Science of the Total Environment 862 (2023) 160914

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evaluation of two different concentration methods for surveillance of human viruses in sewage and their effects on SARS-CoV-2 sequencing

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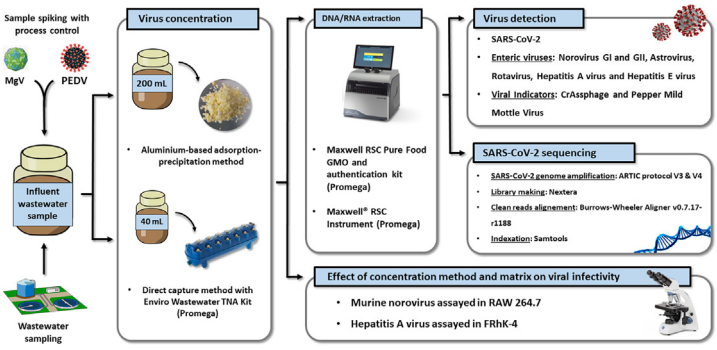
HIGHLIGHTS

- Concentration methods are critical for virus surveillance in sewage.
- Direct capture system (TNA) produces better results in terms of RT-qPCR sensitivity.
- TNA system combined with Artic v4 yields the best SARS-CoV-2 sequencing results.
- Aluminum precipitation would be recommended for infectivity assays.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Paola Verlicchi

Keywords:
Wastewater-based epidemiology
Enteric viruses
Sequencing
SARS-CoV-2

ABSTRACT

During the current COVID-19 pandemic, wastewater-based epidemiology (WBE) emerged as a reliable strategy both as a surveillance method and a way to provide an overview of the SARS-CoV-2 variants circulating among the population. Our objective was to compare two different concentration methods, a well-established aluminum-based procedure (AP) and the commercially available Maxwell® RSC Enviro Wastewater TNA Kit (TNA) for human enteric virus, viral indicators and SARS-CoV-2 surveillance. Additionally, both concentration methods were analyzed for their impact on viral infectivity, and nucleic acids obtained from each method were also evaluated by massive sequencing for SARS-CoV-2. The percentage of SARS-CoV-2 positive samples using the AP method accounted to 100 %, 83.3 %, and 33.3 % depending on the target region while 100 % positivity for these same three target regions was reported using the TNA procedure.

The concentrations of norovirus GI, norovirus GII and HEV using the TNA method were significantly greater than for the AP method while no differences were reported for rotavirus, astrovirus, crAssphage and PMMoV. Furthermore, TNA kit in combination with the Artic v4 primer scheme yields the best SARS-CoV-2 sequencing results. Regarding impact on infectivity, the concentration method used by the TNA kit showed near-complete lysis of viruses. Our results suggest that although the performance of the TNA kit was higher than that of the aluminum procedure, both methods are suitable for the analysis of enveloped and non-enveloped viruses in wastewater by molecular methods.

1. Introduction

Over the last two years, molecular analysis of SARS-CoV-2 in wastewater samples, has become very popular due to the potential for epidemiological

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http://dx.doi.org/10.1016/j.scitotenv.2022.160914
Received 10 June 2022; Received in revised form 9 December 2022; Accepted 9 December 2022
Available online 13 December 2022

surveillance using wastewater collected from wastewater treatment plants (WWTP), sewers or even aircrafts (Ahmed et al., 2022; Davó et al., 2021; Polo et al., 2020). However, the analysis of wastewater for virus surveillance is not new and had been used long before for epidemiological tracking of human enteric viruses such as poliovirus, norovirus, enterovirus, rotavirus, adenovirus and hepatitis A and E viruses (Asghar et al., 2014; Cuevas-Ferrando et al., 2020; Hellmér et al., 2014; Miura et al., 2016; Prevost et al., 2015; Santiso-Bellón et al., 2020).

Human enteric viruses pose one of the highest microbiological risks of water-borne infections (Wyn-Jones and Sellwood, 2001). Due to their excretion in feces, these viruses reach wastewater treatment systems and can contaminate other water sources into which they are discharged. The application of RT-qPCR is currently used as a gold standard method to provide information about levels of these pathogens in wastewater as well as in effluents (Haramoto et al., 2020). However, the low presence of viruses in wastewater in relation to other organisms and the complexity and variability of wastewater samples make viral concentration and nucleic acid extraction methods critical for these types of analyses (Haramoto et al., 2020).

During the current COVID-19 pandemic, several studies have compared different procedures for SARS-CoV-2 detection by RT-qPCR or digital PCR (Rusiñol et al., 2020b; Torii et al., 2022). Moreover, high-throughput sequencing techniques have been used for the analysis of SARS-CoV-2 genomes in wastewaters, evidencing their usefulness in detecting the linage introduction in a population, as well as in profiling new outbreaks, and tracking viral strains (Crits-Christoph et al., 2021; Izquierdo-Lara et al., 2021; Nemudryi et al., 2020; Pérez-Cataluña et al., 2022). Nevertheless, considering the potential of WBE for current and future treats, a broader comparison is needed, not only to establish methods for viral detection and quantification but also to characterize these using high-throughput sequencing techniques.

Therefore, the aim of this study was to evaluate the performance of two concentration methods for the detection of enteric viruses, viral fecal indicators, and SARS-CoV-2. Additionally, nucleic acids obtained from each concentration method were evaluated by targeted sequencing in terms of coverage across the SARS-CoV-2 genome.

2. Materials and methods

2.1. Viral concentration methods

Grab wastewater samples, collected from 6 different WWTPs on August 2021, were inoculated with 100 μL of porcine epidemic diarrhea virus (PEDV) strain CV777 as a coronavirus model and mengovirus (MgV) vMC0 (CECT 100000) as a non-enveloped counterpart. Two hundred milliliters of wastewater samples ($n = 6$) were concentrated through a previously validated aluminum-based adsorption-precipitation method (hereafter referred to as AP) (Pérez-Cataluña et al., 2021; Randazzo et al., 2019). In parallel, 40 mL of wastewater samples ($n = 6$) were processed with the vacuum concentration system using by Enviro Wastewater TNA Kit (Promega Corp., Spain) following the manufacturer's protocol (hereafter referred to as TNA). In brief, 0.5 mL of protease solution was added to 40 mL of wastewater, and samples were incubated statically for 30 min at room temperature (RT) and centrifuged at 3000 $\times g$ for 10 min. Then, in duplicate, 20 mL of the supernatant was transferred to a clean tube and 5.5 mL of Binding Buffer 1, 0.5 mL of Binding Buffer 2, and 24 mL of isopropanol were added. The mixture was passed through a PureYield™ Midi Binding Column (Promega) using a VacMan® Vacuum Manifold (Promega). Five milliliters of Inhibitor Removal Wash (complemented with 40 % isopropanol as specified by the manufacturer's protocol) followed by 20 mL of RNA Wash Solution (complemented with 63 % ethanol 95 % as specified by the manufacturer's protocol) were passed through the column. Finally, the concentrated sample was eluted in 500 μL of nuclease-free water for nucleic acid extraction.

2.2. RNA extraction and virus quantification

Viral extraction from wastewater concentrates, obtained by the AP and the concentration procedure of the TNA kit, was carried out using the Maxwell® RSC Instrument (Promega) with the Maxwell RSC Pure Food GMO and authentication kit (Promega) and the "Maxwell RSC Viral total Nucleic Acid" running program.

Samples concentrated using the AP method were processed as described previously by Pérez-Cataluña et al. (2021). Samples concentrated by the TNA method were subjected to nucleic acid extraction using 500 μL of the eluate. This eluate was mixed with 150 μL of Binding Buffer 1 and 50 μL of Binding Buffer 2, both provided with the TNA kit, vortexed, and incorporated into a Maxwell RSC Cartridge (Promega).

Viral detection of SARS-CoV-2, PEDV, and MgV was performed by RT-qPCR using the One Step PrimeScript™ RT-PCR Kit (Perfect Real Time) (Takara Bio Inc., USA). SARS-CoV-2 detection was achieved by targeting the N1 region of the nucleocapsid gene and the IP4 region of the RNA-dependent RNA polymerase gene (Institut Pasteur, 2020). For N1, two RT-qPCR assays were tested; the One Step PrimeScript™ RT-PCR Kit (Perfect Real Time) was used with N1 primers and conditions described by (CDC, 2020) (hereafter referred to as N1-CDC); and the duplex RT-qPCR kit detection Wastewater SARS-CoV-2 RT-qPCR System (Promega) for SARS-CoV-2 and pepper mild mottle virus (PMMoV) (hereafter referred to as N1-Dup). Membrane gene (M) specific primers were used for PEDV detection as described by Puente et al. (2020). For MgV, detection was carried out using primers and probe described in (ISO 15216-1:2017). Reaction mixes, thermal cycling conditions, and sequences for primers and probes are listed in Pérez-Cataluña et al. (2021).

Levels of norovirus GI and GII, human astrovirus (HAstV), rotavirus (RV), hepatitis A virus (HAV) and hepatitis E viruses (HEV) were determined using the RNA UltraSense One-Step kit (Invitrogen, USA) as previously described (Randazzo et al., 2019).

Occurrence of crAssphage was established using the qPCR Premix Ex Taq™ kit (Takara Bio Inc.) using primers and conditions described by (Stachler et al., 2017).

Different controls were used in all assays: negative process control consisting of PBS; whole process control to monitor the process efficiency of each sample (spiked MgV and PEDV); and positive (reference material) and negative (RNase-free water) RT-qPCR controls. Moreover, undiluted and ten-fold diluted nucleic acid were tested in duplicate to check for inhibitors for all the targeted viruses.

Standard curves were determined according to the Public Health England (PHE) Reference Materials for Microbiology for norovirus GI (batch number 0122-17), norovirus GII (batch number 0247-17) and HAV (batch number 0261-2017) and reported as genomic copies (gc), while standard curves for RV, MgV, and HAstV were generated by amplifying ten-fold serial dilutions of viral suspensions in quintuplicates and calculating the number of PCR units (PCRU). Standard DNA material for crAssphage standard curve generation relied on a customized gBlock gene fragment (Integrated DNA Technologies, Coralville, IA) containing target sequence for CPQ_064 crAssphage primers set (Stachler et al., 2017).

Commercially available Twist Synthetic SARS-CoV-2 RNA Control (Control 2, MN908947.3) was used to prepare standard curves for SARS-CoV-2 quantification.

2.3. Effect of concentration procedure on viral infectivity

To accomplish this, 500 mL of a grab wastewater sample was inoculated with Murine norovirus (MNV-1, kindly provided by Prof. H. W. Virgin, Washington University School of Medicine, USA) and HAV strain HM-175/18f (ATCC VR-1402). In parallel, 500 mL of PBS was also artificially inoculated with both viruses. Two hundred milliliters of wastewater samples ($n = 2$) or PBS ($n = 2$) were concentrated through the AP procedure while 40 mL of wastewater samples ($n = 2$) or PBS ($n = 2$) were processed with the TNA Kit as described above. Then, concentrated samples were ten-fold diluted, and infectious viruses quantified using the Spearman-Karber

method on confluent RAW 264.7 (ATCC TIB-71) and FRhK-4 (ATCC CRL_1688) monolayers for MNV and HAV, respectively (Falcó et al., 2018).

2.4. SARS-CoV-2 sequencing

Genomic sequencing of SARS-CoV-2 present in wastewater samples was carried out following ARTIC protocol versions 3 and 4, as version 4 was released during the study in response to the realization that some V3 primers were located in regions with key mutations. Sequencing libraries were generated using the Nextera Flex kit (Illumina, CA, USA) and sequenced on Illumina MiSeq platform by paired-end reads (2 × 200) (Pérez-Cataluña et al., 2022). Adaptors and nucleotides below Q30 Phred score were cleaned by using *cutadapt* software (Martin, 2011) and *reformat.sh* from bbmap (sourceforge.net/projects/bbmap/), respectively. Obtained clean reads were aligned to the genome of SARS-CoV-2 isolate Wuhan-Hu-1 (MN908947.3) using the Burrows-Wheeler Aligner v0.7.17-r1188 with default parameters (Li and Durbin, 2009) and indexed by samtools (Li et al., 2009). Genomic coverage for each sample was calculated using nucleotide positions with at least 20× depth.

2.5. Statistical analyses

Normal distribution was evaluated with Shapiro-Wilk tests. Significance of the differences in viral detection by RT-qPCR was evaluated using Student's *t*-test for normally distributed data (i.e. norovirus GI and GII, rotavirus, PMMoV, crAssphage, and MgV) and Mann-Whitney-Wilcoxon or Dunn's tests with adjusted *p*-values with the Holm method for not normally distributed data (i.e. PEDV, HAstrV, HEV, SARS-CoV-2).

The statistical analysis of differences in logarithmic reductions after cell culture assays was carried out by the post-hoc Tukey's method (p-value < 0.05) to compare and determine the difference among different concentration procedures.

The statistically significant differences in the results obtained after the genomic analysis (i.e. percentage of reads identified as SARS-CoV-2, percentage of SARS-CoV-2 genome coverage, and mean values of genomic depth) were calculated by pairwise comparisons using Student's *t*-test for the percentage values of SARS-CoV-2 reads and genomic coverage, and with the Mann-Whitney-Wilcoxon test for mean depth values. Differences were considered significant when the p-value was <0.05. All the statistical analyses were made with R Statistical Software (version 3.6.3).

3. Results

3.1. Comparison of the aluminum-based adsorption precipitation method (AP) and the direct capture method (TNA) for viral detection and recovery

Wastewater samples were processed using both the AP method (initial sample volume 200 mL) and the TNA Kit (initial sample volume 40 mL) for their ability to concentrate SARS-CoV-2, human enteric viruses, and viral fecal indicators from wastewater samples.

Wastewater samples were analyzed by targeting two different SARS-CoV-2 genomic fragments (N1 and IP4) to evaluate the sensitivity of each concentration method. The percentage of positive samples using the AP method was 100 %, 83.3 %, and 33.3 %, for N1-Dup, N1-CDC and IP4, respectively, while 100 % positivity for the three targets was reported using the TNA procedure (Fig. 1, Sup. Table S1). Significative differences (p-value = 0.02) were found between SARS-CoV-2 levels targeting IP4 in samples concentrated using the AP method and targeting N1-Dup concentrated by the TNA method (Fig. 1) while no differences were retrieved targeting N1-CDC.

The AP and TNA methods were also evaluated for their relative consistency in quantifying human enteric viruses (Fig. 2) and viral indicators (Fig. 3). HAV was not detected in any sample regardless of the method used (Sup. Table S1). The concentrations of norovirus GI (6.16 ± 0.73 $\log_{10}$ gc/L), norovirus GII (6.88 ± 0.43 $\log_{10}$ gc/L), and HEV (3.87 ± 0.49 $\log_{10}$ gc/L) using the TNA method were significantly greater (p-values of 0.042, 0.007, and 0.036, respectively) than using the AP method (Fig. 2).

No significant differences were found for RV and HAstrV levels. Furthermore, using the AP method, the percentage of positive samples were 50 % and 33.3 %, for HAstrV and HEV respectively, compared to 66.6 % and 100 % of positivity using the TNA method (Fig. 2, Sup. Table S1). Viral indicators showed mean values of 7.82 ± 0.36 $\log_{10}$ gc/L and 9.55 ± 0.25 with the AP method, and 8.32 ± 0.22 $\log_{10}$ gc/L and 9.45 ± 0.21 $\log_{10}$ gc/L with the TNA method, for PMMoV and crAssphage, respectively (Fig. 3). Neither of the two viruses showed significant differences in terms of their detection using the two methods.

Regarding the process controls recoveries, mean values for PEDV were 141.20 % ± 36.03 % and 38.57 ± 5.22 % for the AP and TNA methods, respectively. For MgV, these values were 6.82 ± 4.80 % in the AP method and 33.68 ± 11.62 % in the TNA method. Statistically significant differences were found for MgV (p-value = 0.001) and PEDV (p-value = 0.0008) recoveries, showing higher recoveries with the AP method for PEDV and with the TNA method for MgV (Fig. 3).

3.2. Comparison of the aluminum-based adsorption precipitation method and direct capture method for virus viability

Table 1 shows the infectious viruses recovered after concentration of PBS and sewage samples using both approaches. In samples concentrated using the AP procedure, levels of infectious MNV and HAV were not reduced (p > 0.05). The TNA procedure did not retrieve infectious MNV in PBS and wastewater samples while HAV concentration was statistically (p < 0.05) reduced by 2.5 and 2.1 log in PBS and wastewater samples respectively.

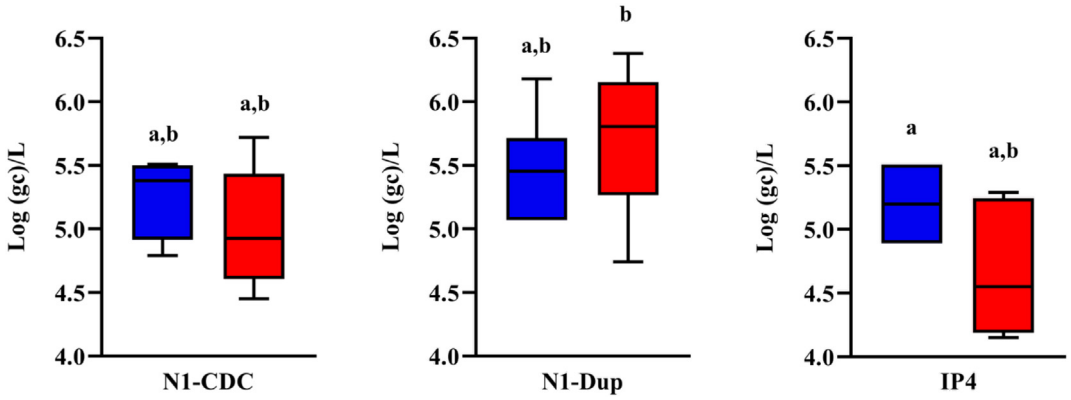

Fig. 1. Levels ($\log_{10}$ gc/L) for three genetic SARS-CoV-2 targets in analyzed wastewaters (n = 6) using the aluminum-based adsorption-precipitation (AP, blue boxes) and the Enviro Wastewater TNA Kit (TNA, red boxes). Different letters denote significant differences (p-value < 0.05) with non-parametric Dunn's test.

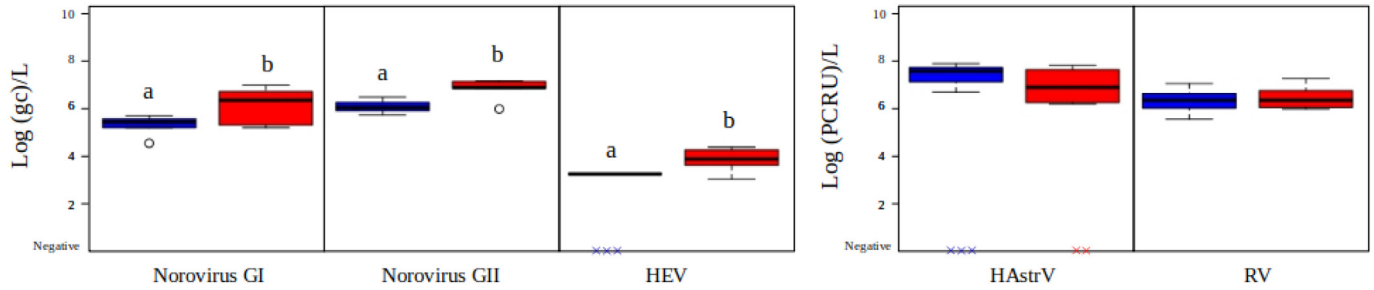

Fig. 2. Levels (log_{10} gc/L for Norovirus GI and GII, and HEV; log PCRU/L for HAstrV and RV) of human enteric viruses in wastewaters ($n = 6$) using the aluminum-based adsorption-precipitation (AP, blue boxes) and the Enviro Wastewater TNA Kit (TNA, red boxes). Different letters denote significant differences (p-value < 0.05) for each virus levels between each concentration method with Student's t-test (norovirus GI and norovirus GII) and Wilcoxon test (HEV). Crosses at the bottom represent negative samples.

3.3. SARS-CoV-2 sequencing

Six grab wastewater samples were concentrated with both concentration methods and nucleic acids were extracted as described above. Additionally, two primer schemes (i.e. Artic V3 and V4, https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019) were used. Fig. 4 shows the results obtained after bioinformatics analyses regarding percentage of viral reads classified as SARS-CoV-2, the

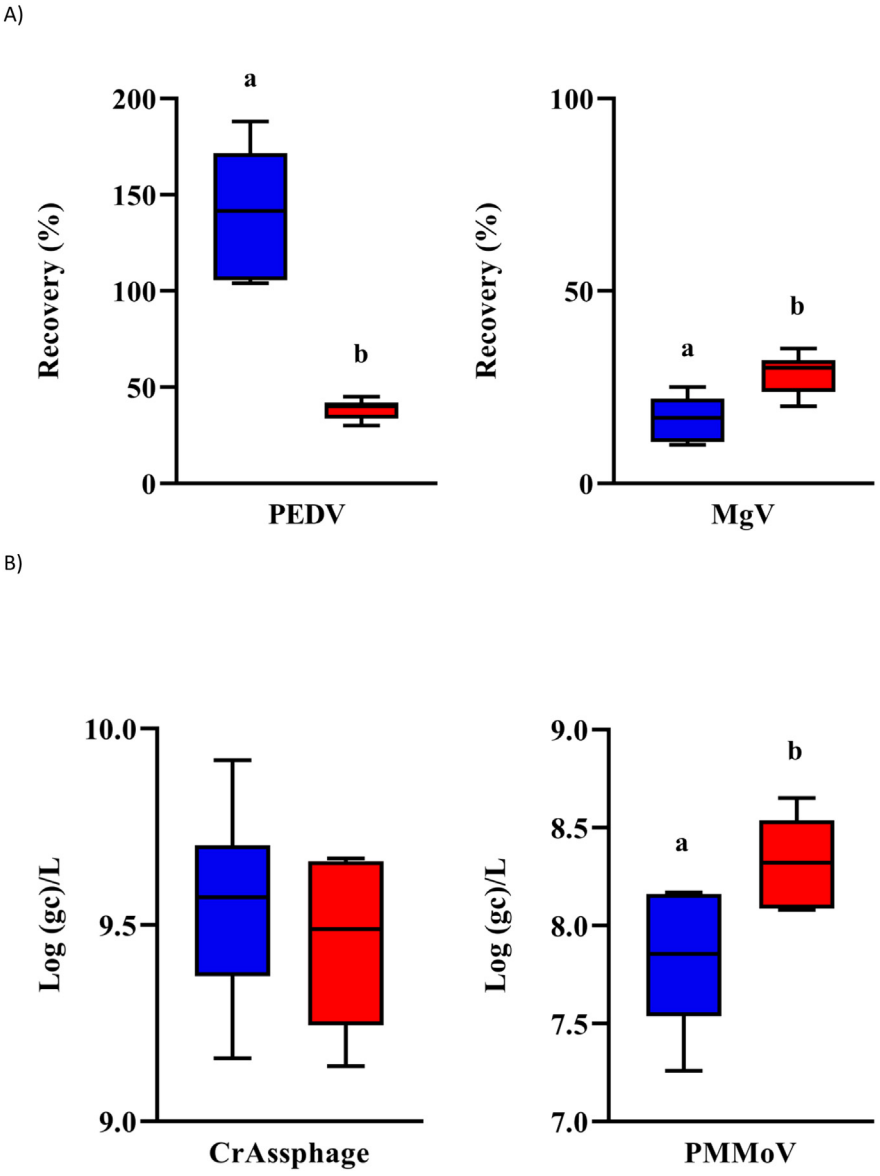

Fig. 3. A) Percentages of PEDV and MgV recoveries, and B) Levels (log_{10} gc/L) of viral indicators PMMoV and crAssphage, in wastewaters ($n = 6$) using the aluminum-based adsorption-precipitation (AP, blue boxes) and the Enviro Wastewater TNA Kit (TNA, red boxes). Different letters denote significant differences (p-value < 0.05) for each virus levels between each concentration method with t-test (MgV and PMMoV) and Wilcoxon test (PEDV).

Table 1
Mean values of murine norovirus (MNV) and hepatitis A virus (HAV) titers (log $TCID_{50}$/mL) and logarithmic reductions obtained for PBS and wastewater samples concentrated using the aluminum-based adsorption-precipitation (AP) and the Enviro Wastewater TNA Kit (TNA). Different letters denote significant differences between treatments.

Concentration method	Sample	MNV		HAV	
		Titer (log $TCID_{50}$/mL)	Log reduction	Titer (log $TCID_{50}$/mL)	Log reduction
	PBS	6.76 ± 0.07 [a]	–	6.04 ± 0.21 [a]	–
AP	PBS	6.64 ± 0.24 [a]	0.13	5.95 ± 0.27 [a]	0.09
	Wastewater	7.14 ± 0.07 [b]	−0.38	5.45 ± 0.10 [b]	0.59
TNA	PBS	<1.15 [c]	>5.61	3.57 ± 0.00 [d]	2.47
	Wastewater	<1.15 [c]	>5.61	3.95 ± 0.00 [c]	2.09

percentage of genome of SARS-CoV-2 covered, and the mean values of coverage depth. The mean percentage of reads identified as SARS-CoV-2 ranged from 20.5 ± 15.0 % in AP-V4 to 55.1 ± 26.7 % in TNA-V4. Statistical analyses showed significative differences in the percentages of SARS-CoV-2 reads between the AP method amplified with the primer scheme V4 and the TNA method (*p*-values of 0.03 for TNA-V3 and 0.008 for TNA-V4), with the reads being lower when the AP-V4 method was used. Regarding the percentage of genome coverage, samples processed with the TNA method and amplified with the V4 primer scheme showed higher genome coverages (83.7 ± 15.5 %) and significant differences (p-value = 0.02) compared with the other methods, with the exception of the TNA method with V3 primer scheme (61.4 ± 26.8 %) which did not show significant differences with respect to the results obtained with TNA-V4 (Figs. 4 and 5). Mean depth values were higher with method TNA-V4 (mean values 727.2 ± 367.8) which showed slight significative differences (p-value = 0.04) with method AP-V3 (318.2 ± 70.7). However, variability was higher in TNA-V4 than in the other analyzed methods.

4. Discussion

Wastewater-based epidemiology has proven to be an effective and useful tool for virus surveillance and outbreak detection, both for enteric viruses and for viruses that can be excreted in feces and urine (Asghar et al., 2014; Cuevas-Ferrando et al., 2020; Hellmér et al., 2014; Miura et al., 2016; Polo et al., 2020; Prevost et al., 2015; Santiso-Bellón et al., 2020). However, the detection of viruses in wastewater entails a previous step of sample concentration due to the low proportion of viruses compared to other microorganisms in these types of samples. Different concentration procedures have already been described and compared; however so far there is no standardized protocol for human enteric virus and SARS-CoV-2 detection (Rusiñol et al., 2020a; Torii et al., 2022). In this study, two different methods for wastewater concentration were evaluated for the detection of human enteric viruses, and viral indicators. Moreover, the performance of these procedures for SARS-CoV-2 detection and characterization by sequencing was evaluated using two different primer schemes.

The aluminum-based adsorption precipitation method (AP) has been used for the detection of enteric viruses in wastewater so far (Cashdollar and Wymer, 2013; Ikner et al., 2012). Furthermore, this method has also been validated for SARS-CoV-2 detection (Pérez-Cataluña et al., 2021) and it is currently used as a reference method in the Spanish COVID-19 wastewater surveillance project for the detection of SARS-CoV-2 and its variants (VATar COVID-19) (Carcereny et al., 2021). On the other hand, the TNA method has been recently validated for SARS-CoV-2 and viral fecal indicators, but no data about its feasibility for enteric virus detection has been published (Jiang et al., 2022; Mondal et al., 2021).

Even though the number of samples analyzed was limited, our results showed differences in viral recoveries of process control viruses (i.e. PEDV and MgV). In the case of PEDV, used as a model of enveloped viruses, the AP method showed higher recovery rates than the TNA method. However, the percentage of SARS-CoV-2 positive samples using the TNA method performed better (Fig. 1, Sup. Table S1). Regarding recovery rates of MgV, used as a model on non-enveloped viruses, higher recoveries were obtained when the TNA method was used. These recovery rates (mean 33.7 %) were similar to the ones obtained by Borgmästars et al. (2021) for MgV and human enteric viruses (Norovirus GI and GII, and HAV) with skimmed milk flocculation (SMF). However, with the SMF technique, 10 L were used for sample concentration, while with the TNA method only 40 mL were processed, simplifying the whole procedure. Moreover, enteric viruses (with the exception of RV and HAstrV) and PMMoV were detected more frequently when the TNA method was used, reinforcing the suitability of the TNA method in the detection of non-enveloped viruses.

Cell culture assays were carried out to evaluate the potential viability/infectivity of the viral particles present in the sewage after both concentration methods were applied. Our results showed that the AP concentration method is more successful for this purpose than TNA, which reported no infectious titers for MNV or infectivity loss of >2 log for HAV after being concentrated. This result could be due to the presence of alcohols (isopropanol and ethanol) in the TNA kit composition affecting viral infectivity. As expected, HAV was more resistant to the alcohols present in the TNA kits. Therefore, with regards to viral infectivity in sewage samples (Cuevas-Ferrando et al., 2021), the present results showed that the concentration methods applied need to be carefully validated.

Due to the limitations that classical techniques used in virus detection sometimes present, such as PCR or cell culture techniques, the use of massive sequencing technologies for the study of viruses in the environment is currently on the rise. For this reason, the European Union urges researchers to analyze SARS-CoV-2 in wastewater using these techniques. However, few studies have analyzed the concentration effects in genome sequencing. Thus, the effect of the two concentration methods as well as

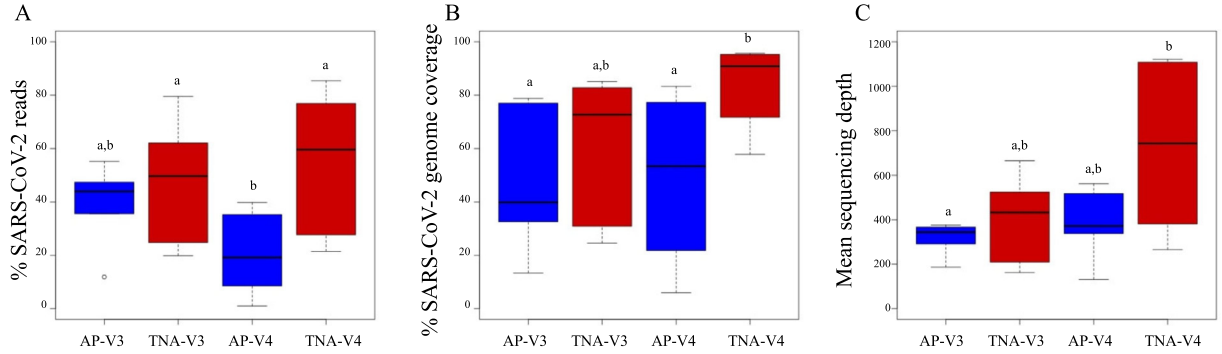

Fig. 4. Values obtained between the different concentrations methods tested in the study of the percentage of SARS-CoV-2 reads (A), SARS-CoV-2 genome coverage (B), and mean genome depth above 20× (C) after amplicon-based sequencing of SARS-CoV-2 with Artic primer scheme version 3 (V3) and 4 (V4). For each analysis (n = 6), boxes with the same letter show differences not statistically significant (p-value < 0.05).

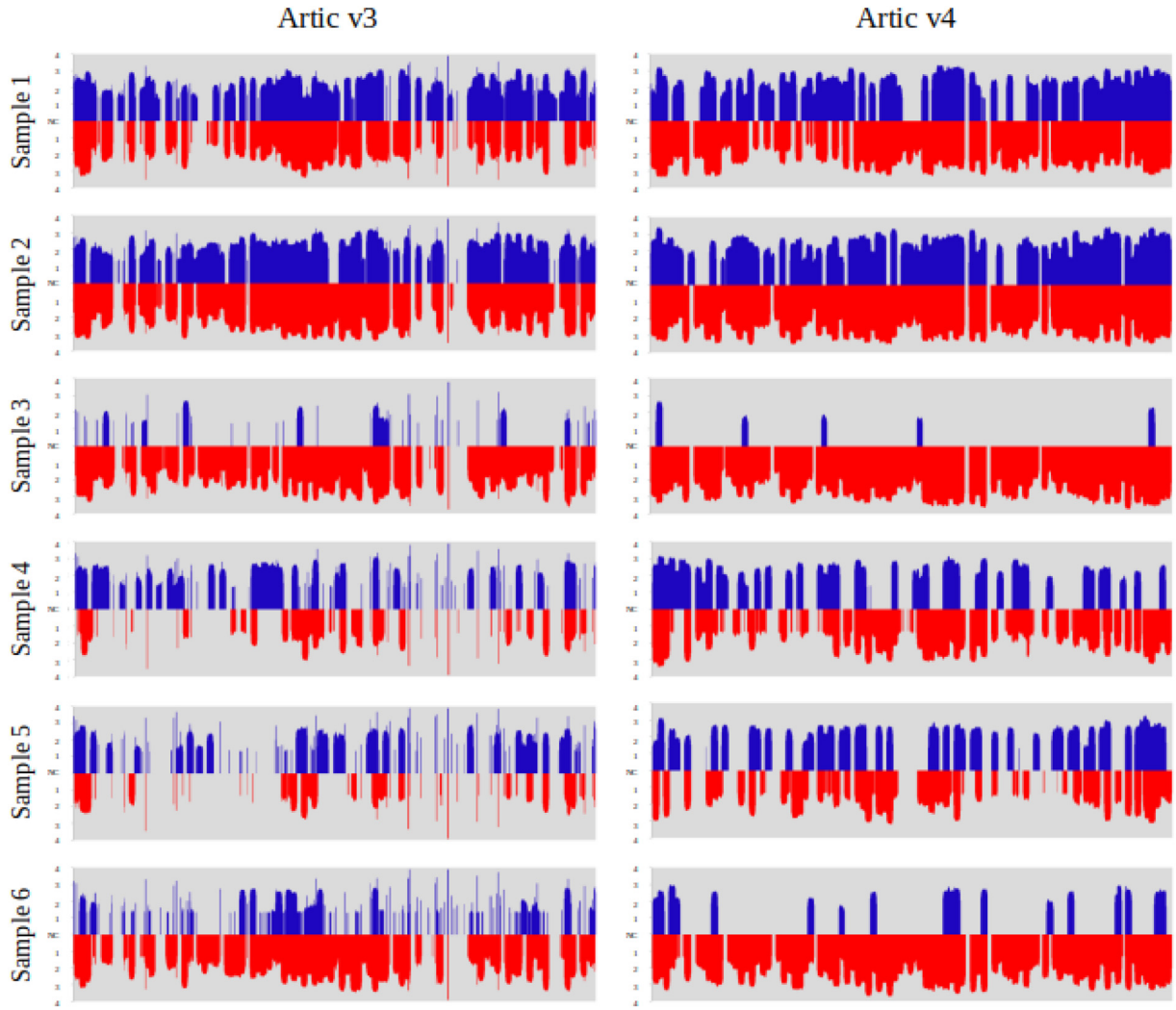

Fig. 5. X-axis: genome coverage of the SARS-CoV-2 reference genome MN908947.3 (only nucleotides with depth higher to 20×) in logarithmic scale (max 4 log) for each sample. Y-axis: logarithm of the depth (>20×) for each nucleotide position of the SARS-CoV-2 genome. NC, not covered. Blue, aluminum-based adsorption-precipitation; Red, Enviro Wastewater TNA Kit.

the primer scheme effect in SARS-CoV-2 genome sequencing was also evaluated. Regarding the percentage of genome coverage, samples processed with the TNA method showed higher genome coverages than with the other studied method. Similar results were obtained for genome coverage in the study performed by Kevill et al. (2022), although the authors did not find significative differences between the methods tested in their study. Values obtained with TNA-V4 regarding genome coverage were higher than the ones reported by Izquierdo-Lara et al. (2021) who showed average values of the percentage of SARS-CoV-2 genome of 51.3 ± 14.7 %. However, these authors performed an ultracentrifugation method for sample concentration that can produce lower virus recoveries, which would also affect sequencing (Hmaïed et al., 2016; Izquierdo-Lara et al., 2021; Prado et al., 2021; Ye et al., 2012). These results suggested that the use of the TNA method combined with the amplification of SARS-CoV-2 genomes using the Artic primer scheme V4 would give better results than with the other methods; although a high intravariability between samples can be produced.

5. Conclusions

WBE has proven to be an effective tool in epidemiological surveillance. However, the different methods used for the analysis of wastewater samples may produce differences in the results obtained. In this work, two sample concentration methods for virus analysis using molecular and cell culture techniques were compared alongside the two most commonly used primer schemes for SARS-CoV-2 genomic sequencing. Our results showed concentration methods are critical for the surveillance of human enteric viruses and SARS-CoV-2. In this sense, the use of the concentration system through the TNA system produces better results in terms of sensitivity and SARS-CoV-2 coverage sequencing. However, this technique completely reduces virus viability, indicating that methods such as aluminum precipitation would be recommended if these samples are to be tested on cell culture. Furthermore, concentration by the TNA method in combination with the Artic v4 primer scheme yields better sequencing results on sewage samples. Our results provide new information on the effects of the methods used for WBE studies, allowing us to improve this tool for use in epidemiology.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160914.

CRediT authorship contribution statement

Inés Girón-Guzmán: Investigation, formal analysis, writing, and reviewing. Azahara Díaz-Reholid: Investigation, formal analysis, writing, and reviewing. Enric Cuevas-Ferrando: Investigation, formal analysis, visualization, writing, and reviewing. Irene Falcó: Investigation, formal analysis, writing, and reviewing. Pablo Cano-Jiménez: Investigations and formal analysis. Iñaki Comas: Supervision, funding acquisition, writing,

and reviewing. Alba Pérez-Cataluña: Investigation, formal analysis, visualization, writing, and reviewing. Gloria Sánchez: Conceptualization, supervision, funding acquisition, writing, and reviewing.

Data availability

Data will be made available on request.

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

This research was supported by project MCEC-WATER (PID2020–116789RB-C42/AEI/FEDER, UE) funded by Spanish Ministry of Science, Innovation and Universities; the European Commission NextGenerationEU fund, through CSIC's Global Health Platform (PTI Salud Global) to GS and IC, the COVID-19 wastewater surveillance project (VATar COVID19) and the Lagoon project (PROMETEO/2021/044). IGG is recipient of a predoctoral contract from the Generalitat Valenciana (ACIF/2021/181), EC-F is recipient of a predoctoral contract from the MICINN Call 2018 (PRE2018-083753) and AP-C was supported by a postdoctoral fellowship from the Generalitat Valenciana (APOSTD/2021/292). We acknowledge MITECO for authorizing the sampling. The authors thank Agustin Garrido Fernández, and Andrea Lopez de Mota for their technical support.

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