comparably simple, is cost-efficient, does not require informed consent, and allows obtaining rapid results (Liu et al., 2022).

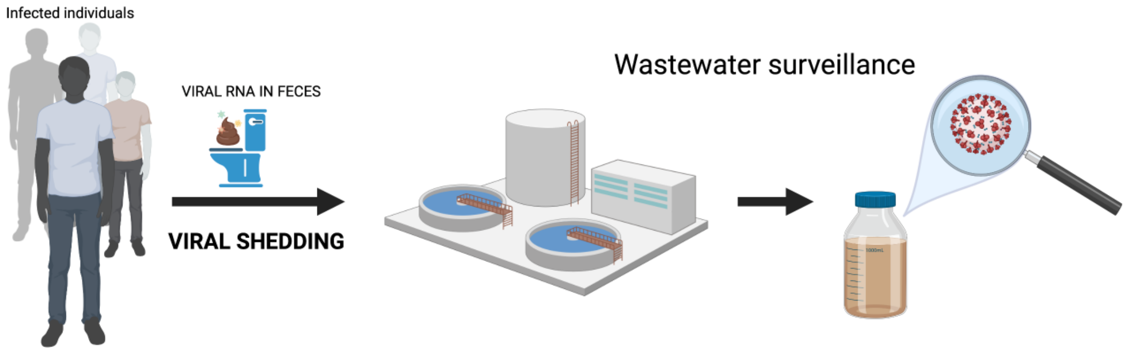


Figure 1 Wastewater-based epidemiology (WBE) diagram

These characteristics make WBE a promising early warning tool to anticipate potential outbreaks, especially for diseases with pre-symptomatic or asymptomatic transmissions, such as COVID-19 (Habtewold et al., 2022).

Most studies that use WBE focus on wastewater collected from wastewater treatment plants (WWTPs) and provide useful city-scale or suburban-scale information (Schang et al., 2021). However, studies focusing on small sewage systems such as long-term care facilities, schools, hospitals, and university campuses are important for timely information for targeted public health actions. Although the application of WBE at smaller scales is attractive, drainage systems from smaller facilities can present challenges for wastewater sampling (Wilson et al., 2022).

Single grab sampling may reduce sensitivity and miss important information, such as viral shedding discharges. Wastewater composition varies substantially depending on the time of day and associated human activities (Schang et al., 2021).

To account for the dynamics of wastewater at smaller scales, automatic samplers are installed to collect 24 h composite samples (Habtewold et al., 2022). However, it is not always possible to use autosamplers at this scale. Such installations are difficult because of high costs of equipment and maintenance, the requirement for specialized skillsets, limited space and difficulty to access the sampling site, the absence of a power supply to operate refrigerated samplers, low wastewater flows, and excessive sampling depths (Schang et al., 2021). Moreover, processing liquid samples involve time-consuming techniques prior to nucleic acid extraction (Kitajima et al., 2020). Therefore, the development of alternative sampling tools is required.

Passive sampling is an inexpensive and practical alternative to active sampling. This method requires the deployment of a device in a targeted sewage catchment for a known period, allowing viruses in water to interact with it. Following the deployment, samples are analyzed by advanced analytical methods (Valenzuela et al., 2020). There are several advantages of using passive samplers. The deployment and collection are easy, fast, and do not usually require a permit to access confined spaces. The devices operate without electricity, so they can be used in any accessible sewage line. In addition, passive samplers are continuously exposed to the water column, reducing sampling errors inherent in collecting discrete water samples (Schang et al., 2021, Wilson et al., 2022).

Liu et al. (2022) successfully developed a novel Moore swab method for wastewater surveillance of COVID-19 at an institutional level. The “Moore swab” approach is an environmental surveillance method that has been used for decades by public health professionals to detect and concentrate enteric pathogens from water. It consists of pieces of gauzes tied with string suspended in flowing water, acting as a filter. The study of Liu et al. showed that this method was sensitive enough to identify one or two COVID-19 cases in a building. However, several limitations were also shown. The Moore swab method only provided results about the presence or absence of cases. No accumulation of SARS-CoV-2 was observed over time, and ideal deployment times could not be established.

Schang et al. (2020) evaluated four designs of passive sampler units (Figure 2) and three adsorption materials (electronegative membranes, cotton buds, and medical gauzes). These samplers, with multiple entry points, are designed to sit in the sewer line and allow the flow of wastewater through the housed materials.

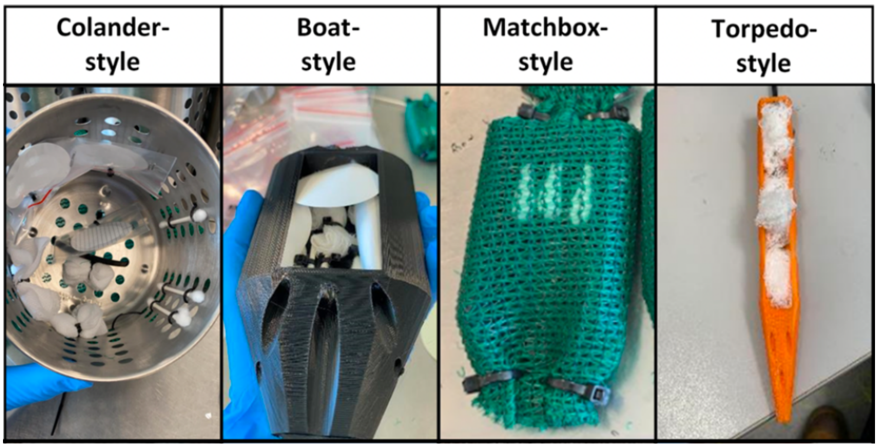


Figure 2 Passive samplers units (colander, boat, matchbox and torpede) used by Schang et al. (2020) before deployment. Adapted from Schang et al. 2020.

They reported greater sensitivities when the daily average SARS-CoV-2 concentration exceeded or equaled 1.8 copy/mL. Torpedo-style units showed the best protection against ragging and clogging of openings. Only 10% of the torpedoes presented ragging materials, and most of the front holes remained unblocked.

Hayes et al. (2021) assessed the performance of four adsorption materials (cotton gauzes, cheesecloth, and electronegative membranes) and three elution mixtures for SARS-CoV-2 analysis using their own 3D-printed devices. This study showed higher performance using electronegative filters and cheesecloth in combination with Tween®20- based elution buffer. Nonetheless, optimal deployment time was not evaluated.

Finally, Habtewold et al. (2022) evaluated the three adsorbent materials developed by Schang et al. using torpedo devices at different deployment times to assess their ability to accumulate SARS-CoV-2. They observed that membranes and cotton buds accumulated RNA gradually, while gauzes had quicker saturation. Specifically, the accumulation of the virus on the membranes was linear up to 48 h, and cotton buds showed more variable ratios. Habtewold et al. suggested electronegative membranes as the best option, considering their high positivity rates, the easy of performance, and accumulation ratios.

Although these studies showed the valuable potential of the application of passive samplers in Wastewater-based epidemiology, further research is needed to validate this methodology. Many questions remain unresolved. For example, the ability of passive sampling for virus quantification or the possible extrapolation to viruses other than SARS-CoV-2.

Enteric viruses are a diverse group of human pathogens transmitted by the fecal-oral route, either by person-to-person contact or by ingestion of contaminated food or water. They are the cause of many gastroenteritis outbreaks. They are a major public health concern globally because they tend to be stable in the environment and are shed at high concentrations in the feces of infected people (Upfold et al., 2021). Their detection in wastewater can also be instrumental in monitoring disease outbreaks and improving public health. Some examples of enteric viruses are Adenovirus (HAdV), Norovirus (NoV), Rotavirus (RoV) and Enterovirus (EV).

On another note, epidemiological studies report a strong relationship between fecal indicators and waterborne diseases when there is wastewater present (McLellan et al. 2015). Fecal viral indicators, such as Human Polyomavirus 2 (JCPyV) or Adenovirus (HAdV), can be used as normalization biomarkers in WBE studies.

In this project, we analyzed SARS-CoV-2 and other human viruses including fecal indicators as well as viral pathogens in two different locations, at city scale and at building level. The presence of the viruses was evaluated in passive samplers (torpedo devices containing electronegative membranes) paired with 24-h composite samples by RT-qPCR.

2. HYPOTHESIS AND OBJECTIVES

The hypothesis of this study is that passive samplers can complement and even replace data obtained with automatic samplers at a qualitative and quantitative level.

The aim of this project is to validate the use of a passive sampling method for the evaluation of the presence of SARS-CoV-2 and other human viruses including fecal viral indicators as well as viral pathogens.

To do that, a comparison between 24-h composite sampling (active sampling) and passive sampling in a wastewater treatment plant and an aged care facility has been made. The variability in the analysis of two replicates of composite sample and torpedo membranes was also analyzed. Moreover, an approach was made to study whether passive sampling could provide semi-quantitative data.

First, we analyzed N1 and N2 regions of SARS-CoV-2, as an additional tool for early detection to complement individual clinical testing in COVID-19 pandemic surveillance. In addition, we analyzed 6 other human viruses: Human Polyomavirus 2 (JCPyV), Human Adenovirus (HAdV), Norovirus (NoV) genogroup I and II, Rotavirus (RoV) and Enterovirus (EV).

3. MATERIALS AND METHODS

3.1 Sampling sites

Two different sampling sites were used in this study to represent different scales. On one side, the Besós wastewater treatment plant with a 2.800.000 population equivalent. It is located on the coast, between the municipalities of Barcelona and Sant Adrià. On the other side, an aged care facility located in Girona, where around 300 people lived.

All sampling events took place between January and March of 2022, during the sixth wave of the COVID-19 pandemic. During the study the prevalence in Catalonia was between 198 and 3.285 cases of COVID-19 per 100.000 inhabitants (Idescat).

3.2 Sampling devices and deployment

Passive sampling devices are composed of a 3D-printed torpedo-shaped shell housing 3 electronegative membranes (cellulose nitrate filters). They were kindly donated by Dr McCarthy, Monash University, Melbourne, Australia.

Torpedo-style units were deployed vertically in the sewer manhole and remained submerged in the wastewater stream. The devices were tied to a nylon rope (Figure 3). Membranes were continuously exposed to flowing wastewater. After 24h, sampling devices were retrieved from the wastewater. It is not possible to calculate the volume of circulating water during sampling. Therefore, passive sampling will not be a quantitative method.

Figure 3 Torpedo-style units (1), electronegative membranes (2) and deployment (3)

To validate the use of passive samplers in urban wastewater, each passive sampling event was paired with the collection of 24h time proportional composite samples from automatic samplers (Figure 4).

Figure 4 Automatic sampler equipment and deployment

Samplers were located at the raw sewer of the WWTP and at the outlet sewer of the aged care facility.

3.3 Pre-processing and storage

Following each deployment period, samples were transported on ice (inside a Zip bag) to the laboratory for analysis (Figure 5). All samples were processed in duplicate on the same day of collection. Although there were 3 biological replicates per passive material, only 2 were processed.

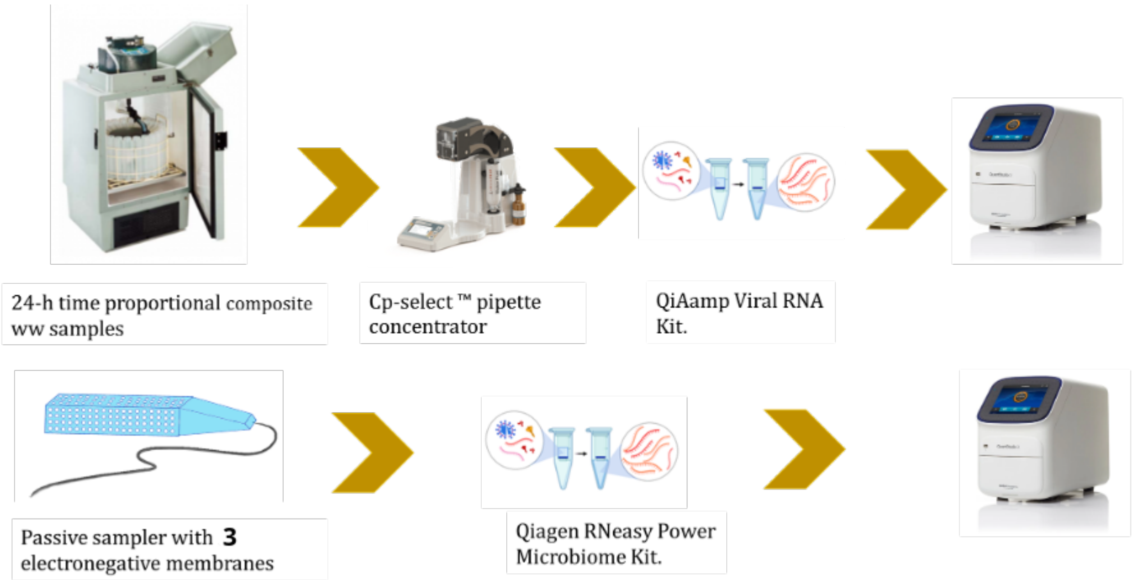


Figure 5 Followed steps for composite and passive samples analysis

3.3.1 Wastewater samples concentration

An automatic ultrafiltration pipette CP-Select (Innovaprep, Drexel, United States) was used to concentrate viruses present in the composite samples from automatic samplers. 80 mL of each sample was filtered following the Standard Operational Procedure under biosecurity 2 conditions. Hollow fiber Polysulfone (PVP) filter tips were used for the ultrafiltration. PBS Elution Fluid cans were used for wet-foam elution, obtaining final volumes of 250-300 mL.

Briefly, the filter tip was introduced on the sampler container. The elution fluid can was previously installed. After pressing “Start run” on the main menu of the device, the vacuum pump started to draw the sample through the tip. For the elution, the option “Extract” was selected, and the concentrated sample was dispensed from the tip into an Eppendorf. Samples were stored at 4°C until extraction was possible.

3.3.2 Membrane processing

One of the torpedo membranes was introduced into a 2 mL Eppendorf and stored at -80°C for future analyses if necessary. The other two membranes were left in Petri plates with 500 µL of DNA/RNA Shield Stabilization Solution and stored at 4°C until the next day.

3.4 Nucleic acid extraction

For water samples, nucleic acid extraction and purification were performed in an automated way using QIAcube Connect (QIAGEN, Hilden, Germany). The QIAmp Viral RNA Mini kit (QIAGEN, Hilden, Germany) was used, following the manufacturer's instructions. In all samples, the volume of concentrate used was 140 µL. The final elution volume was 70 µL. A negative control of extraction with distilled water was added.

The electronegative membranes were cut into pieces and placed into 2 mL bead-beating tubes and then processed using a Qiagen RNeasy® PowerMicrobiome® kit (QIAGEN, Hilden, Germany).

Briefly, a prepared solution containing β-mercaptoethanol was added to the PowerBead Bead Tube, to denature proteins and prevent RNA digestion. Then, a modification was made to the protocol: FastPrep-24™ homogenizer (MP Biomedicals, Santa Ana, United States) was used instead of Vortex (20'' x 2). Next, the sample was centrifuged and the supernatant (450 μL) was transferred to a Collection Tube. IRS solution was added to precipitate non-DNA material. The tube was vortexed, incubated for 5 min on ice, and then centrifuged again. The supernatant (300 μL) was transferred to another tube and two more solutions were added to set up the conditions for RNA and DNA binding to the Spin Filter. After vortexing, the supernatant was loaded into the Spin Column and centrifuged. Then, a wash buffer was added to the column and the process was repeated three times. Finally, 50 µL RNAase-Free Water was added to the Spin Column and it was centrifuged to elute and obtain concentrated RNA/DNA.

One extraction blank was conducted on each day that extractions were conducted.

3.5 RT-qPCR assays

RT-qPCR assays were performed with 10 or 5 µL of direct and diluted (1/10) DNA or RNA extracts respectively in 25 µL reactions using the 96-well Real-Time PCR StepOne™ System (Applied Biosystems, Waltham, United States) and QuantStudio™ Real-Time PCR (Thermo Fisher Scientific, Waltham, United States). Dilutions were prepared in RNase-free water. Duplicate no template controls were run and were always negative. Table 1 specifies the primers and probes that were used.

Table 1 Primers and probes used, bibliographic reference and corresponding sequences

Virus	**Reference**	**Name**	**Sequence (5'→3')**
HAdV	(Bofill-Mas et al., 2006)	AdF	CWTACATGCACATCKCSGG
		AdR	CRCGGGCRAAYTGCACCAG
		AdP1	6-FAM-CCGGGCTCAGGTACTCCGAGGCGTCCT-BHQ1
JCPyV	(Pal et al., 2006)	JE3F	ATGTTTGCCAGTGATGATGAAAA
		JE3R	GGAAAGTCTTTAGGGTCTTCTACCTTT

		JE3P	6-FAM-AGGATCCCAACACTCTACCCCACCTAAAAAGA-BHQ1
NoVGI	(Svraka et al., 2007)	QNIF4	CGCTGGATGCGNTTCCAT
		NV1LCR	CCTTAGACGCCATCATCATTTAC
		TM9	6-FAM-TGGACAGGAGATCGC-MGB
NoVGII	(Loisy et al., 2005)	QNIF4	CGCTGGATGCGNTTCCAT
		NV1LCR	CCTTAGACGCCATCATCATTTAC
		TM9	6-FAM-TGGACAGGAGATCGC-MGB
RoV	(Zeng et al., 2008)	NSP3-F	ACCATCTWCACRTRACCCTCTATGAG
		NSP3-R	GGTCACATAACGCCCCTATAGC
		NSP3-P	6-VIC-AGTTAAAAGCTAACACTGTCAAA- MGB
EV (CVB5)	(Mohamed et al., 2004)	Forward flap	CCCTGAATGCGGCTAATC
		Reverse flap	ATTGTCACCATAAGCAGCCA
		Ebbe5	6-FAM-ACGGACACCCAAAGTAGTCGGTTCCG-BHQ1
SARS-CoV-2 N2	(Corman et al., 2020)	2019-nCoV_N2-F	TTACAAACATTGGCCGCAAA
		2019-nCoV_N2-R	GCGCGACATTCCGAAGAA
		2019-nCoV_N2-P	FAM-ACAATTTGCCCCCAGCGCTTCAG-ZEN/Iowa Black
SARS-CoV-2 N1	(Corman et al., 2020)	2019-nCoV_N1-F	GACCCCAAAATCAGCGAAAT
		2019-nCoV_N1-R	TCTGGTTACTGCCAGTTGAATCTG
		2019-nCoV_N1-P	FAM-ACCCCGCATTACGTTTGGTGGACC-ZEN/Iowa Black

TaqMan™ Environmental Master Mix 2.0 (Thermo Fisher Scientific, Waltham, United States) was used for DNA virus quantification. One-Step RNA UltraSense™ quantitative RT-PCR system (Invitrogen, Waltham, United States) was used for the quantification of RNA viruses at the corresponding amplification profile.

Standard curves were generated using 1/10 serial dilutions of standard RNA/DNA produced for each virus. The R^2 value of each standard curve was greater than 0.98 and the slope was between -3.2 and -3.6. ROX was used as a reference dye. Quantitative cycle (Cq) values were determined using an automatic threshold.

3.6 Statistical analysis

The low sample replicates size precluded statistical comparisons. To analyze significant differences between the two methods, at least 3 replicates are needed.

T-test for means of two paired samples was performed in Microsoft Excel to find out if there was a statistically significant correlation between the load of JCPyV in the passive samplers and the concentrations of JCPyV in the wastewater. The significance level was 0.05. To perform the linear regression, the average of the replicates was calculated, and the values were log-transformed.

4. RESULTS AND DISCUSSION

4.1 Study of the variability between replicates

No previous references were found where differences between biological replicates were studied. Most studies report quantification data as means between replicates.

In this project, the variability between 2 different electronegative membranes and 2 different composite sample replicates from Besós wastewater treatment plant was evaluated by RT-qPCR.

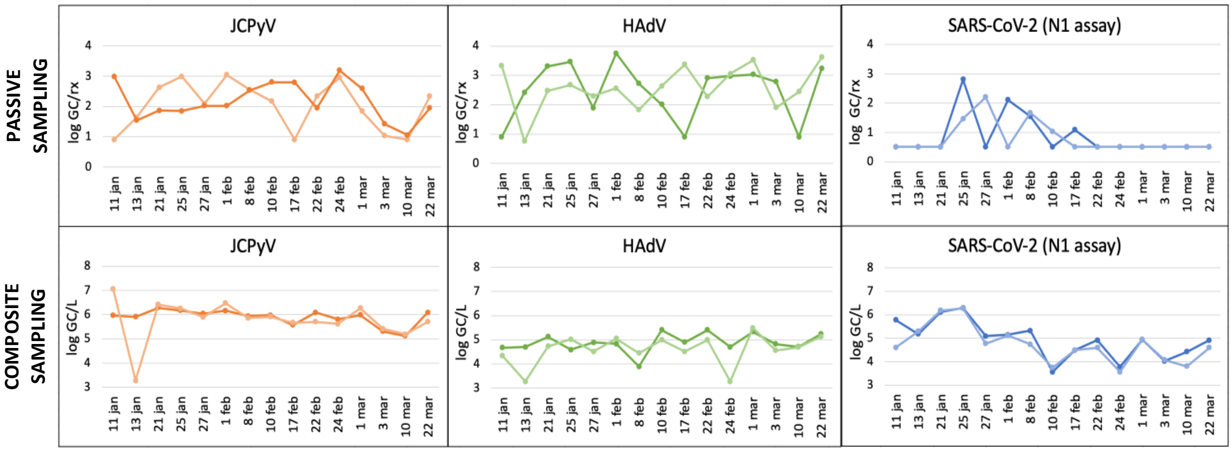


Figure 6 Quantification of JCPyV, HAdV, and SARS-CoV-2 (N1 assay) of two different replicates using a passive sampler device (top row, in log genomic copies/reaction) and 24h composite wastewater samples (bottom row, in log genomic copies/L) from a wastewater treatment plant.

As shown in Figure 6, the two composite sample replicates (bottom row) showed very similar values on all days, following the same trend. Some unusual differences were detected on January 13 in JCPyV and HAdV, which may be due to errors in their laboratory processing.

On the other hand, membrane replicates (top row) show much greater variability. Some causes could be the overlapping of the membranes when deposited inside the sampler, an irregular distribution of the wastewater flow through the passive sampler or the RNA/DNA extraction method used. Exceptional similar values are observed in membrane replicates of JCPyV between February 22 and March 22.

Note that results are referred to as per reaction since it is not possible to calculate the volume of wastewater that circulates through the sampler, being the method qualitative and to some extent (see results section), semi-quantitative.

Despite showing different quantification values, membrane replicates showed similar absence/presence results. Replicates matched 12/15 days in the presence/absence of HAdV and JCPyV and 11/15 days in the presence/absence of SARS-CoV-2. In the case of composite water samples, replicates matched 14/15 days in JCPyV and HAdV, and 13/15 days in SARS-CoV-2. The same trend is observed in the data obtained from sampling in the aged care facility (data not shown).

Subsequent analyzes were conducted with two membranes, to obtain more robust results.

4.2 Qualitative comparison between composite and passive sampling

After converting the quantitative data to absence/presence data, it was possible to conduct a qualitative comparison of the different sampling methods.

In the wastewater treatment plant (Figure 7), positive passive samples ranged from 20% to 100%, depending on the virus analyzed.

Wastewater treatment plant															
N1		N2		JC		HAdV		EV		RoV		NoV GI		NoV GII	
CS	PS	CS	PS	CS	PS	CS	PS	CS	PS	CS	PS	CS	PS	CS	PS
🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩
🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟩	🟩
🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟩	🟥	🟩	🟥
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
🟩	🟩	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟩
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟩	🟩	🟥	🟩	🟩
🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩
🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩
🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟩	🟥	🟩	🟥
🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟩	🟥	🟩	🟥
🟩	🟥	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟩	🟩	🟥	🟩	🟥
🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩
100%	40%	100%	47%	100%	100%	100%	100%	100%	20%	100%	47%	100%	53%	100%	73%

Figure 7 Comparison of composite sampling (CS) vs. passive (PS) in a WWTP. The green square represents a positive sample, and the red represents a negative sample.

Regarding SARS-CoV-2, both N1 and N2 assays showed similar results. 40-47% positives were reported in passive sampling samples, in contrast to 100% positive samples from active sampling.

These results are not consistent with the previous studies. Schang et al. (2021), Hayes et al. (2021), and Habtewold et al. (2022) reported that electronegative membranes outperformed composite sampling for detecting SARS-CoV-2 in WWTP. Their studies suggested that passive samplers may yield positivity like auto sampling for deployment times of 24 h.

Otherwise, Human Polyomavirus 2 (JCPyV) and Human Adenovirus (HAdV) showed 100% positivity in both CS and PS. JCPyV and HAdV are being used as process controls and biomarkers for normalization because of their abundance in wastewater and their correlation with fecal contamination.

These results seem to prove that the passive sampling method works but is less sensitive than the traditional one. However, the fact that it is a non-quantitative method does not allow us to evaluate the sensitivity.

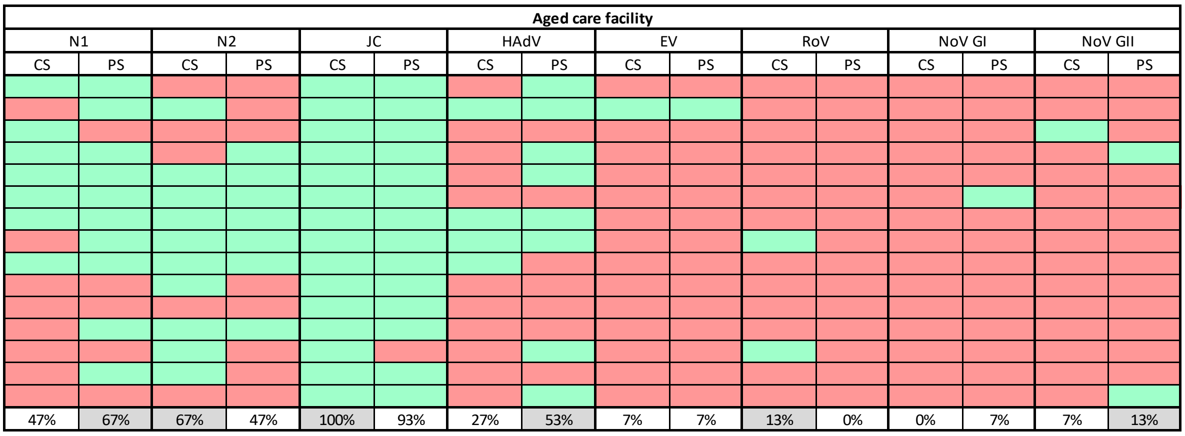
On the other hand, the design of this study does not allow us to differentiate between a problem during virus adsorption and during sample processing. A decreased recovery could be caused by the extraction method used or the presence of RT-qPCR inhibitors.

In other ways, high levels of suspended solids in wastewater may have affected the adsorption of SARS-CoV-2 RNA to the electronegative membranes. Passive sampler performance can also have been altered by temperature, pH, or dissolved solids concentration (Li et al, 2022).

Regarding Enterovirus (EV), Rotavirus (RoV), and Norovirus (NoV) GI and GII assays, a lower percentage of positives is also observed in the passive sampling samples. Automatic sampling showed 100% positivity in all cases. On the other hand, positive samples from passive sampling ranged from 20 to 73%. EV was the assay with the lowest detection rate (20%).

It is expected that different viruses show different results despite being adsorbed and concentrated with the same method, since they present different physical properties. Li et al. (2022) suggested that the different structure between non-enveloped viruses (EV, HAdV, RoV, NoV) and enveloped ones (SARS-CoV-2) could affect their behavior in the affinity for membranes or in the recovery process.

In the aged care facility (Figure 8) different results were obtained. Unlike the WWTP, which is a 2.800.000-equivalent population, the aged care facility represents a smaller group, of about 300 people. Therefore, there is a lower probability of finding people excreting those viruses.



Aged care facility															
N1		N2		JC		HAdV		EV		RoV		NoV GI		NoV GII	
CS	PS	CS	PS	CS	PS	CS	PS	CS	PS	CS	PS	CS	PS	CS	PS
🟩	🟩	🟥	🟥	🟩	🟩	🟥	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
🟥	🟩	🟩	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥
🟩	🟥	🟥	🟥	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟩	🟥
🟩	🟩	🟥	🟩	🟩	🟩	🟥	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟩
🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟩	🟥	🟥
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟩	🟥	🟥	🟥	🟥	🟥
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
🟥	🟥	🟩	🟥	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
🟥	🟥	🟥	🟥	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
🟥	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
🟥	🟥	🟩	🟥	🟩	🟥	🟥	🟩	🟥	🟥	🟩	🟥	🟥	🟥	🟥	🟥
🟥	🟩	🟩	🟥	🟩	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
🟥	🟥	🟥	🟥	🟩	🟩	🟥	🟩	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟩
47%	67%	67%	47%	100%	93%	27%	53%	7%	7%	13%	0%	0%	7%	7%	13%

Figure 8 Comparison of composite sampling (CS) vs. passive (PS) in an aged care facility. The green square represents a positive sample, and the red represents a negative sample.

Passive sampling presented greater sensitivity on the SARS-CoV-2 N1 assay. On the contrary, composite sampling showed higher positivity on the N2 assay. Passive sampling also outperformed composite sampling in HAdV and NoV (GI and GII) assays, and equaled active sampling in the detection of EV. Slightly lower positivity was obtained from passive sampling in RoV assay. In general, similar results are observed from both methods.

Liu et al. (2022) reported similar results where passive samples from hospital wastewater presented higher positivity in SARS-CoV-2 assays (92%) than composite samples (69%). Corchis-Scott et al. (2021) suggested that passive sampling could even detect single COVID-19 cases at building level.

These results might reflect the high temporal variability of wastewater flows and virus concentrations at smaller scales. Composite sampling may omit important information, such as shedding events, even though they took samples very frequently. Otherwise, passive samplers are constantly in contact with the wastewater flow (Schang et al., 2021).

Furthermore, the results suggest that JCPyV is a better fecal indicator than HAdV in building-level analyses.

4.3 Quantitative data from passive samplers

Despite passive sampling is not a quantitative method, an approach has been made to study whether torpedoes can give semi-quantitative results. It is not possible to give any virus concentration value because the volume of water that comes into contact with the membrane is not known.

A comparison between the loading of JCPyV on electronegative membranes and the concentration of JCPyV in the composite wastewater samples has been carried out by means of linear regression (GC/L vs GC/reaction).

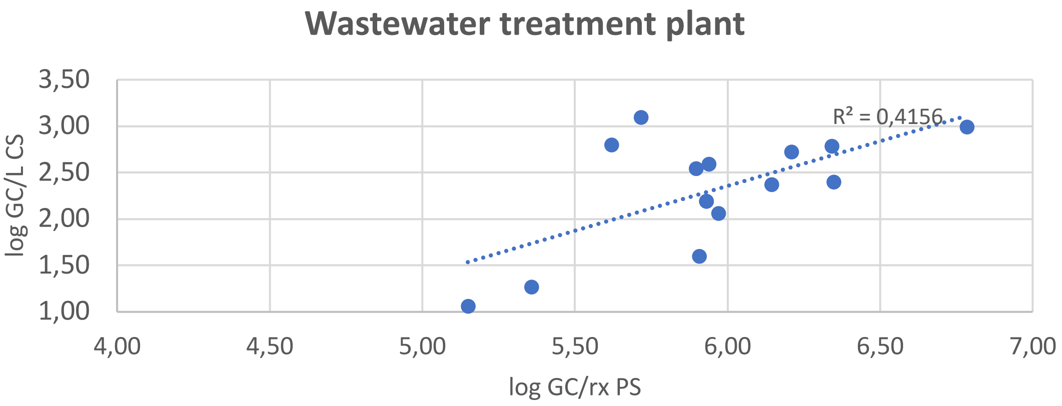


Figure 9 Linear regression comparing JCPyV quantifications in wastewater from WWTP by composite samples (log CG/L) with paired passive samples by (log GC/rx).

A statistically significant correlation (p = 0,003) was obtained between samples from the wastewater treatment plant (Figure 9). The coefficient of determination (R^2) resultant was 0,415. This shows that a higher concentration of viruses in the wastewater results in a higher accumulation of viruses in the passive samplers.

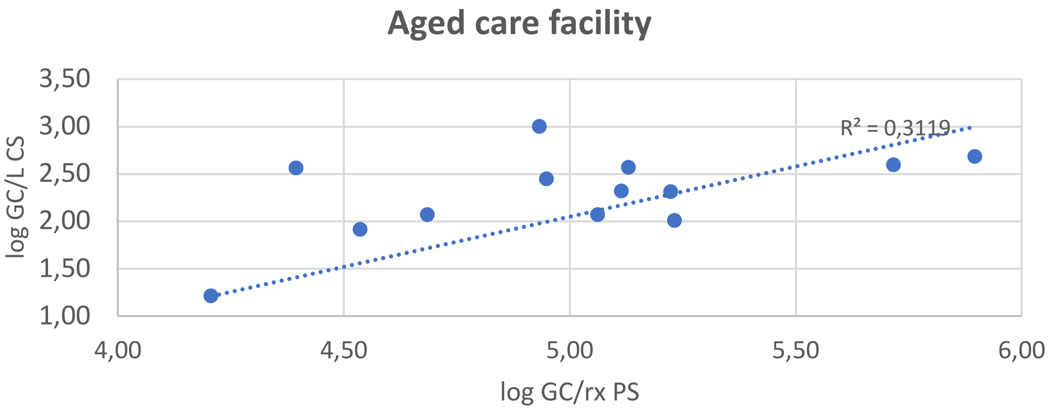


Figure 10 Linear regression comparing JCPyV quantifications in wastewater from an aged care facility by composite samples (log CG/L) with paired passive samples by (log GC/rx).

Samples from the aged care facility also showed a statistically significant correlation (p = 0,011) with an R^2 value of 0,31, as it is shown in Figure 10.

Consistent with our results, Schang et al. (2021) also reported a statistically significant correlation between SARS-CoV-2 RNA concentrations on passive samplers and concentrations on composite wastewater. They obtained a coefficient of determination of $R^2 = 0{,}31$.

Further, Bivins et al. (2022) compiled a dataset of paired measurements of SARS-CoV-2 RNA in wastewater and on passive samplers from three university WBE programs and calculated the correlations. R^2 values obtained were $R^2 = 0{,}27$, $R^2 = 0{,}56$ and $R^2 = 0{,}76$.

All these findings suggest that the loading of viruses on passive sampling materials could be linked to the concentrations of viruses found in wastewater. Therefore, passive samplers, especially electronegative membranes, have the potential to provide useful semi-quantitative data.

5. CONCLUSIONS

From the results obtained, we draw the following conclusions:

- The use of passive torpedo-type samplers requires the analysis of at least two electronegative membranes to obtain robust results.
- At the wastewater treatment plant, composite sampling has shown greater sensitivity than passive sampling by using torpedo devices. Otherwise, equivalent results were observed at a building level.
- Passive samplers with electronegative membranes might yield semi-quantitative data.
- In the event of a new epidemic outbreak, the use of passive samplers can allow the application of targeted actions in facilities with vulnerable people.

6. BIBLIOGRAPHY

1. Bivins, A., Kaya, D., Ahmed, W., Brown, J., Butler, C., Greaves, J., Leal, R., Maas, K., Rao, G., Sherchan, S., Sills, D., Sinclair, R., Wheeler, R. T., & Mansfeldt, C. (2022). Passive sampling to scale wastewater surveillance of infectious disease: Lessons learned from COVID-19. The Science of the total environment, 835, 155347. https://doi.org/10.1016/j.scitotenv.2022.155347
2. Bofill-Mas, S., Albinana-Gimenez, N., Clemente-Casares, P., Hundesa, A., Rodriguez-Manzano, J., Allard, A., Calvo, M., & Girones, R. (2006). Quantification and stability of human adenoviruses and polyomavirus JCPyV in wastewater matrices. Applied and environmental microbiology, 72(12), 7894–7896. https://doi.org/10.1128/AEM.00965-06
3. Corchis-Scott, R., Geng, Q., Seth, R., Ray, R., Beg, M., Biswas, N., Charron, L., Drouillard, K. D., D'Souza, R., Heath, D. D., Houser, C., Lawal, F., … McKay, R. (2021). Averting an Outbreak of SARS-CoV-2 in a University Residence Hall through Wastewater Surveillance. Microbiology spectrum, 9(2), e0079221. https://doi.org/10.1128/Spectrum.00792-21
4. Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M. L. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro surveillance : bulletin Europeen sur les maladies transmissibles, 25(3), 2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045
5. Habtewold, J., McCarthy, D., McBean, E., Law, I., Goodridge, L., Habash, M., & Murphy, H. M. (2022). Passive sampling, a practical method for wastewater-based surveillance of SARS-CoV-2. Environmental Research, 204(Pt B), 112058. https://doi.org/10.1016/j.envres.2021.112058
6. Hayes, EK., Sweeney, CL., Anderson, LE., Li, B., Erjavec, GB. & Gouthro, MT. (2021). A novel passive sampling approach for SARS-CoV-2 in wastewater in a Canadian province with low prevalence of COVID-19. Environ. Sci: Water Res. Technol, 7, 1576– 86. https://doi.org/10.1039/D1EW00207D
7. Hernroth, B. E., Conden-Hansson, A. C., Rehnstam-Holm, A. S., Girones, R., & Allard, A. K. (2002). Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: the first Scandinavian report. Applied and environmental microbiology, 68(9), 4523–4533. https://doi.org/10.1128/AEM.68.9.4523-4533.2002

8. Institut d'Estadística de Catalunya (Idescat). Incidència setmanal de la Covid-19. https://www.idescat.cat/indicadors/?id=conj&n=14356 (accessed June 20, 2022).
9. Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C. P., Hamilton, K. A., Haramoto, E., & Rose, J. B. (2020). SARS-CoV-2 in wastewater: State of the knowledge and research needs. The Science of the total environment, 739, 139076. https://doi.org/10.1016/j.scitotenv.2020.139076
10. Li, J., Verhagen, R., Ahmed, W., Metcalfe, S., Thai, P.K., Kaserzon, S.L., Smith, W.J.M., Schang, C., Simpson, S.L., Thomas, K.V., Mueller, J.F., Mccarthy, D. (2022). In situ calibration of passive samplers for viruses in wastewater. ACS EST Water https://doi.org/10.1021/acsestwater.1c00406
11. Li, X., Kulandaivelu, J., Guo, Y., Zhang, S., Shi, J., O'Brien, J., Arora, S., Kumar, M., Sherchan, S. P., Honda, R., Jackson, G., Luby, S. P., & Jiang, G. (2022). SARS-CoV-2 shedding sources in wastewater and implications for wastewater-based epidemiology. Journal of hazardous materials, 432, 128667. https://doi.org/10.1016/j.jhazmat.2022.128667
12. Liu, P., Ibaraki, M., VanTassell, J., Geith, K., Cavallo, M., Kann, R., Guo, L., & Moe, C. L. (2022). A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level. The Science of the total environment, 807(Pt 3), 151047. https://doi.org/10.1016/j.scitotenv.2021.151047
13. Loisy, F., Atmar, R. L., Guillon, P., Le Cann, P., Pommepuy, M., & Le Guyader, F. S. (2005). Real-time RT-PCR for norovirus screening in shellfish. Journal of virological methods, 123(1), 1–7. https://doi.org/10.1016/j.jviromet.2004.08.023
14. McLellan, S. L., & Eren, A. M. (2014). Discovering new indicators of fecal pollution. Trends in microbiology, 22(12), 697–706. https://doi.org/10.1016/j.tim.2014.08.002
15. Mohamed, N., Elfaitouri, A., Fohlman, J., Friman, G., & Blomberg, J. (2004). A sensitive and quantitative single-tube real-time reverse transcriptase-PCR for detection of enteroviral RNA. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology, 30(2), 150–156. https://doi.org/10.1016/j.jcv.2003.08.016
16. Pal, A., Sirota, L., Maudru, T., Peden, K., & Lewis, A. M., Jr (2006). Real-time, quantitative PCR assays for the detection of virus-specific DNA in samples with mixed populations of polyomaviruses. Journal of virological methods, 135(1), 32–42. https://doi.org/10.1016/j.jviromet.2006.01.018
17. Schang, C., Crosbie, N. D., Nolan, M., Poon, R., Wang, M., Jex, A., John, N., … McCarthy, D. T. (2021). Passive Sampling of SARS-CoV-2 for Wastewater Surveillance.

Environmental science & technology, 55(15), 10432–10441. https://doi.org/10.1021/acs.est.1c01530

18. Svraka, S., Duizer, E., Vennema, H., de Bruin, E., van der Veer, B., Dorresteijn, B., & Koopmans, M. (2007). Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. Journal of clinical microbiology, 45(5), 1389–1394. https://doi.org/10.1128/JCM.02305-06
19. Upfold, N. S., Luke, G. A., & Knox, C. (2021). Occurrence of Human Enteric Viruses in Water Sources and Shellfish: A Focus on Africa. Food and environmental virology, 13(1), 1–31. https://doi.org/10.1007/s12560-020-09456-8
20. Valenzuela, E.F., Menezes, H.C. & Cardeal, Z.L. (2020). Passive and grab sampling methods to assess pesticide residues in water. A review. Environ Chem Lett 18, 1019–1048. https://doi.org/10.1007/s10311-020-00998-8
21. Vincent-Hubert, F., Wacrenier, C., Morga, B., Lozach, S., Quenot, E., Mège, M., Lecadet, C., Gourmelon, M., Hervio-Heath, D., & Le Guyader, F. S. (2021). Passive Samplers, a Powerful Tool to Detect Viruses and Bacteria in Marine Coastal Areas. Frontiers in microbiology, 12, 631174. https://doi.org/10.3389/fmicb.2021.631174
22. Wilson, M., Qiu, Y., Yu, J., Lee, B. E., McCarthy, D. T., & Pang, X. (2022). Comparison of Auto Sampling and Passive Sampling Methods for SARS-CoV-2 Detection in Wastewater. Pathogens (Basel, Switzerland), 11(3), 359. https://doi.org/10.3390/pathogens11030359
23. Zeng, S. Q., Halkosalo, A., Salminen, M., Szakal, E. D., Puustinen, L., & Vesikari, T. (2008). One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. Journal of virological methods, 153(2), 238–240. https://doi.org/10.1016/j.jviromet.2008.08.004