
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Virological Methods 297 (2021) 114272

Contents lists available at ScienceDirect

Journal of Virological Methods

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

# A rapid and simple protocol for concentration of SARS-CoV-2 from sewage

Diana P. Wehrendt [a,b], Mariana G. Massó [c], Adrián Gonzales Machuca [c], Claudia V. Vargas [d], Melina E. Barrios [b,e], Josefina Campos [g], Damián Costamagna [h], Luis Bruzzone [i], Daniel M. Cisterna [g], Néstor Gabriel Iglesias [b,j], Viviana A. Mbayed [b,e,f], Elsa Baumeister [k], Daniela Centrón [b,c], María Paula Quiroga [b,c], Leonardo Erijman [a,b,l,*]

[a] Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr Héctor N. Torres" (INGEBI) Vuelta de Obligado 2490, C1428ADN, Buenos Aires, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] Laboratorio de Investigaciones en Mecanismos de Resistencia a Antibióticos. Instituto de Investigaciones en Microbiología y Parasitología Médica, Facultad de Medicina, Universidad de Buenos Aires (IMPaM, UBA-CONICET), Argentina
[d] Instituto de Investigaciones en Producción Animal (INPA), Argentina
[e] Universidad de Buenos Aires, Facultad de Farmacia y Bioquimica, Argentina
[f] Instituto de Investigaciones en Bacteriología y Virologia Molecular (IBAViM), Argentina
[g] Instituto Nacional de Enfermedades Infecciosas (INEI) ANLIS "Dr Carlos G. Malbrán, Argentina
[h] Autoridad del Agua, Gobierno de la Provincia de Buenos Aires, Argentina
[i] Aguas Bonaerenses SA, Argentina
[j] Laboratorio de Virus Emergentes, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Argentina
[k] Servicio Virosis Respiratorias, Laboratorio Nacional de Referencia de Enfermedades Respiratorias Virales, INEI-ANLIS "Dr Carlos G. Malbrán", Centro Nacional de Influenza de OMS, Argentina
[l] Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

ARTICLE INFO

*Keywords:*
Environmental surveillance
SARS-CoV-2
Sewage
Virus concentration
Wastewater based epidemiology

ABSTRACT

The aim of this study was to set up a simple protocol to concentrate SARS-CoV-2 from sewage, which can be implemented in laboratories with minimal equipment resources. The method avoids the need for extensive purification steps and reduces the concentration of potential inhibitors of RT-qPCR contained in sewage. The concentration method consists of a single step, in which a small volume (40 mL) of sewage sample is incubated with polyaluminum chloride (PAC)(0.00045 N $Al^{3+}$ final concentration). Virus particles adsorbed to the precipitate are collected by low-speed centrifugation, after which the recovered pellet is resuspended with a saline buffer. PAC-concentrated samples are stable for at least one week at 4 °C. Therefore, they may be sent refrigerated to a diagnosis center for RNA extraction and RT-qPCR for SARS-CoV-2 RNA detection if the lab does not have such capabilities. The PAC concentration method produced an average shift of 4.5-units in quantification cycle (Cq) values compared to non-concentrated samples, indicating a 25-fold increase in detection sensitivity. The lower detection limit corresponded approximately to 100 viral copies per ml. Kappa index indicated substantial agreement between PAC and polyethylene glycol (PEG) precipitation protocols (k = 0.688, CI 0.457−0.919). This low-cost concentration protocol could be useful to aid in the monitoring of community circulation of SARS-CoV-2, especially in low- and middle-income countries, which do not have massive access to support from specialized labs for sewage surveillance.

One of the main challenges faced by health authorities during the Coronavirus disease 2019 (COVID-19) pandemics has been testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on a large enough scale. It has been shown that analysis of sewage can complement clinical testing by providing a fair representation of the incidence of SARS-CoV-2 within a community, including pre-symptomatic and asymptomatic individuals (Foladori et al., 2020). For these reasons, SARS-CoV-2 sewage surveillance has the potential to provide early warning of the emergence of new outbreaks (De Araujo et al., 2021; Mao et al., 2020; Medema et al., 2020; Róka et al., 2021;

* Corresponding author at: Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr Héctor N. Torres" (INGEBI) Vuelta de Obligado 2490, C1428ADN, Buenos Aires, Argentina.
*E-mail address:* erijman@dna.uba.ar (L. Erijman).

https://doi.org/10.1016/j.jviromet.2021.114272
Received 27 May 2021; Received in revised form 24 August 2021; Accepted 25 August 2021
Available online 26 August 2021

Zhu et al., 2021). Sewage screening may also be a useful tool in evaluating the efficacy of the incipient vaccination campaign (Gross, 2021).

Methods for the detection of SARS-CoV-2 (RNA extraction and RT-qPCR detection) in sewage samples are the same as those used for testing nasopharyngeal samples (Jayamohan et al., 2021). However, viral particles in sewage are usually very diluted and therefore require a concentration step before RNA extraction. To be considered effective and applicable, the concentration method has to provide highly efficient viral recovery and must be repeatable within a laboratory, as well as reproducible between laboratories (Block and Schwartzbrod, 1989). There is no single standardized method for the concentration of SARS-CoV-2 in sewage. Instead, there are a wide variety of techniques to recover SARS-CoV-2 from wastewater used by different laboratories around the world (Cervantes-Avilés et al., 2021; Kantor et al., 2021). The commonly used methods to successfully concentrate viral particles from sewage include adsorption-elution with negatively charged membrane, centrifugal concentrators, ultracentrifugation, PEG precipitation and aluminum-driven flocculation (Ahmed et al., 2020; Barril et al., 2021; LaTurner et al., 2021; Philo et al., 2021). Except the last one, these procedures require instrumentation, such as high speed refrigerated centrifuges, which are not always readily available in non-research laboratories, including those in local municipal wastewater treatment plants. This restricts the possibility of many villages and small towns to gain the information on virus circulation in the community that is afforded by sewage surveillance.

On the other hand, aluminum hydroxide adsorption-precipitation is a general method used to concentrate viruses from water, wastewater, and eluates from adsorbent filters (AWWA, 2018). The technique shows acceptable efficiency recovery for SARS-CoV-2 (Barril et al., 2021; Randazzo et al., 2020), though it requires extensive purification procedures, especially for sewage samples containing high amounts of suspended solids. This is a drawback because the solid component of sewage may contain a higher number of SARS-CoV-2 RNA copies than the corresponding liquid phase (Graham et al., 2021; Michael-Kordatou et al., 2020).

We describe here a simple method, applicable to sewage within an ample range of Biochemical Oxygen Demand (BOD) strength, which avoids the need for extensive purification steps and reduces the concentration of potential inhibitors of RT-qPCR contained in sewage. The concentration protocol is simple enough to be performed in any low-resource laboratory.

The concentration method consists of a single step, in which a small volume (40 mL) of sewage sample is incubated with poly-aluminum chloride (PAC). This inorganic polymer has been used worldwide in water and wastewater treatment for decades as a primary coagulant aid for clarification and phosphorus removal. Total aluminum content of PAC products, expressed as $Al_2O_3$, ranges from about 6–24% by weight in aqueous solutions. The mechanisms of adsorption appear to involve electrostatic interactions between the negatively charged virus surface and the positively charged aluminum hydroxide surfaces and coordination of the virus surface by hydroxo-aluminum complexes (AWWA, 2018). Virus particles adsorbed to the precipitate are collected by low-speed centrifugation, after which the recovered pellet is resuspended with a saline buffer (Fig. 1). The concentrated samples are stable for at least 1 week at 4 °C, and therefore can be sent refrigerated to a diagnosis center, where SARS-CoV-2 should be tested in the same way as

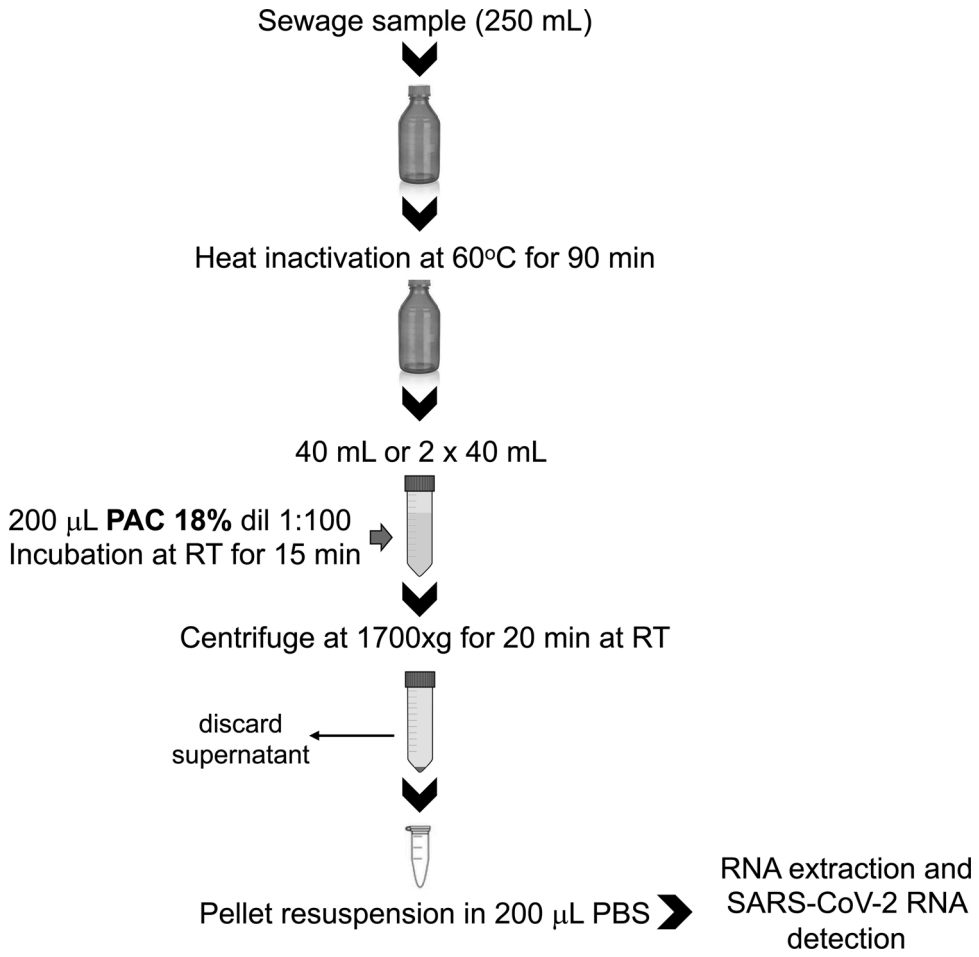


**Fig. 1.** Concentration protocol flow diagram for virus concentration from sewage samples. RT: room temperature. Concentrated samples can be stored for at least one week at 4 °C before RNA extraction.

on a clinical sample.

A total of 60 sewage samples were collected weekly in duplicate, during a 15-weeks period at pumping stations of two wastewater treatment plants located in the Buenos Aires Metropolitan Area (AK, 20,000 population equivalents (pe) and SV, 15,000 pe), and at sewer manholes of two apartment block complexes (SP, 3500 pe and SF, 5000 pe). Sewage strength varied considerably between locations. Biological oxygen demand of collected samples ranged from 248 ± 33 mg/L in SF to 630 ± 116 mg/L in SP (Table S1). Grab samples (250 mL) were transported within 3 h of collection to two different laboratories and processed immediately upon arrival. For the purpose of operator protection, samples were initially incubated in the same glass bottles used for sampling at 60 °C during 90 min for heat inactivation of the virus. Duplicate samples were concentrated using either PEG/NaCl concentration protocol (Wu et al., 2020) or the modified PAC concentration protocol. Briefly, for the PEG/NaCl concentration method 20 g PEG8000 and 4.5 g NaCl were added to 200 mL of pre-inactivated wastewater samples and mixed at 4 °C for 1 h. Samples were centrifuged for 1 h at 4 °C at 12000xg and the resultant pellet was resuspended in 1 mL of Trizol (Invitrogen) and RNA was purified according to the supplier's protocol. The extracted RNA was further purified using the High Pure Viral Nucleic acid kit (Roche) following manufacturer instructions and eluted in 50 µL RNase-free water.

For the PAC concentration protocol, 200 µL of a 1/100 PAC solution ($Al_2O_3$ 18 %, KLARAID IC1176 L, Suez Water Technologies) were added to 40 mL of pre-inactivated wastewater samples (1/20,000 PAC final dilution). The pH was adjusted, if necessary, within the range of 6–7. Incubation was carried out for 15 min with agitation at room temperature. Samples were centrifuged at 1,700xg for 20 min at room temperature and the resultant pellet was resuspended in 200 µL of 1X phosphate buffer saline (PBS: NaCl 8 *g*/L, KCl 0.2 *g*/L, $Na_2HPO_4$ 1.44 *g*/L, $KH_2PO_4$ 0.24 *g*/L, pH 7.4). This concentration step costs less than one US cent per sample. RNA purification was performed using the Viral Nucleic Extraction Kit II (Geneaid), following manufacturer instructions, with the addition of a short spin after the lysis step, to prevent column clogging. RNA was eluted in 50 µL RNase- free water.

SARS-CoV-2 RNA was detected using the DisCoVery SARS-CoV-2 RT-PCR detection kit (APBiotech), using 5 µL of purified RNA in a final reaction volume of 25 µL. The targets of this kit are SARS-CoV-2 nucleocapsid (N) and orf1 genes. The kit also includes human RNase P as an internal control.

A kappa index (Cohen, 1960) was calculated to measure the agreement between tests performed after the PAC concentration protocol and the PEG concentration method, which had been run in parallel on the same samples. The result was kappa = 0.688; SE = 0.118; 95 % confidence interval 0.457 to 0.919; n = 60), indicating substantial agreement between the two concentration methods.

The sensitivity of SARS-CoV-2 detection did not increase when 80 mL of samples (n = 28) were concentrated separately and pooled before RNA extraction (Fig. 2), likely due to the simultaneous concentration of PCR inhibitors. We did not detect any relationship between relative Cq shifts and the measured sewage characteristics.

To evaluate the relative fold increase gained by using the PAC concentration method, RT-qPCR was also performed on RNA extracted from 200 µL of 28 inactivated sewage samples without any concentration procedure (thereafter called non-concentrated samples). Cq values were compared to those obtained using RNA purified from the same samples, previously concentrated and resuspended in 200 µL of PBS. The concentration method produced a shift of an average of 4.55±1.72 in Cq values for SARS-CoV-2 genes compared to non-concentrated samples (Fig. 2), indicating a 25-fold increase in detection sensitivity.

The human RNase P gene was detected in all analyzed samples, including those negative for SARS-CoV-2. The adequacy of RNase P gene as an internal amplification for human stool samples has been previously assessed (Babiker et al., 2021; Coryell et al., 2021).

To estimate the detection limit of the method, a 40 mL sewage sample taken during the early stage of the COVID-19 pandemics, i.e. negative for SARS-CoV-2, was artificially seeded with a 10-µL droplet of a SARS-CoV-2 virus culture inactivated with beta-propiolactone ($2.33 \times 10^5$ tissue culture infectious dose TCID50/mL, corresponding to approximately $4 \times 10^9$ copies/µL, isolated at the "Dr Carlos G. Malbrán" Institute). The inoculated sample was 10-fold serially diluted in sewage

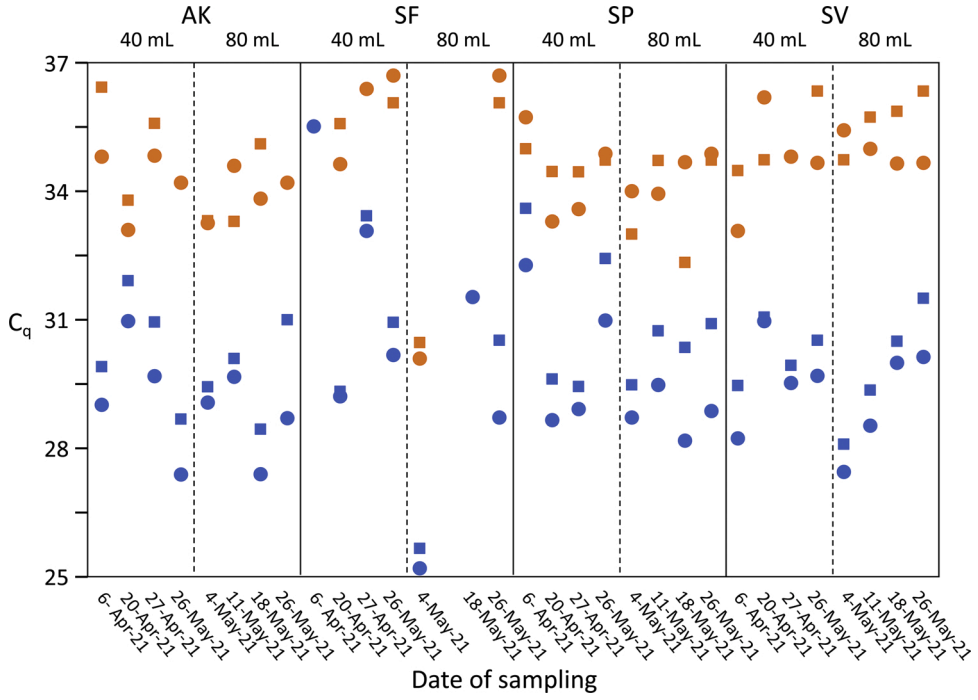


**Fig. 2.** RT-qPCR charts for each treatment plant (AK and SV) and sewer manholes (SF and SP) before (orange) and after PAC concentration (light blue). RNA was extracted after concentration from 40 mL or 80 mL of sewage sample, as indicated. $C_q$ above 40 was considered a non-detection. Circles and squares represent the targets of the RT-qPCR, respectively SARS-CoV-2 nucleocapsid (N) and orf1 genes.

samples negative for SARS-CoV-2. Each dilution was subsequently subjected to the concentration protocol followed by RNA extraction and RT-qPCR detection. The lower detection limit was the $10^{-7}$ dilution, corresponding to an initial viral genome concentration of approximately 100 copies /mL.

By starting with a low initial volume of raw sewage (40 mL), we were able to perform RNA extraction directly from the pellet obtained after precipitation, and therefore the use of beef extract in the final purification step (AWWA, 2018) could be omitted. Nevertheless, because PAC is a nonspecific coagulant, other substances such as humic substances contained in the sewage (Mendoza et al., 2020) may still be concentrated with the virus. The presence of such impurities may not be removed in the RNA extraction step and might cause the concentrated sample to be inhibitory to the RT-qPCR assay. Therefore, although virus precipitation could be potentially increased by using larger amounts of $Al(OH)_3$ and larger sample volumes, the optimum values of sample volume and PAC concentration was a rather delicate balance for maximum virus recovery with minimal enzyme inhibition. We note that the physical and chemical properties of the numerous PAC products can vary, including their content of other inorganic salts, which can affect its properties. Therefore, it is recommended to validate the optimum PAC concentration for each different batch of commercial PAC.

COVID-19 incidence in the metropolitan area that includes the catchment area of the wastewater treatment plants showed a significantly negative correlation with the Cq of SARS-CoV-2 detection (Spearman's correlation r =-0.635, p = 0.00463). Despite the differences in sewage characteristics, the method could consistently detect the presence of SARS-CoV-2 in the four samples, even under relatively low COVID-19 incidence (<45 reported active cases / 100,000 people). Importantly, we did not detect major changes in Cq when concentrated samples were stored at 4 °C in PBS for at least one week before RNA extraction (Table S2).

In conclusion, we describe here a rapid and simple protocol that can efficiently concentrate SARS-CoV-2 from sewage, which can be straightforwardly performed in a laboratory with low infrastructure. Importantly, our data indicate that this low-cost method is suitable to concentrate efficiently SARS-CoV-2 from sewage with different levels of settleable solids. Concentrated samples can be sent refrigerated to a diagnosis center, where they may be handled as any other sample received for SARS-CoV-2 detection. This protocol could be useful to aid in the monitoring of community circulation of SARS-CoV-2, especially in low- and middle-income countries, which do not have massive access to support from specialized labs for sewage surveillance.

## CRediT authorship contribution statement

**Diana P. Wehrendt:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Mariana G. Massó:** Validation, Investigation. **Adrián Gonzales Machuca:** Validation, Investigation. **Claudia V. Vargas:** Validation, Investigation. **Melina E. Barrios:** Validation, Investigation. **Josefina Campos:** Investigation, Resources. **Damián Costamagna:** Resources. **Luis Bruzzone:** Resources. **Daniel M. Cisterna:** Investigation, Resources. **Néstor Gabriel Iglesias:** Validation, Investigation, Resources. **Viviana A. Mbayed:** Validation, Investigation, Resources. **Elsa Baumeister:** Investigation, Resources, Funding acquisition. **Daniela Centrón:** Conceptualization, Validation, Investigation, Resources, Funding acquisition. **María Paula Quiroga:** Validation, Investigation. **Leonardo Erijman:** Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgments

This work was funded by the Programa de Articulación y Fortalecimiento Federal de las Capacidades en Ciencia y Tecnología COVID-19 (Proyecto BSAS-32; Ministerio de Ciencia, Tecnología e Innovación de la Nación (MINCyT) to LE and by Grant IP No. 13 COVID 19 from ANPCyT to EB. We thank all members of the network for the detection of Coronavirus in the Environment (MINCyT) for helpful discussions. We appreciate the essential collaboration of Aguas Bonaerenses SA (ABSA), Autoridad del Agua (ADA) and Organismo Provincial para el Desarrollo Sostenible (OPDS). Carlos Zapata and Matias Goñi are gratefully acknowledged for the collection and transportation of samples.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.jviromet.2021.114272.

## References

Ahmed, W., Bertsch, P.M., Bivins, A., Bibby, K., Farkas, K., Gathercole, A., Haramoto, E., Gyawali, P., Korajkic, A., McMinn, B.R., Mueller, J.F., Simpson, S.L., Smith, W.J.M., Symonds, E.M., Thomas, K.V., Verhagen, R., Kitajima, M., 2020. Comparison of virus concentration methods for the RT-qPCR-based recovery of murine hepatitis virus, a surrogate for SARS-CoV-2 from untreated wastewater. Sci. Total Environ. 739, 139960 https://doi.org/10.1016/j.scitotenv.2020.139960.

AWWA, 2018. In: Rice, E.W., Baird, R., Eaton, A.D. (Eds.), Virus Concentration by Aluminum Hydroxide Adsorption-Precipitation, Chapter Detection of Enteric Viruses. American Water Works Association, Denver, CO.

Babiker, A., Ingersoll, J.M., Adelman, M.W., Webster, A.S., Broder, K.J., Stittleburg, V., Waggoner, J.J., Kraft, C.S., Woodworth, M.H., 2021. Validation of high-sensitivity severe acute respiratory syndrome coronavirus 2 testing for stool-toward the new normal for fecal microbiota transplantation. Clin. Transl. Gastroenterol. 12, e00363 https://doi.org/10.14309/ctg.0000000000000363.

Barril, P.A., Pianciola, L.A., Mazzeo, M., Ousset, M.J., Jaureguiberry, M.V., Alessandrello, M., Sánchez, G., Oteiza, J.M., 2021. Evaluation of viral concentration methods for SARS-CoV-2 recovery from wastewaters. Sci. Total Environ. 756, 144105 https://doi.org/10.1016/j.scitotenv.2020.144105.

Block, J.C., Schwartzbrod, L., 1989. Viruses in water systems. Detection and Identification. VCH Publishers, New York.

Cervantes-Avilés, P., Moreno-Andrade, I., Carrillo-Reyes, J., 2021. Approaches applied to detect SARS-CoV-2 in wastewater and perspectives post-COVID-19. J. Water Process Eng. 40, 101947 https://doi.org/10.1016/j.jwpe.2021.101947.

Cohen, J., 1960. A coefficient of agreement for nominal scales. Educ. Psychol. Meas. 20, 37–46.

Coryell, M.P., Iakiviak, M., Pereira, N., Murugkar, P.P., Rippe, J., Williams, D.B., Heald-Sargent, T., Sanchez-Pinto, L.N., Chavez, J., Hastie, J.L., Sava, R.L., Lien, C.Z., Wang, T.T., Muller, W.J., Fischbach, M.A., Carlson, P.E., 2021. A method for detection of SARS-CoV-2 RNA in healthy human stool: a validation study. The Lancet Microbe 2, E259–E266. https://doi.org/10.1016/S2666-5247(21)00059-8.

De Araujo, J.C., Gavazza, S., Leao, T.L., Florencio, L., Da Silva, H.P., De Oliveira Albuquerque, J., De Lira Borges, M.A., De Oliveira Alves, R.B., Alves Rodrigues, R. H., Dos Santos, E.B., 2021. SARS-CoV-2 sewage surveillance in low-income countries: potential and challenges. J. Water Health 19, 1–19. https://doi.org/10.2166/WH.2020.168.

Foladori, P., Cutrupi, F., Segata, N., Manara, S., Pinto, F., Malpei, F., Bruni, L., La Rosa, G., 2020. SARS-CoV-2 from faeces to wastewater treatment: What do we know? A review. Sci. Total Environ. 743, 140444 https://doi.org/10.1016/j.scitotenv.2020.140444.

Graham, K.E., Loeb, S.K., Wolfe, M.K., Catoe, D., Sinnott-Armstrong, N., Kim, S., Yamahara, K.M., Sassoubre, L.M., Mendoza Grijalva, L.M., Roldan-Hernandez, L., Langenfeld, K., Wigginton, K.R., Boehm, A.B., 2021. SARS-CoV-2 RNA in wastewater settled solids is associated with COVID-19 cases in a large urban sewershed. Environ. Sci. Technol. 55, 488–498. https://doi.org/10.1021/acs.est.0c06191.

Gross, M., 2021. Wastewater warnings. Curr. Biol. 31, PR267–R269. https://doi.org/10.1016/j.cub.2021.03.007.

Jayamohan, H., Lambert, C.J., Sant, H.J., Jafek, A., Patel, D., Feng, H., Beeman, M., Mahmood, T., Nze, U., Gale, B.K., 2021. SARS-CoV-2 pandemic: a review of molecular diagnostic tools including sample collection and commercial response with associated advantages and limitations. Anal. Bioanal. Chem. 413, 49–71. https://doi.org/10.1007/s00216-020-02958-1.

Kantor, R.S., Nelson, K.L., Greenwald, H.D., Kennedy, L.C., 2021. Challenges in measuring the recovery of SARS-CoV-2 from wastewater. Environ. Sci. Technol. 55, 3514–3519. https://doi.org/10.1021/acs.est.0c08210.

LaTurner, Z.W., Zong, D.M., Kalvapalle, P., Gamas, K.R., Terwilliger, A., Crosby, T., Ali, P., Avadhanula, V., Santos, H.H., Weesner, K., Hopkins, L., Piedra, P.A., Maresso, A.W., Stadler, L.B., 2021. Evaluating recovery, cost, and throughput of different concentration methods for SARS-CoV-2 wastewater-based epidemiology. Water Res. 197, 117043 https://doi.org/10.1016/j.watres.2021.117043.

Mao, K., Zhang, K., Du, W., Ali, W., Feng, X., Zhang, H., 2020. The potential of wastewater-based epidemiology as surveillance and early warning of infectious disease outbreaks. Curr. Opin. Environ. Sci. Heal. 17, 1–7. https://doi.org/10.1016/j.coesh.2020.04.006.

Medema, G., Been, F., Heijnen, L., Petterson, S., 2020. Implementation of environmental surveillance for SARS-CoV-2 virus to support public health decisions: opportunities and challenges. Curr. Opin. Environ. Sci. Heal. 17, 49–71. https://doi.org/10.1016/j.coesh.2020.09.006.

Mendoza, L.M., Mladenov, N., Kinoshita, A.M., Pinongcos, F., Verbyla, M.E., Gersberg, R., 2020. Fluorescence-based monitoring of anthropogenic pollutant inputs to an urban stream in Southern California. USA. Sci. Total Environ. 718, 137206 https://doi.org/10.1016/j.scitotenv.2020.137206.

Michael-Kordatou, I., Karaolia, P., Fatta-Kassinos, D., 2020. Sewage analysis as a tool for the COVID-19 pandemic response and management: the urgent need for optimised protocols for SARS-CoV-2 detection and quantification. J. Environ. Chem. Eng. 8, 104306 https://doi.org/10.1016/j.jece.2020.104306.

Philo, S.E., Keim, E.K., Swanstrom, R., Ong, A.Q.W., Burnor, E.A., Kossik, A.L., Harrison, J.C., Demeke, B.A., Zhou, N.A., Beck, N.K., Shirai, J.H., Meschke, J.S., 2021. A comparison of SARS-CoV-2 wastewater concentration methods for environmental surveillance. Sci. Total Environ. 760, 144215 https://doi.org/10.1016/j.scitotenv.2020.144215.

Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A., Sánchez, G., 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res. 181, 115942 https://doi.org/10.1016/j.watres.2020.115942.

Róka, E., Khayer, B., Kis, Z., Kovács, L.B., Schuler, E., Magyar, N., Málnási, T., Oravecz, O., Pályi, B., Pándics, T., Vargha, M., 2021. Ahead of the second wave: early warning for COVID-19 by wastewater surveillance in Hungary. Sci. Total Environ. 786, 147398 https://doi.org/10.1016/j.scitotenv.2021.147398.

Wu, F., Zhang, J., Xiao, A., Gu, X., Lee, W.L., Armas, F., Kauffman, K., Hanage, W., Matus, M., Ghaeli, N., Endo, N., Duvallet, C., Poyet, M., Moniz, K., Washburne, A.D., Erickson, T.B., Chai, P.R., Thompson, J., Alm, E.J., 2020. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. mSystems 5. https://doi.org/10.1128/msystems.00614-20.

Zhu, Y., Oishi, W., Maruo, C., Saito, M., Chen, R., Kitajima, M., Sano, D., 2021. Early warning of COVID-19 via wastewater-based epidemiology: potential and bottlenecks. Sci. Total Environ. 767, 145124 https://doi.org/10.1016/j.scitotenv.2021.145124.