Environmental Science
Water Research & Technology

COMMUNICATION

Cite this: *Environ. Sci.: Water Res. Technol.*, 2021, **7**, 1545

Standardizing data reporting in the research community to enhance the utility of open data for SARS-CoV-2 wastewater surveillance†

Jill S. McClary-Gutierrez, [a] Zachary T. Aanderud,[b] Mitham Al-faliti,[c] Claire Duvallet, [d] Raul Gonzalez, [e] Joe Guzman,[f] Rochelle H. Holm, [g] Michael A. Jahne, [h] Rose S. Kantor,[i] Panagis Katsivelis,[j] Katrin Gaardbo Kuhn,[k] Laura M. Langan, [l] Cresten Mansfeldt,[m] Sandra L. McLellan, [n] Lorelay M. Mendoza Grijalva,[o] Kevin S. Murnane, [pqr] Colleen C. Naughton,[s] Aaron I. Packman,[t] Sotirios Paraskevopoulos, [u] Tyler S. Radniecki, [v] Fernando A. Roman Jr, [s] Abhilasha Shrestha,[w] Lauren B. Stadler, [x] Joshua A. Steele, [y] Brian M. Swalla,[z] Peter Vikesland, [aa] Brian Wartell,[ab] Carol J. Wilusz, [ac] Judith Chui Ching Wong, [ad] Alexandria B. Boehm, [o] Rolf U. Halden, [aeafag] Kyle Bibby [a] and Jeseth Delgado Vela [*c]

Received 2nd April 2021,
Accepted 13th June 2021

DOI: 10.1039/d1ew00235j

rsc.li/es-water

SARS-CoV-2 RNA detection in wastewater is being rapidly developed and adopted as a public health monitoring tool worldwide. With wastewater surveillance programs being implemented across many different scales and by many different stakeholders, it is critical that data collected and shared are accompanied by an appropriate minimal amount of meta-information to enable meaningful interpretation and use of this new

Water impact

Extensive wastewater surveillance data are being generated during the COVID-19 pandemic; however, there is no consensus on the meta-information that should be reported with wastewater SARS-CoV-2 concentrations. Complete and consistent data are important for regional, national, and international data synthesis. The minimum recommended meta-information here aims to set a framework for wastewater surveillance data reporting.

[a] *Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, IN, USA*
[b] *Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT, USA*
[c] *Department of Civil and Environmental Engineering, Howard University, Washington, DC, USA. E-mail: jeseth.delgadovela@howard.edu*
[d] *Biobot Analytics, Inc., Cambridge, MA, USA*
[e] *Hampton Roads Sanitation District, Virginia Beach, VA, USA*
[f] *Orange County Public Health Laboratory, Newport Beach, CA, USA*
[g] *Christina Lee Brown Envirome Institute, University of Louisville, 302 E. Muhammad Ali Blvd., Louisville, KY, 40202, USA*
[h] *U.S. Environmental Protection Agency, Cincinnati, OH, USA*
[i] *Department of Civil and Environmental Engineering, University of California, Berkeley, CA, USA*
[j] *Venthic Technologies, Athens, Greece*
[k] *Department of Biostatistics and Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA*
[l] *Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX, USA*
[m] *Department of Civil, Environmental, and Architectural Engineering, University of Colorado Boulder, Boulder, CO, USA*
[n] *School of Freshwater Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, USA*
[o] *Department of Civil & Environmental Engineering, Stanford University, Stanford, CA, USA*
[p] *Department of Pharmacology, Toxicology & Neuroscience, Louisiana State University Health – Shreveport, Shreveport, LA, USA*
[q] *Department of Psychiatry, Louisiana State University Health – Shreveport, Shreveport, LA, USA*
[r] *Louisiana Addiction Research Center, Louisiana State University Health – Shreveport, Shreveport, LA, USA*
[s] *Civil and Environmental Engineering, University of California, Merced, CA, USA*
[t] *Department of Civil and Environmental Engineering, Northwestern Center for Water Research, Northwestern University, Evanston, IL, USA*
[u] *KWR Water Research Institute, Nieuwegein, The Netherlands*
[v] *School of Chemical, Biological, and Environmental Engineering, Oregon State University, Corvallis, OR, USA*
[w] *Division of Environmental and Occupational Health Sciences, School of Public Health, University of Illinois Chicago, Chicago, IL, USA*
[x] *Department of Civil & Environmental Engineering, Rice University, Houston, TX, USA*
[y] *Southern California Coastal Water Research Project, Costa Mesa, CA, USA*
[z] *IDEXX Laboratories, Inc., Westbrook, ME, USA*
[aa] *Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA, USA*
[ab] *Department of Environmental Engineering, University of Maryland, Baltimore, MD, USA*
[ac] *Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA*
[ad] *Environmental Health Institute, National Environment Agency, Singapore*
[ae] *Biodesign Center for Environmental Health Engineering, Biodesign Institute, Arizona State University, Tempe, AZ, USA*
[af] *OneWaterOneHealth, Arizona State University Foundation, Tempe, AZ, USA*
[ag] *AquaVitas, LLC, Scottsdale, AZ, USA*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1ew00235j

information source and intercomparison across datasets. While some databases are being developed for specific surveillance programs locally, regionally, nationally, and internationally, common globally-adopted data standards have not yet been established within the research community. Establishing such standards will require national and international consensus on what meta-information should accompany SARS-CoV-2 wastewater measurements. To establish a recommendation on minimum information to accompany reporting of SARS-CoV-2 occurrence in wastewater for the research community, the United States National Science Foundation (NSF) Research Coordination Network on Wastewater Surveillance for SARS-CoV-2 hosted a workshop in February 2021 with participants from academia, government agencies, private companies, wastewater utilities, public health laboratories, and research institutes. This report presents the primary two outcomes of the workshop: (i) a recommendation on the set of minimum meta-information that is needed to confidently interpret wastewater SARS-CoV-2 data, and (ii) insights from workshop discussions on how to improve standardization of data reporting.

Introduction

Following early reports of SARS-CoV-2 RNA detection in sewage,[1,2] there has been high interest in the application of wastewater surveillance for monitoring the COVID-19 pandemic. Many academic researchers, government agencies, and commercial scientists have developed methods for detecting SARS-CoV-2 in wastewater and applied these methods to inform COVID-19 pandemic public health response.[3,4] Ensuring prompt, appropriate access to complete and organized data following FAIR data principles (findable, accessible, interoperable, and reusable) is critical for scientific advancement,[5] and the COVID-19 pandemic has highlighted the utility of publicly available datasets, such as transit data for assessing lockdown impacts and genome sequencing data for tracking viral transmission dynamics.[6,7] Large-scale wastewater surveillance efforts, including those in the United States,[8,9] the European Union,[10,11] Canada,[12] Australia,[13] and Turkey,[14] are developing data reporting structures for their own internal databases. However, it is not always clear how reporting structures for these databases were developed, and many researchers, utilities, and public health officials are generating additional wastewater surveillance data outside of these programs.[15] As such, there is not yet a consensus on what meta-information should accompany these measurements to enable a careful and judicious data interpretation, nor a central and open repository for all wastewater surveillance data, though efforts are underway to develop these resources.[11,16]

Here we provide initial guidance on minimum appropriate meta-information related to infrastructure characteristics, collection and processing procedures, and quantification methods to accompany SARS-CoV-2 wastewater surveillance. We recognize that specific data applications may require additional information depending on the purpose of a research study or surveillance program; however, our objective is that the guidance developed here, using an open community-led format and with input from many ongoing SARS-CoV-2 wastewater surveillance efforts, will advance a more standardized and accessible reporting protocol. This will enable more robust comparisons across studies and create more reusable and interoperable long-term resources for future applications of wastewater surveillance.

Methods

The Research Coordination Network (RCN) on Wastewater Surveillance for SARS-CoV-2 is a U.S. National Science Foundation (NSF) funded initiative launched in August 2020 to advance research and education in the detection and longitudinal monitoring of SARS-CoV-2 and COVID-19 via wastewater analysis. In February 2021, this NSF RCN convened a workshop with the goal of reaching an agreement on the minimum information that must accompany wastewater SARS-CoV-2 measurements for the data to be broadly useful for wastewater-based epidemiology applications. Participants in the workshop were nominated in response to an open call and subsequently selected to represent the wide array of technical backgrounds and expertise that is relevant to wastewater surveillance. The 28 participants represented various professional sectors, including academia, government agencies, private companies, wastewater utilities, public health laboratories, and research institutes, and included international perspective from four countries.

Prior to the workshop, moderators developed a list of 47 possible meta-information variables based on the U.S. Centers for Disease Control & Prevention (CDC) National Wastewater Surveillance System (NWSS)[8] data reporting structure (Table S1†). As there are already well-established community guidelines on necessary data reporting for quantitative PCR (qPCR) and digital PCR (dPCR) – the MIQE[17] and dMIQE[18] guidelines, respectively – variables covered in these guidelines were specifically excluded from the workshop discussion. Using a survey, workshop participants were asked to rank each variable on a 5-point scale from "unnecessary" (1) to "essential" (5) based on the question: "How important is this variable for appropriate interpretation of SARS-CoV-2 wastewater monitoring data?" Participants were also provided the opportunity to suggest additional reporting variables, which resulted in suggestions of 23 new variables (Table S1†). During the workshop, participants were provided with the aggregate rankings of each variable. They were then divided into four groups (wastewater treatment plant & infrastructure, sample collection, sample processing, and target quantification), where groups discussed the preliminary rankings and identified a final set of variables within their category that are essential for interpreting SARS-CoV-2 wastewater surveillance data. Participants were asked to focus on only the minimum meta-information they would require to interpret an unfamiliar dataset and to consider practicality in

measuring or obtaining the data for determining essential variables. Each group then presented the results of their discussion to the full set of workshop participants, explained their rationale, and incorporated contributions from other participants. This resulted in an initial agreement from the entire group of workshop participants. Following the workshop, preliminary variable rankings, discussion group rationale, and notes from workshop discussions were combined by workshop moderators to devise a final set of recommended minimum meta-information, which are described below.

Minimum meta-information for data reporting

Based on variable rankings and discussion during the convened workshop, we recommend a minimum set of information that should be included with SARS-CoV-2 wastewater measurements (Table 1). Here we explain why certain variables were selected and provide recommendations for the level of detail that should be included for each variable.

Wastewater treatment plant & infrastructure

Many wastewater surveillance efforts are focused on sampling at wastewater treatment plants, either at the primary influent or primary sludge locations, because these sites provide community-level coverage, are easily sampled, and are generally well-mixed.[19] However, there are also important applications for wastewater surveillance in sewer regions upstream of treatment plants or at specific facilities or buildings.[20] Regardless of the scale of sampling, it is critical to define the approximate population served and whether the sampling location is a combined or separated sewer system, as stormwater flows in combined sewers can dilute target waste streams and affect data interpretation. Reporting of mean daily flow rates is particularly helpful for estimating the population contributing to a sample, viral loading rates, or infection prevalence in the population. However, measuring or estimating flow rates at upstream sewer sites is often not feasible, and treatment plant influent flow rates are not directly relevant to sampling of primary sludge. We therefore encourage reporting of flow rates when possible but recognize that these data are often not available. While specific GPS coordinates of sample sites would be valuable for cross-referencing with other databases, this level of spatial specificity may lead to privacy concerns in some cases. Therefore, we determined that location information should be reported at the county or municipality level to allow comparison to other public health data while maintaining a degree of sampling anonymity. Wastewater-based surveillance can also be applied to non-sewered waste streams, such as septic tanks, pit latrines, or drainage ditches in areas lacking piped sanitation infrastructure. While our discussion focused specifically on sewered systems, the general scope of variables identified in Table 1 are likely still applicable and could be adapted to data collected from non-sewered systems.

Sample collection

The type of samples collected and the manner in which they are collected are critical for understanding the quality of data that can be obtained from a wastewater sample. The impacts of grab sampling versus composite sampling on resulting data utility are not yet clear,[21] but prior work suggests that daily fluctuations in wastewater flows and possibly defecation rates may impact results.[22–24] Therefore, identifying the type of sample (grab, composite), the duration of compositing, and the sampling date and time are important for data interpretation, as well as comparison to other public health data sources. During workshop discussion, participants noted that sampling dates are not uniformly reported, particularly for composite samples that can span multiple dates. This ambiguity is especially problematic for comparing wastewater-based data to other public health data sets. It is therefore recommended that laboratories collecting composite samples at minimum report the start date and start time of the composite sampling program, as this information, along with the sampling duration, identifies the complete time period captured by the composite sample. Additionally, when reporting the sample matrix collected, it should be noted whether the wastewater was collected after any pre-treatment, such as chlorination or ferric chloride addition, which may impact the results obtained by laboratory analysis of SARS-CoV-2 RNA.[25]

Sample processing

If samples are not processed immediately, SARS-CoV-2 RNA targets may undergo decay during storage, and storage temperature can impact the extent of decay, especially if samples undergo freeze–thaw cycles.[26,27] Laboratories should therefore report the temperature of sample storage prior to processing, including any freeze–thaw cycles. Exact temperatures during sample shipping may not be available, but qualitative shipping conditions (*e.g.*, on ice, dry ice, refrigerated, *etc.*) should still be specified as available. Many different methods can be used to concentrate and isolate viral RNA from wastewater, and it is therefore important to identify the major processing approach and the results of negative processing controls (*i.e.*, extraction blanks). We advocate for the inclusion of a reference to specific concentration and extraction protocols when reporting SARS-CoV-2 wastewater surveillance data, as protocol differences will be important for comparisons across laboratories. In cases where protocols are not yet published in the peer-reviewed literature or defined by a kit manufacturer, open-source resources such as *protocols.io* can be used to document and reference laboratory-specific protocols. While the utility of spiked-in recovery standards is not yet universally agreed upon,[28] and SARS-CoV-2 concentration data can be interpreted without this information, we nonetheless recommend the additional reporting of recovery controls and recovery efficiency when possible to facilitate comparisons between studies, samples, and methods.

Table 1 List of minimum information to accompany measurements of SARS-CoV-2 in wastewater

Category	Variable	Description
Wastewater treatment plant & infrastructure	Sample location type	Primary influent, primary sludge, street line manhole, pump station, septic, on-facility (university campus, correctional facility, *etc.*), other
	Population served	Estimated population contributing to sample location
	Combined or separated system?	Combined, separated, mixed
	Primary county/municipality served	County/municipality, state/province, country
	Flow	Mean daily flow on day(s) of for sample collection. List "N/A" if this information is not available (*e.g.* for sewer or building samples)
Sample collection	Sample collection type	Grab, composite (flow-weighted or time-weighted, including composite duration), other
	Sample matrix	Raw wastewater, pre-treated wastewater (including pre-treatment type), wastewater solids, other
	Sample date	Date (MM/DD/YYYY) of sample collection from sewer system; if composite, composite sampling start date
	Sample time	Time of sample collection from sewer system; if composite, composite sampling start time
Sample processing	Pre-concentration storage temperature	Degrees Celsius (if available), on ice, dry ice, refrigerated, frozen. Specify number of freeze–thaw cycles, if any
	Concentration method & citation	PEG[a] precipitation, ultrafiltration, none, HA filtration, ultracentrifugation, nanotrap beads, other; include protocol citation
	Recovery control name & efficiency	BCoV,[b] BRSV,[c] MHV,[d] OC43,[e] other; include recovery efficiency if a control was used. List "none" if no recovery control was used
	Extraction method & citation	Kit-based (include kit name), TRIzol, MagBead, other; include protocol citation
	Amount of sample processed	Starting volume [mL] or mass [g] of raw sample processed
	Extraction blanks results	Signal not detected, signal detected (% positive), blanks not used
Target quantification	PCR type	qPCR, dPCR, other
	SARS-CoV-2 concentrations	Concentration back-calculated to raw sample volume/mass basis (*e.g.* copies per L wastewater or copies per g dry weight sludge)
	Identification of samples below LOD[f]/LOQ[g]	Flag as below LOD[f] (BLOD) or below LOQ[g] (BLOQ)
	SARS-CoV-2 target gene(s)	Gene target and primers/assay
	Endogenous wastewater control name & concentration	PMMoV,[h] crAssphage, HF183,[i] other; include concentration if measured. List "none" if no endogenous wastewater control was used
	Required MIQE[17]/dMIQE[18] guidelines	Includes specifications for assessing RNA quantification & integrity, reaction conditions, no-template controls, positive controls, assay efficiencies (for qPCR), LOD,[f] LOQ,[g] inhibition testing, and others. See references[17,18] for complete lists

[a] Polyethylene glycol. [b] Bovine coronavirus. [c] Bovine respiratory syncytial virus. [d] Murine hepatitis virus. [e] Human coronavirus OC43. [f] Limit of detection. [g] Limit of quantification. [h] Pepper mild mottle virus. [i] Human *Bacteroides* marker HF183.

Finally, the amount of sample (volume or mass) analyzed has to be specified to enable the calculation of analyte concentrations in sewage and determination of methodological detection limits.

Target quantification

Most current wastewater surveillance efforts rely on reverse transcription qPCR (RT-qPCR) or reverse transcription dPCR (RT-dPCR) to quantify specific SARS-CoV-2 gene targets. As qPCR and dPCR experiments use different strategies for target quantification, it is important to identify the type of PCR used to measure SARS-CoV-2 concentrations and to report the minimal information that has been previously established for these quantification methods (*i.e.*, MIQE[17] and dMIQE[18] standards). These standards include specifications for reporting on no-template controls, positive controls, assay efficiencies, limits of detection and quantification, and inhibition testing, which are all critical for appropriate interpretation of qPCR and dPCR results. For SARS-CoV-2 concentrations, we recommend reporting concentrations in terms of copies per liter of sewage or per gram dry weight of sewage solids without normalization to recovery controls or endogenous wastewater controls. Endogenous wastewater controls are additional targets within a sample that are associated with typical human inputs to sewer systems and may serve as both an indication of fecal matter content and as a recovery control.[29] It is not yet clear if normalization to endogenous wastewater controls improves SARS-CoV-2 RNA data interpretation,[30,31] and a consensus on exactly how recovery or endogenous control data should be used to adjust SARS-CoV-2 gene copy concentrations has not been established. We therefore encourage the reporting of endogenous wastewater controls and their concentrations, including stating if no endogenous controls were evaluated, alongside SARS-CoV-2 RNA concentrations when available. Even if laboratories choose to normalize or adjust data, by specifying that raw concentrations for SARS-CoV-2, recovery controls, and endogenous controls also be reported separately, we believe data reported can be more robust to

changes in analysis strategies. Finally, as is true for any dilute target, methodological limits of detection and quantification can substantially impact data interpretation, and identification of sample measurements that fall below these limits is therefore necessary.

Summary and perspective

The variables described in the previous section and summarized in Table 1 represent a recommendation of the minimum information that should be reported with SARS-CoV-2 wastewater concentration data. Table 1 also generally describes additional variables required by MIQE[17] or dMIQE[18] guidelines, and these guidelines must also be referenced to ensure complete data reporting. Additionally, as the research and knowledge continue to advance, future refinements of the recommendations and inclusion of additional meta-information may be warranted. For example, two types of processing controls — recovery controls and endogenous wastewater controls — are used by many laboratories to monitor method performance and may be useful for comparison across laboratories. We have included these controls in our recommended minimum meta-information to encourage their use and reporting, but also recognize that utility of these controls remains uncertain and data reported without these parameters is still useful for local surveillance with consistent methods. While our discussions focused on PCR methods, the overall structure of variables we have included in this guidance could easily be broadened or adapted to include other types of data.

The final set of minimum required variables in Table 1 is similar to the required variables for reporting in the CDC NWSS database. While the initial set of variables provided during the workshop was based on the CDC NWSS data reporting structure, workshop participants were not provided with any additional information on NWSS data requirements. By arriving at a similar set of variables, our recommendation reinforces existing data standards for wastewater surveillance and provides a useful framework for laboratories to share their data in a way that will improve interoperability across datasets and databases.

Conclusion

We recommend that laboratories include the minimum data listed in Table 1 when reporting SARS-CoV-2 RNA measurements in wastewater, whether for scientific publication or public dashboards. We also encourage laboratories to make their data publicly available whenever feasible, ideally through deposition into public repositories, as this can greatly facilitate efficient technology development and method optimization. Some wastewater surveillance data may be subject to non-disclosure agreements or other sharing restrictions based on privacy concerns, and laboratories should work in collaboration with institutional review boards, health agencies, and other stakeholders to carefully ensure ethical data sharing. The framework provided here is purposefully simple and can be modified to accommodate different circumstances. We also stress that this framework is meant to address the minimum meta-information necessary for reporting only. Additional meta-information is valuable and may indeed be necessary for more complex data applications, such as modeling. Wastewater surveillance is a rapidly developing technique with applications beyond the present COVID-19 pandemic. As academic researchers, government agencies, and private companies continue to innovate and invest in this technology, the framework provided here can serve as a basis for harmonizing data reporting across applications.

Disclaimer

The research presented was not performed or funded by EPA and was not subject to EPA's quality system requirements. The views expressed in this article are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.

Author contributions

J. S. M., A. B. B., R. U. H., K. B., and J. D. V. conceived & designed the study. Z. T. A., C. D., R. G., J. G., R. H. H., M. J., R. S. K., P. K., K. G. K., L. M. L., C. M., S. L. M., K. S. M., C. C. N., A. I. P., S. P., T. S. R., A. S., L. S., J. A. S., B. M. S., P. V., B. W., C. W., J. C. C. W., and A. B. B. ranked variables and contributed to workshop discussion. J. S. M, M. A., L. M. M. G., F. A. R., K. B., and J. D. V. moderated and interpreted workshop discussion. J. S. M. wrote the initial draft. All authors provided critical review and approval of the final draft.

Conflicts of interest

C. D. is an employee of Biobot Analytics, Inc. P. K. is the founder of Venthic Technologies. B. M. S. is an employee of IDEXX Laboratories, Inc. R. U. H. is a cofounder of AquaVitas, LLC and the nonprofit project OneWaterOneHealth of the Arizona State University Foundation.

Acknowledgements

This work was supported by National Science Foundation grants 2038087 (J. S. M., A. B. B., R. U. H., K. B., J. D. V.), 2027752 (K. B.), 2023057 (A. B. B.), 2029025 (J. D. V.), and 2028564 (R. U. H.). We would like to thank Aaron Bivins for contributing to initial variable rankings and Angela Coulliette-Salmond and Wiley Jennings for their contributions to variable rankings and workshop discussions.

References

1 G. Medema, L. Heijnen, G. Elsinga, R. Italiaander and A. Brouwer, Presence of SARS-Coronavirus-2 RNA in sewage

and Correlation with Reported COVID-19 Prevalence in the Early Stage of the epidemic in The Netherlands, *Environ. Sci. Technol. Lett.*, 2020, **7**, 511–516.

2 W. Randazzo, P. Truchado, E. Cuevas-Ferrando, P. Simón, A. Allende and G. Sánchez, SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area, *Water Res.*, 2020, **181**, 115942.

3 W. Ahmed, P. M. Bertsch, N. Angel, K. Bibby, A. Bivins, L. Dierens, J. Edson, J. Ehret, P. Gyawali, K. Hamilton, I. Hosegood, P. Hugenholtz, G. Jiang, M. Kitajima, H. T. Sichani, J. Shi, K. M. Shimko, S. L. Simpson, W. J. M. Smith, E. M. Symonds and K. V. Thomas, DSC, R. Verhagen, J. Zaugg and J. F. Mueller, Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travelers, *J. Travel Med.*, 2020, 1–11.

4 M. Kitajima, W. Ahmed, K. Bibby, A. Carducci, C. P. Gerba, K. A. Hamilton, E. Haramoto and J. B. Rose, SARS-CoV-2 in wastewater: State of the knowledge and research needs, *Sci. Total Environ.*, 2020, **739**, 139076.

5 M. D. Wilkinson, M. Dumontier, Ij. J. Aalbersberg, G. Appleton, M. Axton, A. Baak, N. Blomberg, J.-W. Boiten, L. Bonino da Silva Santos, P. E. Bourne, J. Bouwman, A. J. Brookes, T. Clark, M. Crosas, I. Dillo, O. Dumon, S. Edmunds, C. T. Evelo, R. Finkers, A. Gonzalez-Beltran, A. J. G. Gray, P. Groth, C. Goble, J. S. Grethe, J. Heringa, P. A. C. 't Hoen, R. Hooft, T. Kuhn, R. Kok, J. Kok, S. J. Lusher, M. E. Marone, A. Mons, A. L. Packer, B. Persson, P. Rocca-Serra, M. Roos, R. van Schaik, S.-A. Sansone, E. Schultes, T. Sengstag, T. Slater, G. Strawn, M. A. Swertz, M. Thompson, J. van der Lei, E. van Mulligen, J. Velterop, A. Waagmeester, P. Wittenburg, K. Wolstencroft, J. Zhao and B. Mons, The FAIR Guiding Principles for scientific data management and stewardship, *Sci. Data*, 2016, **3**, 160018.

6 J. Hadfield, C. Megill, S. M. Bell, J. Huddleston, B. Potter, C. Callender, P. Sagulenko, T. Bedford and R. A. Neher, Nextstrain: real-time tracking of pathogen evolution, *Bioinformatics*, 2018, **34**, 4121–4123.

7 T. Bedford, A. L. Greninger, P. Roychoudhury, L. M. Starita, M. Famulare, M. L. Huang, A. Nalla, G. Pepper, A. Reinhardt, H. Xie, L. Shrestha, T. N. Nguyen, A. Adler, E. Brandstetter, S. Cho, D. Giroux, P. D. Han, K. Fay, C. D. Frazar, M. Ilcisin, K. Lacombe, J. Lee, A. Kiavand, M. Richardson, T. R. Sibley, M. Truong, C. R. Wolf, D. A. Nickerson, M. J. Rieder, J. A. Englund, J. Hadfield, E. B. Hodcroft, J. Huddleston, L. H. Moncla, N. F. Müller, R. A. Neher, X. Deng, W. Gu, S. Federman, C. Chiu, J. S. Duchin, R. Gautom, G. Melly, B. Hiatt, P. Dykema, S. Lindquist, K. Queen, Y. Tao, A. Uehara, S. Tong, D. MacCannell, G. L. Armstrong, G. S. Baird, H. Y. Chu, J. Shendure and K. R. Jerome, Cryptic transmission of SARS-CoV-2 in Washington state, *Science*, 2020, **370**, 571–575.

8 *Centers for Disease Control and Prevention*, National Wastewater Surveillance System (NWSS), https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html, (accessed 5 December 2020).

9 *genomeweb*, AquaVitas Partners With HHS, CDC on Wastewater Coronavirus Study, https://www.genomeweb.com/infectious-disease/aquavitas-partners-hhs-cdc-wastewater-coronavirus-study#.YF0WMq9KiUk, (accessed 15 June 2021).

10 European Commission, *Commission Recommendation of 17.3.2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU*, 2021, vol. C.

11 *NORMAN*, NORMAN SCORE Database - SARS-CoV-2 in sewage (SC2S), https://www.norman-network.com/nds/sars_cov_2/, (accessed 25 March 2021).

12 *Canadian Water Network*, COVID-19 Wastewater Coalition, https://cwn-rce.ca/covid-19-wastewater-coalition/, (accessed 25 March 2021).

13 *Water Research Australia*, ColoSSoS: Collaboration on Sewage Surveillance of SARS-CoV-2, https://www.waterra.com.au/research/communities-of-interest/covid-19/, (accessed 25 March 2021).

14 B. A. Kocamemi, H. Kurt, A. Sait, H. Kadi, F. Sarac, I. Aydın, A. M. Saatci and B. Pakdemirli, Routine SARS-CoV-2 wastewater surveillance results in Turkey to follow Covid-19 outbreak, *medRxiv*, 2020, DOI: 10.1101/2020.12.21.20248586.

15 C. C. Naughton, F. A. Roman Jr., A. G. F. Alvarado, A. Q. Tariqi, M. A. Deeming, K. Bibby, A. Bivins, J. B. Rose, G. Medema, W. Ahmed, P. Katsivelis, V. Allan, R. Sinclair, Y. Zhang and M. N. Kinyua, Show us the Data: Global COVID-19 Wastewater Monitoring Efforts, Equity and Gaps, *medRxiv*, 2021, 1–15, DOI: 10.1101/2021.03.14.21253564.

16 *Global Water Pathogens Project*, Wastewater SARS Public Health Environmental REsponse (Wastewater SPHERE): A Global Data Center by GWPP, https://www.waterpathogens.org/about-w-sphere, (accessed 1 April 2021).

17 S. A. Bustin, V. Benes, J. A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M. W. Pfaffl, G. L. Shipley, J. Vandesompele and C. T. Wittwer, The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments, *Clin. Chem.*, 2009, **55**, 611–622.

18 A. S. Whale, W. De Spiegelaere, W. Trypsteen, A. A. Nour, Y.-K. Bae, V. Benes, D. Burke, M. Cleveland, P. Corbisier, A. S. Devonshire, L. Dong, D. Drandi, C. A. Foy, J. A. Garson, H.-J. He, J. Hellemans, M. Kubista, A. Lievens, M. G. Makrigiorgos, M. Milavec, R. D. Mueller, T. Nolan, D. M. O'Sullivan, M. W. Pfaffl, S. Rödiger, E. L. Romsos, G. L. Shipley, V. Taly, A. Untergasser, C. T. Wittwer, S. A. Bustin, J. Vandesompele and J. F. Huggett, The Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020, *Clin. Chem.*, 2020, **66**, 1012–1029.

19 The Water Research Foundation, in *International Water Research Summit on Environmental Surveillance for the Genetic Signal of SARS-CoV-2 in Sewersheds*, 2020, pp. 1–27.

20 F. Hassard, L. Lundy, A. C. Singer, J. Grimsley and M. Di Cesare, Innovation in wastewater near-source tracking for rapid identification of COVID-19 in schools, *Lancet Microbe*, 2021, **2**, e4–e5.

21 D. Gerrity, K. Papp, M. Stoker, A. Sims and W. Frehner, Early-pandemic wastewater surveillance of SARS-CoV-2 in Southern Nevada: Methodology, occurrence, and incidence/prevalence considerations, *Water Res.: X*, 2021, **10**, 100086.

22 C. Ort, M. G. Lawrence, J. Rieckermann and A. Joss, Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: Are your conclusions valid? A critical review, *Environ. Sci. Technol.*, 2010, **44**, 6024–6035.

23 S. D. Supowit, I. B. Roll, V. D. Dang, K. J. Kroll, N. D. Denslow and R. U. Halden, Active Sampling Device for Determining Pollutants in Surface and Pore Water - the In Situ Sampler for Biphasic Water Monitoring, *Sci. Rep.*, 2016, **6**, 21886.

24 K. W. Heaton, J. Radvan, H. Cripps, R. A. Mountford, F. E. M. Braddon and A. O. Hughes, Defecation frequency and timing, and stool form in the general population: A prospective study, *Gut*, 1992, **33**, 818–824.

25 C. Schrader, A. Schielke, L. Ellerbroek and R. Johne, PCR inhibitors - occurrence, properties and removal, *J. Appl. Microbiol.*, 2012, **113**, 1014–1026.

26 B. M. Pecson, E. Darby, C. N. Haas, Y. M. Amha, M. Bartolo, R. Danielson, Y. Dearborn, G. Di Giovanni, C. Ferguson, S. Fevig, E. Gaddis, D. Gray, G. Lukasik, B. Mull, L. Olivas, A. Olivieri and Y. Qu, SARS-CoV-2 Interlaboratory Consortium, Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the U.S., *Environ. Sci.: Water Res. Technol.*, 2021, **7**(3), 504–520.

27 L. V. Parker and C. J. Martel, *Long-Term Survival of Enteric Microorganisms in Frozen Wastewater*, 2002.

28 R. S. Kantor, K. L. Nelson, H. D. Greenwald and L. C. Kennedy, Challenges in Measuring the Recovery of SARS-CoV - 2 from Wastewater, *Environ. Sci. Technol.*, 2021, **55**(6), 3514–3519.

29 K. E. Graham, S. K. Loeb, M. K. Wolfe, D. Catoe, N. Sinnott-Armstrong, S. Kim, K. M. Yamahara, L. M. Sassoubre, L. M. Mendoza Grijalva, L. Roldan-Hernandez, K. Langenfeld, K. R. Wigginton and A. B. Boehm, SARS-CoV-2 RNA in Wastewater Settled Solids Is Associated with COVID-19 Cases in a Large Urban Sewershed, *Environ. Sci. Technol.*, 2021, **55**, 488–498.

30 S. Feng, A. Roguet, J. S. McClary-Gutierrez, R. J. Newton, K. Nathan, J. G. Meiman and S. L. McLellan, Evaluation of sampling frequency and normalization of SARS-CoV-2 wastewater concentrations for capturing COVID-19 burdens in the community, *medRxiv*, 2021, DOI: 10.1101/2021.02.17.21251867.

31 F. Wu, J. Zhang, A. Xiao, X. Gu, W. L. Lee, F. Armas, K. Kauffman, W. Hanage, M. Matus, N. Ghaeli, N. Endo, C. Duvallet, M. Poyet, K. Moniz, A. D. Washburne, T. B. Erickson, P. R. Chai, J. Thompson and E. J. Alm, SARS-CoV-2 Titers in Wastewater Are Higher than Expected from Clinically Confirmed Cases, *mSystems*, 2020, **5**, e00614–e00620.