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Challenges and emerging perspectives of an international SARS-CoV-2 epidemiological surveillance in wastewater

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Abstract: SARS-CoV-2 is a new type of coronavirus capable to infect humans and cause the severe acute respiratory syndrome COVID-19, a disease that has been causing huge impacts across the Earth. COVID-19 patients, including mild, pre-symptomatic and asymptomatic cases, were often seen to contain infectious fragments of SARS-CoV-2 in feces and urine samples. Therefore, studies to detect the new coronavirus in wastewater, which collect and concentrate human excreta, have been extremely useful as a viral tracking tool in communities. This type of monitoring, in addition to serve as a non-invasive early warning of COVID-19 outbreaks, would provide better predictions about the SARS-CoV-2 spread and strongly contribute to maintenance the global health. Although current methods to detect viruses in wastewater, based on molecular RT-PCR and RT-qPCR techniques, were considered as reliable and provided accurate qualitative and quantitative results, they have been facing considerable challenges concerning the SARS-CoV-2 surveillance. In this review, the methods used to detect the SARS-CoV-2 in wastewater and the challenges to implement an international viral monitoring network were described. The article also addressed the emerging perspectives associated with the SARS-CoV-2 epidemiological surveillance in this environment and the importance of a worldwide collaboration to generate and disseminate the detection results.

Key words: Collective health, epidemiology, molecular epidemiology, preventive medicine, public health, sewage monitoring.

INTRODUCTION

SARS-CoV-2 is a new type of coronavirus capable to infect humans and cause the severe acute respiratory syndrome COVID-19, a disease that has been responsible for serious infections in the human respiratory system (Shi et al. 2020). Although a controversial origin, SARS-CoV-2, which phylogenetically belongs to the *Coronaviridae* family, *Betacoronavirus* genus and *Sarbecovirus* subgenus, was firstly detected on December 31 of 2019 in the city of Wuhan, China (Andersen et al. 2020, Tian et al. 2020). According to studies, the SARS-CoV-2 virus has been transmitted through the direct contact with secretions, such as saliva, respiratory droplets, and through aerosol particles transported and dispersed through the air (Correia et al. 2020, Meselson 2020, Morawska & Cao 2020). Reports also indicated the viral transmission by the indirect contact through contaminated surfaces (Enyoh et al. 2020, Mouchtouri et al. 2020, Ong et al. 2020). The best practices to limit the transmission of the disease have been protective measures, such as personal hygiene, use of face masks, eye protectors, surface disinfections, adequate ventilation of closed spaces, physical distancing and immunization by vaccines (Chu et al. 2020, Dagotto et al. 2020, Ding et al. 2021, Fathizadeh et al. 2020, Who 2020a).

COVID-19 disease was seen to cause manifestations in several human systems, including neurological, cardiovascular, visual, renal, immune, musculoskeletal and gastrointestinal (Cipollaro et al. 2020, Diao et al. 2020, Hong et al. 2020, Lin et al. 2020, Pontelli et al. 2020, Zheng et al. 2020, Chen et al. 2021). Symptomatic patients, in general, have indicated clinical symptoms such as taste and smell dysfunctions, fever, dry cough, fatigue, rhinorrhea, dyspnea, lethargy, muscle pain, headache, diarrhea, vomiting and, in some cases, severe pneumonia (Eliezer et al. 2020, Sun et al. 2020, Yang et al. 2020). COVID-19, due to its high spread and lethality rate, was considered as a global health emergency by the World Health Organization in late January 2020 and acquired pandemic proportions in March 2020 (Who 2020b). In the year of 2020, there were 219 countries, areas or territories affected by the COVID-19, approximately 47 500 000 confirmed cases and 1 220 000 deaths (Who 2020c).

COVID-19 patients, including mild, pre-symptomatic and asymptomatic cases were often seen to contain fragments of SARS-CoV-2 in stool and urine samples (Furukawa et al. 2020, Jeong et al. 2020, Jiang et al. 2020, Li et al. 2020, Long et al. 2020, Wei 2020). The fragments, usually viral genome particles that were detected by molecular biology methods, have presumed an active replication of SARS-CoV-2 in those environments (Brönimann et al. 2020, Qian et al. 2020). The presence of viral fragments in those samples, infectious in some cases, has evidenced the possibility of the SARS-CoV-2 transmission through the direct contact or by the aerosols generated by feces and urine of infected patients (Jeong et al. 2020, Kang et al. 2020, Kashi et al. 2020, Parasa et al. 2020, Patel 2020, Van Doorn et al. 2020, Xiao et al. 2020).

The presence of SARS-CoV-2 fragments in stool and urine samples has also brought the importance of the viral detection in wastewater. The first report was made by Medema et al. (2020a), which detected the SARS-CoV-2 in wastewater samples collected on March 4 of 2020 in the Netherlands. Other studies have also indicated the presence of SARS-CoV-2 in archived wastewater samples collected in the respective months of March, November and December of 2019 in Spain, Brazil and Italy, dates prior to the first official case reported in China (Chavarria-Miró et al. 2020, Fongaro et al. 2020, La Rosa et al. 2020a). The findings, although requires detailed studies and verifications (Nikolaenko 2020), corroborated clinical, immunological and molecular evidences that indicated that the SARS-CoV-2 was circulating before the supposed (Apolone et al. 2020, Basavaraju et al. 2020, Birtolo et al. 2020, Deslandes et al. 2020, Gerbaud et al. 2020, Paolo et al. 2020, Valenti et al. 2020, Amendola et al. 2021).

The occurrence of SARS-CoV-2 in wastewater was also reported in samples collected in February of 2020 in England, China and Denmark, and in March of the same year in the United States, France, Japan, Pakistan, Australia and Israel (Jorgensen et al. 2020, Martin et al. 2020, Wang et al. 2020a, Ahmed et al. 2020a, Bar Or et al. 2020, Hata et al. 2020, Sharif et al. 2020, Wu et al. 2020a, Wurtzer et al. 2020). Other studies have indicated the presence of the new coronavirus in wastewater samples collected in April of 2020 in Germany, Greece and the Czech Republic, in May of 2020 in the United Arab Emirates, India, Turkey and Chile, and in July of the same year in Bangladesh (Ahmed et al. 2020b, Ampuero et al. 2020, Arora et al. 2020, Hasan et al. 2020, Kocamemi et al. 2020, Mlejnkova et al. 2020, Petala et al. 2020, Westhaus et al. 2021). The locations and the chronology of the SARS-CoV-2 detections in wastewater were respectably summarized in Table I and shown in Figure 1.

Table I. Reports of SARS-CoV-2 detection in wastewater and sludge.

Country	Approximate location	Sampling date	Reference
Spain	Barcelona	2019/03/12	Chavarria-Miró et al. (2020)
Brazil	Florianópolis, Santa Catalina	2019/11/27	Fongaro et al. (2020)
Italy	Milan, Lombardy and Turin, Piedmont	2019/12/18	La Rosa et al. (2020a)
England	South East Region	2020/02/11	Martin et al. (2020)
China	Hangzhou, Zhejiang	2020/02/19*	Wang et al. (2020a)
Denmark	Solrød, Zealand.	2020/02/24	Jorgensen et al. (2020)
Italy	Milan, Lombardy	2020/02/24	La Rosa et al. (2020b)
Spain	Valencia region	2020/02/24	Randazzo et al. (2020a)
China	Wuhan, Hubei	2020/02/26	Zhang et al. (2020)
United States	Massachusetts	2020/03/03	Wu et al. (2020a)
Netherlands	Den Haag, Zuid-Holland	2020/03/04	Medema et al. (2020a)
France	Paris	2020/03/05	Wurtzer et al. (2020)
United States	Southeastern Virginia	2020/03/11	Gonzalez et al. (2020)
Spain	Murcia	2020/03/12	Randazzo et al. (2020b)
United States	Massachusetts	2020/03/18	Wu et al. (2020b)
Japan	Ishikawa-ken	2020/03/19	Hata et al. (2020)
United States	New Haven, Connecticut	2020/03/19	Peccia et al. (2020)
Pakistan	Quetta district	2020/03/20	Sharif et al. (2020)
Australia	Southeast Queensland	2020/03/27	Ahmed et al. (2020a)
United States	Santa Clara County, California	2020/03/29	Graham et al. (2020)
United States	Bozeman, Montana	2020/03/30	Nemudryi et al. (2020)
Israel	Jerusalem	2020/03/30	Bar Or et al. (2020)
Spain	Ourense, north-western Spain	2020/04/06	Balboa et al. (2020)
United States	Southern Louisiana	2020/04/08	Sherchan et al. (2020)
Germany	North Rhine-Westphalia	2020/04/08	Westhaus et al. (2021)
United States	Detroit, Michigan	2020/04/08	Miyani et al. (2020)
China	Dongxihu district	2020/04/11	Zhao et al. (2020)
Italy	Milano Metropolitan Area	2020/04/14	Rimoldi et al. (2020)
Japan	Yamanashi-ken	2020/04/14	Haramoto et al. (2020)
Brazil	Niterói, Rio de Janeiro	2020/04/15	Prado et al. (2021)
Spain	A Coruña, NW Spain	2020/04/15	Vallejo et al. (2020)
Israel	Hura	2020/04/21	Ali et al. (2020)
Greece	Thessaloniki	2020/04/21	Petala et al. (2020)
Czech Republic	Various Regions	2020/04/26*	Mlejnkova et al. (2020)
United States	Norfolk, Virginia	2020/05/02	Curtis et al. (2020)
United Arab Emirates	Different locations	2020/05/03	Hasan et al. (2020)
India	Jaipur	2020/05/04	Arora et al. (2020)
United States	Syracuse, New York	2020/05/06	Green et al. (2020)
Turkey	Istanbul	2020/05/07	Kocamemi et al. (2020)
France	Montpellier	2020/05/07	Trottier et al. (2020)
India	Ahmedabad, Gujarat	2020/05/08	Kumar et al. (2020)
United States	Houston, Texas	2020/05/11	Stadler et al. (2020)
Israel	Ashkelon	2020/05/17	Yaniv et al. (2020)
Chile	Santiago	2020/05/25	Ampuero et al. (2020)
Japan	Tokyo	2020/07/07	Torii et al. (2021)
India	Hyderabad metropolitan city	2020/07/08	Manupati et al. (2020)
Bangladesh	Noakhali	2020/07/10	Ahmed et al. (2020b)
Pakistan	Lahore, Punjab	2020/07/13	Yaqub et al. (2020)

***Approximate date.**

Analyzes in wastewater, which collect and concentrate human excreta, have often been used to qualitatively or quantitatively monitor the presence of chemical compounds, pollutants and pathogens in communities (Choi et al. 2018, Daughton 2018). The approach has been used as a quick, non-invasive and economical diagnosis to generate near real time information about people's habits and behaviors (Mao et al. 2020a, Sims & Kasprzyk-Hordern 2020). This type of monitoring, so called Wastewater Based Epidemiology (WBE), has provided the understanding of the circulation dynamics of different types of substances and pathogens in populations and has been commonly used as a health surveillance tool (Figure 2) (Orive et al. 2020, Messina 2020).

Wastewater surveillance, when focused on the detection of viral pathogens, such as the SARS-CoV-2, has the potential to enormously contribute to maintain the public health. The approach has been considered as a fast, economical, non-invasive and robust form of viral tracking and epidemiological control (Sodré et al. 2020, Souza et al. 2020, Thompson et al. 2020). The detection of SARS-CoV-2 in wastewater, e.g., would provide better predictions about the spread of COVID-19 in the communities, promote the generation of rapid alerts on emerging and reemerging outbreaks of COVID-19 and the application of better viral containment measures (Bogler et al. 2020, Kitajima et al. 2020, Lodder & De Roda Husman 2020).

Surveillance of SARS-CoV-2 in wastewater would enable the enumeration of people who

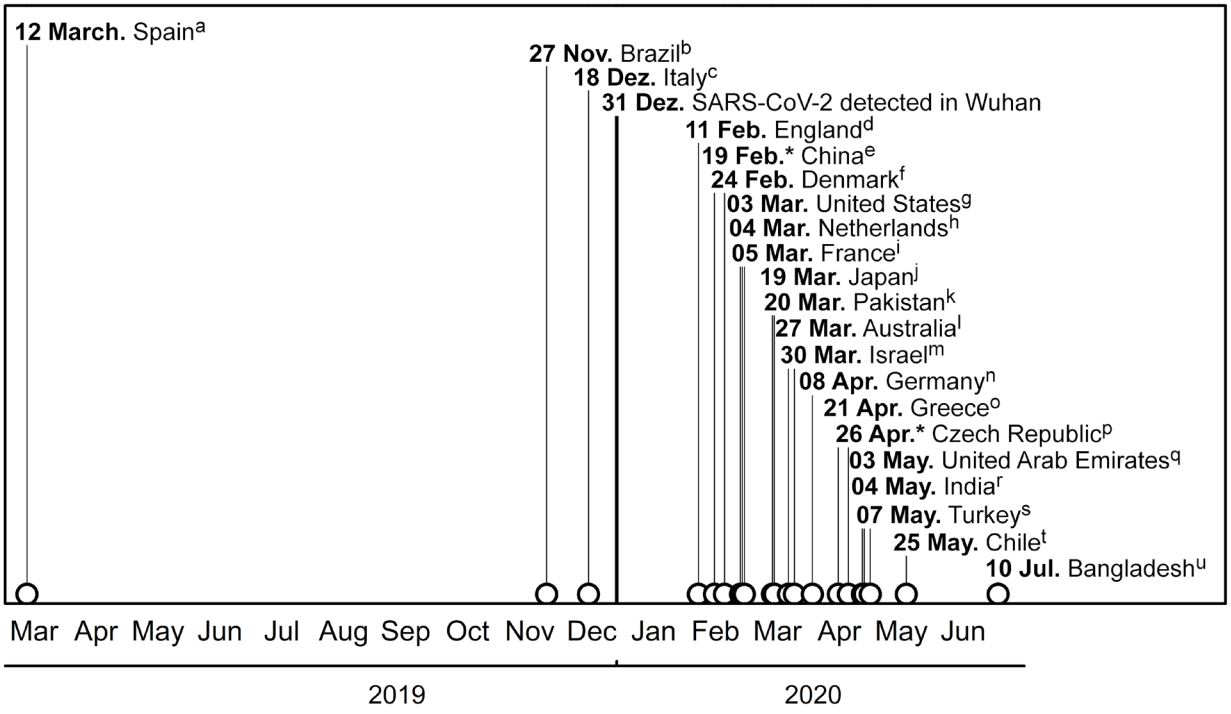

Figure 1. Time course of SARS-CoV-2 detections in wastewater
References: a: Chavarria-Miró et al. (2020), b: Fongaro et al. (2020), c: La Rosa et al. (2020a), d: Martin et al. (2020), e: Wang et al. (2020a), f: Jorgensen et al. (2020), g: Wu et al. (2020a), h: Medema et al. (2020a), i: Wurtzer et al. (2020), j: Hata et al. (2020), k: Sharif et al. (2020), l: Ahmed et al. (2020a), m: Bar Or et al. (2020), n: Westhaus et al. (2021), o: Petala et al. (2020), p: Mlejnkova et al. (2020), q: Hasan et al. (2020), r: Arora et al. (2020), s: Kocamemi et al. (2020), t: Ampuero et al. (2020), u: Ahmed et al. (2020b), *: Approximate date".

do not have access to health care, as well as pre-symptomatic and asymptomatic cases, which are not detected by clinical diagnoses and may still spread the COVID-19 (La Rosa et al. 2020b, Larsen & Wigginton 2020, Lee et al. 2020, Nabi et al. 2020). The approach could be used to assess the genetic diversity of SARS-CoV-2 variants that are circulating in communities, infer a viral ancestry and estimate their prevalence across time and space scales (Crits-Christoph et al. 2020, Izquierdo Lara et al. 2020, Nemudryi et al. 2020). In addition, SARS-CoV-2 surveillance in wastewater could be used to assess the efficiency of viral disinfection systems and coordinate resources to administer vaccines (Bogler et al. 2020, Messina 2020, Sodré et al. 2020, Stadler et al. 2020, Zhang et al. 2020).

In this context, given the importance of promoting studies aimed at better predictions about the SARS-CoV-2 spread in populations and approaches to ensure the maintenance of the global health, this review article aimed to present the procedures used to detect the SARS -CoV-2 in wastewater, and describe the challenges and the emerging perspectives inherent to the epidemiological surveillance of the new coronavirus in this environment.

MATERIALS AND METHODS

The literature review was written based on articles indexed in the Google Scholar, PubMed, ScienceDirect, Web of Science, Scopus and MedRxiv databases. The terms used in the

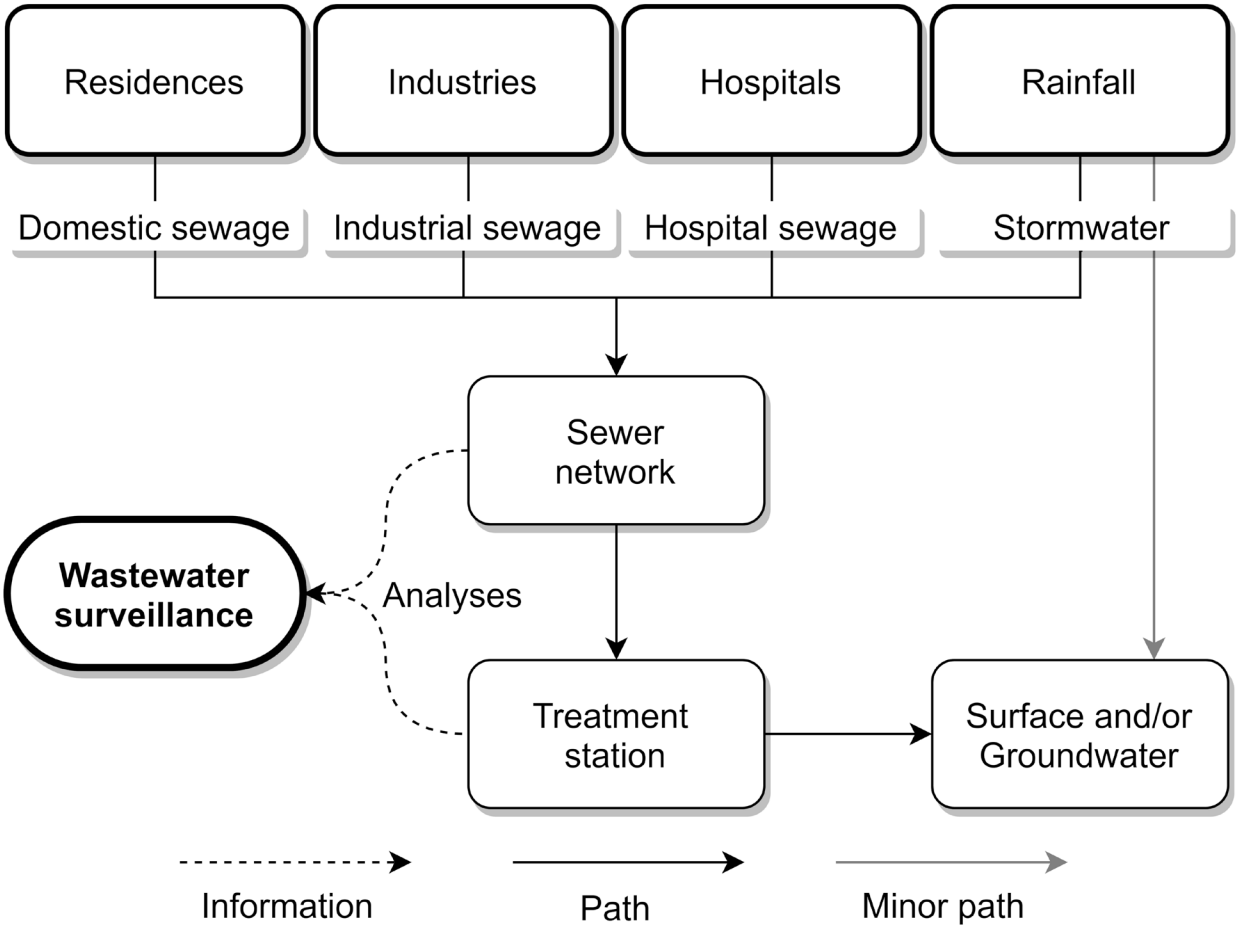

Figure 2. Schematic diagram of wastewater-based epidemiology as a surveillance strategy.

surveys were a combination of "SARS-CoV-2", "presence", "detection", "wastewater", "sewage", "monitoring", "epidemiological", "surveillance", "challenges", "issues", "problems", "emerging perspectives" and "trends". The surveys were conducted until January 31, 2021 and included public open-access and institutional available articles published in English or Portuguese. After the acquirement of the documents, they were screened according to their titles, abstracts and contents in order to eliminate duplicates and verify their adequacy to the theme proposed in this article. Then, the documents were classified according to the following topics: (1) Detection and surveillance of SARS-CoV-2 in wastewater, (2) Methods to detect the SARS-CoV-2 in wastewater, (2a) Sampling, (2b) Processing, (2c) Data interpretation, (2d) Results dissemination, (3) Challenges and issues related to the SARS-CoV-2 surveillance in wastewater and (4) Emerging perspectives related to the SARS-CoV-2 monitoring in wastewater. Afterwards, 180 articles that presented great quality were chosen to be used as the theoretical basis in the preparation of this article and included in the references.

RESULTS AND DISCUSSION

Detection methods of SARS-CoV-2 in wastewater

The methods used to detect the SARS-CoV-2 in wastewater have been made through the use of molecular biology techniques, which enable the copy and the analysis of genetic material fragments of the virus through *in-vitro* replications, called "Polymerase Chain Reaction" (PCR) (Toze 1999). The copies of the SARS-CoV-2 viral genome fragments, which are composed by Ribonucleic acid (RNA) (Rangan et al. 2020), have been initiated with the production of complementary DNA strands (cDNA) from the viral RNA. PCR methods that amplify fragments of RNA, called "Reverse Transcription Polymerase Chain Reaction" (RT-PCR), have been made by the use of a specific enzyme, called Reverse Transcriptase (Corpuz et al. 2020).

Most studies on wastewater samples, however, have been done using real-time RT-PCR methods, called RT-qPCR. Unlike RT-PCR, RT-qPCR methods have enabled the amplification of the nucleotide acids, as well as the simultaneous quantification of the target sequences (Mackay et al. 2002). Another great advantage of RT-qPCR techniques, according to Corpuz et al. (2020), have been the elimination of the agarose gel electrophoresis stage. Real-time RT-PCR analyses, also called quantitative RT-PCR, have been considered as the "gold standard" in the detection of low amounts of genetic material (Hamouda et al. 2021). Depending on the degree of the epidemic, RT-qPCR methods were able to detect concentrations of up to 1.9 copies of SARS-CoV-2 gene particles per 100 mL of wastewater (Ahmed et al. 2020a).

Challenges of an epidemiological surveillance of SARS-CoV-2 in wastewater

Methods that use RT-PCR and RT-qPCR techniques to detect viruses in wastewater, in general, have demonstrated high reliability, specificity and sensitivity. According to reports, they were capable to successfully determine the diversity and abundance of different viruses in wastewater samples, including human Herpesvirus 6 and 8, Salivirus, Hepatitis A and E, Aichi, Noroviruses, Sapovirus, Rotavirus, Zika and Poliovirus (Nakamura et al. 2015, Haramoto et al. 2018, Azhdar et al. 2019, Beyer et al. 2020, McCall et al. 2020, Muirhead et al. 2020). Although the feasibility of the detection techniques was already proven, the achievement of useful information for the epidemiological monitoring of SARS-CoV-2 in wastewater, however, depends

on several relevant factors (Figure 3) (Ahmed et al. 2020c, Medema et al. 2020b).

The methods, firstly, require adequate sampling, preservation and processing procedures (Orive et al. 2020). The detection results have also required appropriate validations and interpretations, made through quality control plans and epidemiological models that cover a large number of variables (Pecson et al. 2020, Medema et al. 2020b). In addition, an effective SARS-CoV-2 surveillance system in wastewater has required specialized laboratories, with modern infrastructures, adequate levels of biosafety and qualified professionals (Orive et al. 2020). In the following topics, the challenges inherent to the implementation of a SARS-CoV-2 epidemiological surveillance system in wastewater were described. The methodological emerging perspectives, which have focused on more accurate estimates of viral incidence and prevalence, and the importance of a global collaboration to generate and disseminate the detection results were also detailed.

Sampling, preservation and transport

The results of the SARS-CoV-2 detection analyzes in wastewater depend on adequate sampling. The proceeding has been crucial to adequately represent the characteristics of an entire population. In the sampling stage, both spatial and temporal resolutions have been considered, including the mode, volume and frequency of the procedure. In general, the researches have been collecting volumes between 50 to 1000 mL of residual water, however, only an aliquot has been used in the RT-qPCR analyzes (Hamouda et al. 2021). Although most of the authors have sampled at specific part of the days, the collections have been preferably made in fixed aliquots collected at defined time intervals during the day. This type of sampling, called 24 h composite, has been used to represent the average characteristics of wastewater during the day, thus, include relevant pulses of wastewater (Foladori et al. 2020).

The collected samples have also required adequate preservation, responsible for conserving the SARS-CoV-2 viability and viral load (Foladori et al. 2020). Although there is

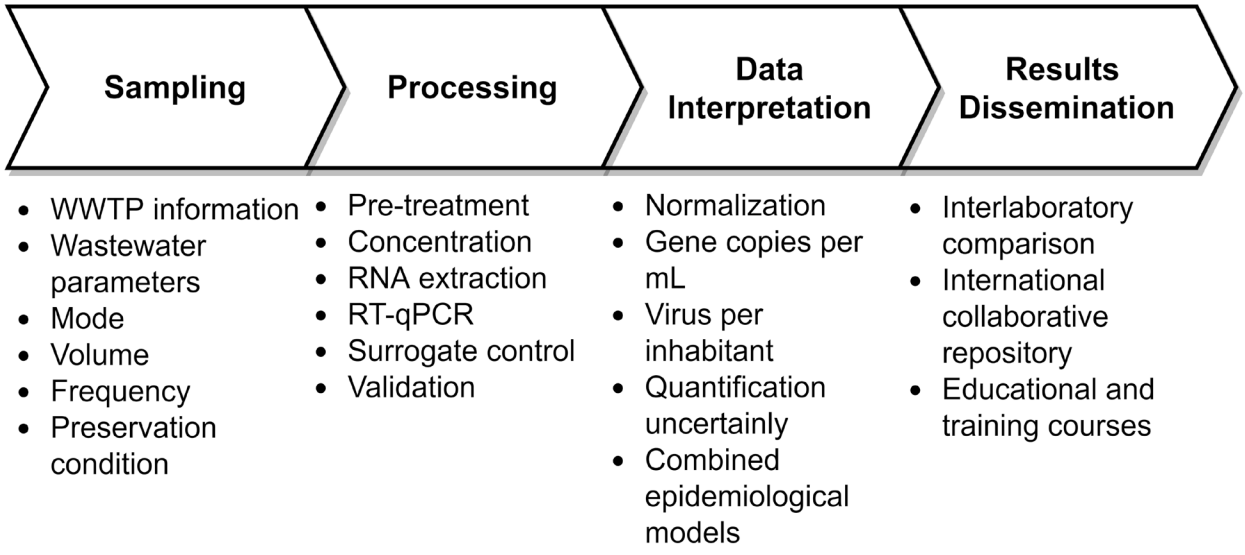

Figure 3. Main steps of a SARS-CoV-2 epidemiological surveillance strategy in wastewater.

no standardized procedure, in general, the samples have been kept at a temperature of 4°C and the analytical procedure done within 2 to 3 days (Ahmed et al. 2020c). Some authors, due to long distances between the sampling site and the laboratory, lack of supplies or lockdowns, have frozen the wastewater samples at temperatures of -80°C until the analytical procedures (Medema et al. 2020b). Freezing and archiving wastewater samples have been widely recommended in order to promote future studies aimed at estimating the spread and the SARS-CoV-2 ancestry (Dolfing 2020).

Concentration, extraction and amplification

The detection results have also required adequate sample processing, which include the steps of concentration, extraction and amplification of the viral RNA (Ahmed et al. 2020c). The concentration step, called primary concentration, has aimed the recovering of the largest amount of viral RNA and removing the impurities from the samples (Rusiñol et al. 2020). Although there is no standardization, in general, it has been preferable methods with great viral recovery efficiency, high repeatability and reproducibility. The methods have also been highly recommended to be as simple, fast and economical as possible (Michael-Kordatou et al. 2020, Lu et al. 2020). According to reports, four main methods have been used to concentrate the SARS-CoV-2: ultrafiltration, precipitation, ultracentrifugation and filtration by electronegative membranes (Hamouda et al. 2021). As a safety measure, SARS-CoV-2 inactivation steps by pasteurization have also been recommended before the sample processing stages (Jorgensen et al. 2020, Pecson et al. 2020, Whitney et al. 2020, Wu et al. 2020b).

The extraction and amplification steps have included the extraction of the genetic material from the concentrated sample, production of cDNA strands from the viral RNA using the reverse transcriptase, and the amplification of the template sequences by RT-qPCR. The extraction stage has been proceeded through the use of commercial kits based on organic extraction techniques, mostly by solutions such as phenol-guanidine isothiocyanate, silica-membrane rotation column or through the use of paramagnetic particles (Michael-Kordatou et al. 2020). The amplification step, in turn, has been using specific gene sequences that detect and copy unique fragments of the SARS-CoV-2 viral genome in the RT-PCR reaction, so called primers.

In general, the amplifications have been made by the use of primers that targeted the viral RNA polymerase gene RdRP, the envelope protein gene E, the nucleocapsid proteins N, N1 and N2, and the spike protein gene S (Corpuz et al. 2020, Foladori et al. 2020). The RT-qPCR reactions, due to their quantitative characteristic, have required hybridization probes, which are DNA sequences marked with fluorescent dyes (reporters), such as 6-carboxyfluorescein (FAM), carboxyrhodamine (ROX) and tetrachlorofluorescein (TET) (Michael-Kordatou et al. 2020). The main primers and hybridization probes used in the SARS-CoV-2 detections in wastewater were indicated on Table II.

The qualities of the kits, primers and the reagents used in the extraction and amplification procedures have significantly influenced the detection results of SARS-CoV-2 in wastewater (Ye et al. 2016, Ahmed et al. 2020c). The methods used in those stages, moreover, were prone to be highly sensitive to the presence of inhibiting compounds in the complex matrix of residual water. The compounds, including metals, fats, proteins, humic and fulvic acids, nucleases, organic and inorganic material, were capable to inhibit the reverse transcription and the PCR

Table II. Main primers and probes used to detect and quantify the SARS-CoV-2 in wastewater.

Target gene	Name	Sequence (5' to 3')	Reference
RdRp	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Corman et al. (2020)
RdRp	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Corman et al. (2020)
RdRp	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Corman et al. (2020)
RdRp	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Corman et al. (2020)
E (envelope protein)	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Corman et al. (2020)
E (envelope protein)	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Corman et al. (2020)
E (envelope protein)	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Corman et al. (2020)
N (nucleocapsid protein)	N_Sarbeco_F	CACATTGGCACCCGCAATC	Corman et al. (2020)
N (nucleocapsid protein)	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Corman et al. (2020)
N (nucleocapsid protein)	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Corman et al. (2020)
N1 (nucleocapsid protein)	2019-nCoV_N1-F	GACCCCAAAATCAGCGAAAT	CDCP (2020)
N1 (nucleocapsid protein)	2019-nCoV_N1-R	TCTGGTTACTGCCAGTTGAATCTG	CDCP (2020)
N1 (nucleocapsid protein)	2019-nCoV_N1-P	FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1	CDCP (2020)
N2 (nucleocapsid protein)	2019-nCoV_N2-F	TTACAAACATTGGCCGCAAA	CDCP (2020)
N2 (nucleocapsid protein)	2019-nCoV_N2-R	GCGCGACATTCCGAAGAA	CDCP (2020)
N2 (nucleocapsid protein)	2019-nCoV_N2-P	FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1	CDCP (2020)
S (spike protein)	WuhanCoV-spk1-f	TTGGCAAAATTCAAGACTCACTTT	Shirato et al. (2020)
S (spike protein)	WuhanCoV-spk2-r	TGTGGTTCATAAAAATTCCTTTGTG	Shirato et al. (2020)
S (spike protein)	NIID_WH-1_F24381	TCAAGACTCACTTTCTTCCAC	Shirato et al. (2020)
S (spike protein)	NIID_WH-1_R24873	ATTTGAAACAAAGACACCTTCAC	Shirato et al. (2020)

FAM: 6-carboxyfluorescein, BBQ: *blackberry quencher*, BHQ-1: *Black Hole Quencher 1.*

reaction (Hjelmsø et al. 2017, Graham et al. 2020, Michael-Kordatou et al. 2020, Sims & Kasprzyk-Hordern 2020). In order to mitigate the inhibitory effects, purification procedures have been used, such as the solvent extraction, cation exchange resins, column chromatography and silica columns, magnetic silica beads and the dilution of the samples (Graham et al. 2020, Jorgensen et al. 2020, Michael-Kordatou et al. 2020).

Interpretation and validation

The viral detection results, when used as an epidemiological surveillance tool, have been based on the assumption that there was a quantitative relationship between the concentration of the SARS-CoV-2 RNA in wastewater and the circulation of the virus in the population (Medema et al. 2020b). The interpretation of those results, therefore, has required the estimation of the size of the population that was contributing for the actual volume of the residual water, and the rationalization of parameters that compensated the variability of non-human inflow of wastewater, like domestic appliances, industrial effluents, stormwater, combined sewage (Mao et

al. 2020a, Medema et al. 2020b). The calculations regarding the size of the contributing population, called *de facto population*, in general, have used exogenous and endogenous markers that propitiated the estimation of the combination of residents, passengers and occasional visitors of a given location (Gracia-Lor et al. 2017).

Exogenous markers, like caffeine, nicotine, artificial sweeteners, pharmaceuticals (atenolol and hydrochlorothiazide) and endogenous markers (linked to human metabolism), like ammonia, 5-hydroxyindoleacetic acid (5-HIAA), coprostanol and creatinine, have been used to estimate the size of a target population and normalize the detection results (Gracia-Lor et al. 2017, Choi et al. 2018, Westhaus et al. 2021). In SARS-CoV-2 studies, researchers have used ubiquitous viruses of human intestinal tracts, such as crAssphage and pepper mild mottle virus (PMMoV) (Polo et al. 2020, D'Aoust et al. 2021, Jafferali et al. 2021). Authors have also used the amount of water, electricity consumption, and data regarding the use of cell phones at the collection time (Medema et al. 2020b). Noteworthy, there is no consensus concerning a standard normalizing indicator and, in general, in order to infer the actual size of a population, the number of viral copies of SARS-CoV-2 has been related to the number of inhabitants registered at the studied site (Medema et al. 2020b).

The quality of the detection results has been validated through the use of positive process controls, generally non-pathogenic viral strains that structurally and morphologically resembled SARS-CoV-2, like bovine coronavirus, *Pseudomonas* bacteriophage φ6, murine hepatitis virus or non-infectious nucleotide sequence Hep G Armored RNA (Ahmed et al. 2020d, Gonzalez et al. 2020, LaTurner et al. 2020, Alygizakis et al. 2021, Torii et al. 2021). The researchers have also conducted confirmatory sequencing of the PCR products, included negative controls that evidenced the presence of false positives and cross-contaminations, besides the addition of multiple replicates that ensured the variability and the efficiency of the detection procedures (Ahmed et al. 2020c, Medema et al. 2020b, Alygizakis et al. 2021).

Viral infectivity

The results of the RT-PCR and RTqPCR tests, based on the detection of SARS-CoV-2 nucleic acids, have not been able to distinguish infectious from non-infectious particles (Maal-Bared et al. 2020). Virological methods, based on *in vitro* cell culture techniques, have been used to provide estimates regarding the number of infectious viruses in wastewater (Rimoldi et al. 2020, Westhaus et al. 2021). In general, the protocols used to characterize the infectivity status of enveloped viruses, such as SARS-CoV-2, have been made by the use of techniques and reagents that could not disrupt the sensitive lipid bilayer that surrounded the virus (Wigginton et al. 2015, Ye et al. 2016). The procedures, although requiring laborious techniques, specialized equipment and laboratories with high degrees of biosafety, have been crucial to assess the risk that a sample poses to animal hosts and human health (Polo et al. 2020).

Emerging perspectives

The perspectives regarding the detection and surveillance of SARS-CoV-2 in wastewater, in general, have aimed at more precise analytical methods, with greater simplicity, practicality, economy and safety. In the sampling stage, according to studies, it has been preferable the use automatic samplers and pragmatic methodological protocols in the selection of the sampling sites and the collecting periods, ensuring accurate calculations and representativeness of the results (Colosi et al.

2020, Yeager et al. 2020, Alygizakis et al. 2021). The genetic materials used in the detection analysis could be obtained from the sludge precipitate of wastewater treatment plants (Balboa et al. 2020, D'aoust et al. 2021). The strategy was responsible for an increase of the SARS-CoV-2 detection sensitivity in comparison to samples collected at the influent of treatment plants (Graham et al. 2020). In addition, researchers have developed methods to extract the viral RNA directly from the sample, hence, reducing the number of steps and the time required for processing the analyzes (Parra Guardado et al. 2020, Whitney et al. 2020).

More accurate techniques for detecting the viral genome, such as the Nested RT-PCR, which uses two sets of primers in two successive polymerase reactions, and the RT digital droplet PCR (RT-ddPCR), which partitions the samples into thousands of nanodroplets through oil-water emulsions, have been successfully applied in the detection of SARS-CoV-2 in wastewater (Alygizakis et al. 2021). The methods, although laborious, expensive and more prone to influences caused by reaction inhibitors, especially in complex matrices such as wastewater, have provided results with greater sensitivity and specificity (Cassinari et al. 2020, Falzone et al. 2020, Wang et al. 2020b, D'aoust et al. 2021). The methods have also provided the confirmation of the quality of the detections, decreased false-negative results, and provided useful information concerning the viral nucleotide variability (Ahmed et al. 2020c, Hata et al. 2020, Martin et al. 2020, Zhou et al. 2020).

The complete SARS-CoV-2 genome sequencing and metagenomic analyzes from the total genetic material of the samples, which use next generation sequencing techniques (NGS), have been considered as promising, capable to perform high resolution genotyping of the predominant strains circulating in a community and to overcome certain RT-PCR limitations, such as the presence of false negatives due to viral nucleotide polymorphisms that can corrupt the primers and probes binding sites (Nemudryi et al. 2020, Sims & Kasprzyk-Hordern 2020, Giri et al. 2021). The approaches have also enabled the detection of new viral strains, the estimation of the ancestry of SARS-CoV-2 strains, the elucidation of their gene products, molecular mechanisms, and their genotype variability across spatial and temporal scales (Wigginton et al. 2015, Haramoto et al. 2020, Izquierdo Lara et al. 2020, Chiara et al. 2021).

Hybrid immunological methods of detecting SARS-CoV-2 structural proteins, based on PCR amplification techniques of sequences attached to specific antibodies, were seen to be highly sensitive and specific. The methods were able to generate satisfactory results even in complex wastewater matrices (Feng et al. 2020, Neault et al. 2020). Fast, transportable and economical detection devices have been developed in order to simplify, improve and reduce the detection costs of SARS-CoV-2. The devices, based on different types of technologies, such as isothermal amplification, isothermal amplification incorporated with CRISPR technology, microfluidic systems and paper-based biosensors, have aimed at the detection of SARS-CoV-2 at the sampling site, without the need to transport material and use centralized laboratories (Corpuz et al. 2020, Feng et al. 2020, Ghernaout & Elboughdiri 2020, Mao et al. 2020b, Ongerth & Danielson 2020, Patel et al. 2020, Giri et al. 2021). The approaches have been able to generate near real time detection results and promote analyzes with greater practicality and efficiency (Bhalla et al. 2020, Tymm et al. 2020). Those devices, although under development, have great potential to be used in the environmental monitoring of SARS-CoV-2

and other pathogen taxa. The techniques could provide a valuable tool for the authorities to assess and act quickly towards epidemic outbreaks (Farkas et al. 2020).

Epidemiological models, crucial in the analysis and prediction of future scenarios, have been mainly constructed by the use of differential equations or stochastic procedures that related the number of copies of SARS-CoV-2 genes detected in wastewater with the number of infected individuals in defined regions of interest (Medema et al. 2020b, Piccolomini & Zama 2020). The models, in order to promote more accurate estimates, have considered the effect of several chemical-physical-biological factors as a function of the SARS-CoV-2 viral load variability, such as flowrate, temperature, pH, presence of other microorganisms, amount of organic matter and chemicals dissolved in the wastewater (Hart & Hadden 2020, Petala et al 2020, Polo et al. 2020, Stadler et al. 2020, Hamouda et al. 2021). The mathematical structures, indeed, have a great potential to provide better estimates about the true number of infected individuals in a population if combined with other epidemiological models, such as serological data, rhinopharyngeal swabs diagnoses, clinical records and hospital admissions (Medema et al. 2020b, Piccolomini & Zama 2020, Kaplan et al. 2021).

Adequation and international collaboration

The methods used to detect and analyze the monitoring data demand specialized laboratories, with modern infrastructures and adequate levels of biosafety. The decentralization of certified laboratories used to carry out detection tests, which in the current situation are mainly centrally located nearby the main capitals, would tend to provide better quality and speed of results (Silva Reis & Santos et al. 2020, Magno et al. 2020). In order to foment a diagnostic service network, it has been advised to use university laboratories, public research institutes and certified third-party laboratories. Considering the high degree of complexity of the analytical procedures, it has also been recommended the qualification of the personnel involved in the monitoring and surveillance tasks (Orive et al. 2020, Magno et al. 2020).

Due to the need to provide sensitive, representative and reproductive results, the methods used to detect SARS-CoV-2 have been advised to follow plans that guarantee the quality of the researches and diagnostics (Huggett et al. 2020). The Quality Assurance Project Plan (QAPP), e.g., have focused on an interlaboratory sharing and comparison of results through the use quality control procedures. The strategy has aimed at covering the entire monitoring process, from collection, handling, sample processing, data management and validation (Pecson et al. 2020). The efforts may support a global collaborative repository of SARS-CoV-2 surveillance in wastewater that could be used to generate comparable results across different geographical and temporal scales (https://www.covid19wbec.org/) (Bivins et al. 2020). The approach has a great potential to contribute in the refinement of wastewater monitoring techniques and to foment a useful global surveillance network (Collivignarelli et al. 2020, Larsen & Wigginton 2020, Michael-Kordatou et al. 2020).

Public health decisions have been advised to include educational and training courses to aware citizens and workers about the SARS-CoV-2's risks and protection measures (Saraiva Soares et al. 2020, Stadler et al. 2020). Sewage networks and wastewater treatment plants, if necessary, must be decentralized and readjusted for better safety, quality of disinfection and waste disposal (Adelodun et al. 2020, Ali et al. 2020, Maal-Bared

et al. 2020, Rollemberg et al. 2020). According to researches, greater investments have been widely recommended in environmental monitoring, water supply and sanitation areas. Moreover, it has been also advised greater commitments by government agencies and policies aimed at public health, which are surely indispensable for better preventive measures and maintenance of a worldwide quality of life (Da Silva Ferreira et al. 2020, Ghernaout & Ghernaout 2020, La Rosa et al. 2020c, Sodré et al. 2020).

CONCLUSION

Due to the fact that patients with COVID-19, including mild, asymptomatic and pre-symptomatic cases, have often been seen to contain infectious fragments of SARS-CoV-2 in stool and urine samples, monitoring the new coronavirus in wastewater, which collect and concentrate human excreta, has shown a great potential to be used as an epidemiological surveillance tool. The approach, called WBE, would provide better predictions about the spread of SARS-CoV-2 and foment the implementation of better viral containment strategies, including rapid alerts concerning possible emerging and reemerging outbreaks of COVID-19. Surveillance of SARS-CoV-2 in wastewater would also propitiate the enumeration of people who do not have access to health care, evaluate the genetic diversity of SARS-CoV-2 variants circulating in communities, the effectiveness of disinfection systems and the allocation of resources to administer vaccines.

An effective SARS-CoV-2 surveillance system in wastewater, however, depends on many relevant factors, including adequate sampling, preservation and processing procedures. Moreover, the detection results have required appropriate interpretations and validations, vital to generate an interlaboratory comparable results and predict future scenarios. In order to promote better viral containment measures, the adequacy of the diagnostic infrastructures, disinfection systems, and qualification of professionals involved in the monitoring and operation of wastewater treatment plants have been strongly recommended. Above all, considering the degree of dangerousness and the uncertainties around the SARS-CoV-2 pandemic, it has been urgently needed greater investments in environmental monitoring, sanitation and water supply sectors, as well as greater commitment from government agencies, public health policies and citizens.

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REFERENCES

ADELODUN B, AJIBADE FO, IBRAHIM RG, BAKARE HO & CHOI K-S. 2020. Snowballing transmission of COVID-19 (SARS-CoV-2) through wastewater: Any sustainable preventive measures to curtail the scourge in low-income countries? Sci Total Environ 742: 140680.

AHMED F ET AL. 2020b. First detection of SARS-CoV-2 genetic material in the vicinity of COVID-19 isolation centre through wastewater surveillance in Bangladesh (preprint). Sci Total Environ 1(776): 145724.

AHMED W ET AL. 2020a. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci Total Environ 728: 138764.

AHMED W ET AL. 2020c. Surveillance of SARS-CoV-2 RNA in wastewater: Methods optimization and quality control are crucial for generating reliable public health information. Curr Opin Environ Sci Health 17: 82-93.

AHMED W ET AL. 2020d. 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.

ALI HA, YANIV K, BAR-ZEEV E, CHAUDHURY S, SHAGA M, LAKKAKULA S, RONEN Z, KUSHMARO A & NIR O. 2020. Tracking SARS-CoV-2 RNA through the wastewater treatment process (preprint). ACS EST Water 1(5): 1161-1167.

ALYGIZAKIS N ET AL. 2021. Analytical methodologies for the detection of SARS-CoV-2 in wastewater: Protocols and future perspectives. TrAC Trends Anal Chem 134: 116125.

AMENDOLA A, BIANCHI S, GORI M, COLZANI D, CANUTI M, BORGHI E, RAVIGLIONE MC, ZUCCOTTI GV & TANZI E. 2021. Evidence of SARS-CoV-2 RNA in an Oropharyngeal Swab Specimen, Milan, Italy, Early December 2019. Emerg Infect Dis 27: 648-650.

AMPUERO M, VALENZUELA S, VALIENTE-ECHEVERRIA F, SOTO-RIFO R, BARRIGA GP, CHNAIDERMAN J, ROJAS C, GUAJARDO-LEIVA S, DIEZ B & GAGGERO A. 2020. SARS-CoV-2 Detection in Sewage in Santiago, Chile - Preliminary results. (preprint). medRxiv 2020.07.02.20145177.

ANDERSEN KG, RAMBAUT A, LIPKIN WI, HOLMES EC & GARRY RF. 2020. The proximal origin of SARS-CoV-2. Nat Med 26: 450-452.

APOLONE G ET AL. 2020. Unexpected detection of SARS-CoV-2 antibodies in the prepandemic period in Italy. Tumori 300891620974755.

ARORA S, NAG A, SETHI J, RAJVANSHI J, SAXENA S, SHRIVASTAVA SK & GUPTA AB. 2020. Sewage surveillance for the presence of SARS-CoV-2 genome as a useful wastewater-based epidemiology (WBE) tracking tool in India. Water Sci Technol 82: 2823-2836.

AZHDAR Z, GHADERI M & MOUSAVI-NASAB SD. 2019. Optimization of RT-qPCR for Detection of Aichi Virus in Sewage and River Water Samples in Karaj, Iran. Arch Iran Med 22: 242-246.

BALBOA S, MAURICIO-IGLESIAS M, RODRIGUEZ S, MARTÍNEZ-LAMAS L, VASALLO FJ, REGUEIRO B & LEMA JM. 2020. The fate of SARS-CoV-2 in WWTPs points out the sludge line as a suitable spot for monitoring. Sci Total Environ 10(772): 145268.

BAR OR I ET AL. 2020. Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance (preprint). medRxiv 2020.04.26.20073569.

BASAVARAJU SV ET AL. 2020. Serologic testing of U.S. blood donations to identify SARS-CoV-2-reactive antibodies: December 2019-January 2020. Clin Infect Dis 72: e1004-e1009.

BEYER S, SZEWZYK R, GNIRSS R, JOHNE R & SELINKA H-C. 2020. Detection and Characterization of Hepatitis E Virus Genotype 3 in Wastewater and Urban Surface Waters in Germany. Food Environ Virol 12: 137-147.

BHALLA N, PAN Y, YANG Z & PAYAM AF. 2020. Opportunities and Challenges for Biosensors and Nanoscale Analytical Tools for Pandemics: COVID-19. ACS Nano 14: 7783-7807.

BIRTOLO LI, MAESTRINI V, SEVERINO P, CHIMENTI C, AGNES G, TOCCI M, COLAIACOMO MC, FRANCONE M, MANCONE M & FEDELE F. 2020. Coronavirus disease 2019 in Rome: was it circulating before December? Journal of Cardiovascular Medicine 21: 835-836.

BIVINS A ET AL. 2020. Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19. Environ Sci Technol 54: 7754-7757.

BOGLER A ET AL. 2020. Rethinking wastewater risks and monitoring in light of the COVID-19 pandemic. Nat Sustain 3: 981-990.

BRÖNIMANN S, REBHAN K, LEMBERGER U, MISRAI V, SHARIAT SF & PRADERE B. 2020. Secretion of severe acute respiratory syndrome coronavirus 2 in urine. Curr Opin Urol 30: 735-739.

CASSINARI K ET AL. 2020. Assessment of multiplex digital droplet RT-PCR as an accurate diagnosis tool for SARS-CoV-2 detection in nasopharyngeal swabs and saliva samples (preprint). Clin Chem 67(5): 736-741.

CDCP - CENTERS FOR DISEASE CONTROL AND PREVENTION. 2020. 2019-Novel Coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html.

CHAVARRIA-MIRÓ G, ANFRUNS-ESTRADA E, GUIX S, PARAIRA M, GALOFRÉ B, SÁANCHEZ G, PINTÓ R & BOSCH A. 2020. Sentinel surveillance of SARS-CoV-2 in wastewater anticipates the occurrence of COVID-19 cases (preprint). medRxiv 2020.06.13.20129627.

CHEN X, LAURENT S, ONUR OA, KLEINEBERG NN, FINK GR, SCHWEITZER F & WARNKE C. 2021. A systematic review of neurological symptoms and complications of COVID-19. J Neurol 268: 392-402.

CHIARA M ET AL. 2021. Next generation sequencing of SARS-CoV-2 genomes: challenges, applications and opportunities. Brief Bioinform 22: 616-630.

CHOI PM ET AL. 2018. Wastewater-based epidemiology biomarkers: Past, present and future. Trends Analyt Chem 105: 453-469.

CHU DK ET AL. 2020. Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. The Lancet 395: 1973-1987.

CIPOLLARO L, GIORDANO L, PADULO J, OLIVA F & MAFFULLI N. 2020. Musculoskeletal symptoms in SARS-CoV-2 (COVID-19) patients. J Orthop Surg Res 15: 1-7.

COLLIVIGNARELLI MC, COLLIVIGNARELLI C, CARNEVALE MIINO M, ABBÀ A, PEDRAZZANI R & BERTANZA G. 2020. SARS-CoV-2 in sewer systems and connected facilities. Process Saf Environ Prot 143: 196-203.

COLOSI LM ET AL. 2020. Development of wastewater pooled surveillance of SARS-CoV-2 from congregate living settings (preprint). medRxiv 2020.10.10.20210484.

CORMAN VM ET AL. 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 25: 1-8.

CORPUZ MVA, BUONERBA A, VIGLIOTTA G, ZARRA T, BALLESTEROS F, CAMPIGLIA P, BELGIORNO V, KORSHIN G & NADDEO V. 2020. Viruses in wastewater: occurrence, abundance and detection methods. Sci Total Environ 745: 140910.

CORREIA G, RODRIGUES L, GAMEIRO DA SILVA M & GONÇALVES T. 2020 Airborne route and bad use of ventilation systems as non-negligible factors in SARS-CoV-2 transmission. Med Hypotheses 141: 109781.

CRITS-CHRISTOPH A ET AL. 2020. Genome sequencing of sewage detects regionally prevalent SARS-CoV-2 variants. mBio 12(1): e02703-027020.

CURTIS K, KEELING D, YETKA K, LARSON A & GONZALEZ R. 2020. Wastewater SARS-CoV-2 Concentration and Loading Variability from Grab and 24-Hour Composite Samples (preprint). medRxiv 2020.07.10.20150607.

D'AOUST PM ET AL. 2021. Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. Water Res 188: 116560.

DA SILVA FERREIRA AD, PEREIRA PIMENTEL C, MOSCON A, NEVES CURTY T & DUTRA DE OLIVEIRA M. 2020. Sars-cov-2 no esgoto: métodos de detecção e tratamento. Ifes Ciência 6: 15-22.

DAGOTTO G, YU J & BAROUCH DH. 2020. Approaches and Challenges in SARS-CoV-2 Vaccine Development. Cell Host Microbe 28: 364-370.

DAUGHTON CG. 2018. Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM). Sci Total Environ 619: 748-764.

DESLANDES A, BERTI V, TANDJAOUI-LAMBOTTE Y, ALLOUI C, CARBONNELLE E, ZAHAR JR, BRICHLER S & COHEN Y. 2020. SARS-CoV-2 was already spreading in France in late December 2019. Int J Antimicrob Agents 55: 106006.

DIAO B ET AL. 2020. Human Kidney is a Target for Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection. Nat Commun 12: 2506.

DING Z ET AL. 2021. Toilets dominate environmental detection of severe acute respiratory syndrome coronavirus 2 in a hospital. Sci Total Environ 753: 141710.

DOLFING J. 2020. The Importance of Sewage Archiving in Coronavirus Epidemiology and Beyond. Environ Sci Technol 54: 7740-7741.

ELIEZER M, HAUTEFORT C, HAMEL A-L, VERILLAUD B, HERMAN P, HOUDART E & ELOIT C. 2020. Sudden and Complete Olfactory Loss of Function as a Possible Symptom of COVID-19. JAMA Otolaryngol Head Neck Surg 146: 674.

ENYOH CE ET AL. 2020. Indirect exposure to novel coronavirus (SARS-CoV-2): an overview of current knowledge. J Teknol Lab 9: 67-77.

FALZONE L, MUSSO N, GATTUSO G, BONGIORNO D, PALERMO C, SCALIA G, LIBRA M & STEFANI S. 2020. Sensitivity assessment of droplet digital PCR for SARS-CoV-2 detection. Int J Mol Med 46: 957-964.

FARKAS K, MANNION F, HILLARY LS, MALHAM SK & WALKER DI. 2020. Emerging technologies for the rapid detection of enteric viruses in the aquatic environment. Curr Opin Environ Sci Health 16: 1-6.

FATHIZADEH H, MAROUFI P, MOMEN-HERAVI M, DAO S, KÖSE Ş, GANBAROV K, PAGLIANO P, ESPOSITO S & KAFIL HS. 2020. Protection and disinfection policies against SARS-CoV-2 (COVID-19). Infez Med 28: 185-191.

FENG W ET AL. 2020. Molecular Diagnosis of COVID-19: Challenges and Research Needs. Anal Chem 92: 10196-10209.

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.

FONGARO G ET AL. 2020. SARS-CoV-2 in human sewage in Santa Catalina, Brazil, November 2019 (preprint). Sci Total Environ 15(778): 146198.

FURUKAWA NW, BROOKS JT & SOBEL J. 2020. Evidence Supporting Transmission of Severe Acute Respiratory

Syndrome Coronavirus 2 While Presymptomatic or Asymptomatic. Emerg Infect Dis 26: e201595.

GERBAUD L, GUIGUET-AUCLAIR C, BREYSSE F, ODOUL J, OUCHCHANE L, PETERSCHMITT J, DEZFOULI-DESFER C & BRETON V. 2020. Hospital and Population-Based Evidence for COVID-19 Early Circulation in the East of France. IJERPH 17: 7175.

GHERNAOUT D & ELBOUGHDIRI N. 2020. Environmental Engineering for Stopping Viruses Pandemics. OALib 07: 1-17.

GHERNAOUT D & GHERNAOUT B. 2020. Controlling COVID-19 Pandemic through Wastewater Monitoring. OALib 07: 1-20.

GIRI B, PANDEY S, SHRESTHA R, POKHAREL K, LIGLER FS & NEUPANE BB. 2021. Review of analytical performance of COVID-19 detection methods. Anal Bioanal Chem 413: 35-48.

GONZALEZ R, CURTIS K, BIVINS A, BIBBY K, WEIR MH, YETKA K, THOMPSON H, KEELING D, MITCHELL J & GONZALEZ D. 2020. COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. Water Res 186: 116296.

GRACIA-LOR E ET AL. 2017. Measuring biomarkers in wastewater as a new source of epidemiological information: Current state and future perspectives. Environ Int 99: 131-150.

GRAHAM K ET AL. 2020. SARS-CoV-2 in wastewater settled solids is associated with COVID-19 cases in a large urban sewershed. Environ Sci Technol 55(1): 488-498.

GREEN H, WILDER M, MIDDLETON FA, COLLINS M, FENTY A, GENTILE K, KMUSH B, ZENG T & LARSEN DA. 2020. Quantification of SARS-CoV-2 and cross-assembly phage (crAssphage) from wastewater to monitor coronavirus transmission within communities (preprint). medRxiv 2020.05.21.20109181.

HAMOUDA M, MUSTAFA F, MARAQA M, RIZVI T & ALY HASSAN A. 2021. Wastewater surveillance for SARS-CoV-2: Lessons learnt from recent studies to define future applications. Sci Total Environ 759: 143493.

HARAMOTO E, KITAJIMA M, HATA A, TORREY JR, MASAGO Y, SANO D & KATAYAMA H. 2018. A review on recent progress in the detection methods and prevalence of human enteric viruses in water. Water Res 135: 168-186.

HARAMOTO E, MALLA B, THAKALI O & KITAJIMA M. 2020. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Sci Total Environ 737: 140405.

HART OE & HALDEN RU. 2020. Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges. Sci Total Environ 730: 138875.

HASAN SW, IBRAHIM Y, DAOU M, KANNOUT H, JAN N, LOPES A, ALSAFAR H & YOUSEF AF. 2020. Detection and quantification of SARS-CoV-2 RNA in wastewater and treated effluents: Surveillance of COVID-19 epidemic in the United Arab Emirates. Sci Total Environ 142929.

HATA A, HONDA R, HARA-YAMAMURA H & MEUCHI Y. 2020. Detection of SARS-CoV-2 in wastewater in Japan by multiple molecular assays-implication for wastewater-based epidemiology (WBE). Sci Total Environ 758: 143578.

HJELMSØ MH ET AL. 2017. Evaluation of Methods for the Concentration and Extraction of Viruses from Sewage in the Context of Metagenomic Sequencing. PLoS ONE 12: e0170199.

HONG N, YU W, XIA J, SHEN Y, YAP M & HAN W. 2020. Evaluation of ocular symptoms and tropism of SARS-CoV-2 in patients confirmed with COVID-19. Acta Ophthalmol 98.

HUGGETT JF ET AL. 2020. Cautionary Note on Contamination of Reagents Used for Molecular Detection of SARS-CoV-2. Clin Chem 66: 1369-1372.

IZQUIERDO LARA RW ET AL. 2020. Monitoring SARS-CoV-2 circulation and diversity through community wastewater sequencing. Emerg Infect Dis 27(5): 1405-1415.

JAFFERALI MH, KHATAMI K, ATASOY M, BIRGERSSON M, WILLIAMS C & CETECIOGLU Z. 2021. Benchmarking virus concentration methods for quantification of SARS-CoV-2 in raw wastewater. Sci Total Environ 755: 142939.

JEONG HW ET AL. 2020. Viable SARS-CoV-2 in various specimens from COVID-19 patients. Clin Microbiol Infect 26: 1520-1524.

JIANG X, LUO M, ZOU Z, WANG X, CHEN C & QIU J. 2020. Asymptomatic SARS-CoV-2 infected case with viral detection positive in stool but negative in nasopharyngeal samples lasts for 42 days. J Med Virol 92: 1807-1809.

JORGENSEN AU, GAMST J, HANSEN LV, KNUDSEN IIH & JENSEN SKS. 2020. Eurofins Covid-19 Sentinel TM Wastewater Test Provide Early Warning of a potential COVID-19 outbreak (preprint). medRxiv 2020.07.10.20150573.

KANG M ET AL. 2020. Probable Evidence of Fecal Aerosol Transmission of SARS-CoV-2 in a High-Rise Building. Ann Intern Med M20-0928.

KAPLAN EH, WANG D, WANG M, MALIK AA, ZULLI A & PECCIA J. 2021. Aligning SARS-CoV-2 indicators via an epidemic model: application to hospital admissions and RNA

detection in sewage sludge. Health Care Manag Sci 24: 320-329.

KASHI AH, DE LA ROSETTE J, AMINI E, ABDI H, FALLAH-KARKAN M & VAEZJALALI M. 2020. Urinary Viral Shedding of COVID-19 and its Clinical Associations: A Systematic Review and Meta-analysis of Observational Studies. Urol J 17: 433-441.

KITAJIMA M, AHMED W, BIBBY K, CARDUCCI A, GERBA CP, HAMILTON KA, HARAMOTO E & ROSE JB. 2020. SARS-CoV-2 in wastewater: State of the knowledge and research needs. Sci Total Environ 739: 139076.

KOCAMEMI BA, KURT H, SAIT A, SARAC F, SAATCI AM & PAKDEMIRLI B. 2020. SARS-CoV-2 Detection in Istanbul Wastewater Treatment Plant Sludges (preprint). medRxiv 2020.05.12.20099358.

KUMAR M, PATEL AK, SHAH AV, RAVAL J, RAJPARA N, JOSHI M & JOSHI CG. 2020. First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. Sci Total Environ 746: 141326.

LA ROSA G, BONADONNA L, LUCENTINI L, KENMOE S & SUFFREDINI E. 2020c. Coronavirus in water environments: Occurrence, persistence and concentration methods - A scoping review. Water Res 179: 115899.

LA ROSA G, IACONELLI M, MANCINI P, BONANNO FERRARO G, VENERI C, BONADONNA L, LUCENTINI L & SUFFREDINI E. 2020b. First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci Total Environ 736: 139652.

LA ROSA G, MANCINI P, BONANNO FERRARO G, VENERI C, IACONELLI M, BONADONNA L, LUCENTINI L & SUFFREDINI E. 2020a. SARS-CoV-2 has been circulating in northern Italy since December 2019: evidence from environmental monitoring (preprint). medRxiv 2020.06.25.20140061.

LARSEN DA & WIGGINTON KR. 2020. Tracking COVID-19 with wastewater. Nat Biotechnol 38: 1151-1153.

LATURNER ZW ET AL. 2020. Evaluating recovery, cost, and throughput of different concentration methods for SARS-CoV-2 wastewater-based epidemiology. Water Res 197: 117043.

LEE S ET AL. 2020. Clinical Course and Molecular Viral Shedding Among Asymptomatic and Symptomatic Patients With SARS-CoV-2 Infection in a Community Treatment Center in the Republic of Korea. JAMA Intern Med 180: 1447.

LI W ET AL. 2020. Virus shedding dynamics in asymptomatic and mildly symptomatic patients infected with SARS-CoV-2. Clin Microbiol Infect 26: 1556.e1-1556.e6.

LIN L ET AL. 2020. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. Gut 69: 997-1001.

LODDER W & DE RODA HUSMAN AM. 2020. SARS-CoV-2 in wastewater: potential health risk, but also data source. Lancet Gastroenterol Hepatol 5: 533-534.

LONG Q-X ET AL. 2020. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 26: 1200-1204.

LU D, HUANG Z, LUO J, ZHANG X & SHA S. 2020. Primary concentration – The critical step in implementing the wastewater based epidemiology for the COVID-19 pandemic: A mini-review. Sci Total Environ 747: 141245.

MAAL-BARED R ET AL. 2020. Implications of SARS-CoV-2 on current and future operation and management of wastewater systems. Water Environ Res 93: 502-515.

MACKAY IM. 2002. Real-time PCR in virology. Nucleic Acids Research 30: 1292-1305.

MAGNO L, ROSSI TA, MENDONÇA-LIMA FW, SANTOS CC, CAMPOS GB, MARQUES LM, PEREIRA M, PRADO NM & DOURADO I. 2020. Desafios e propostas para ampliação da testagem e diagnóstico para COVID-19 no Brasil. Ciênc saúde coletiva 25: 3355-3364.

MANUPATI H, KIRAN U, KUNCHA SK, KOPPERI H, GOKULAN CG & MISHRA RK. 2020. Comprehensive Surveillance of SARS-CoV-2 Spread Using Wastewater-based Epidemiology Studies (preprint). medRxiv 2020.08.18.20177428.

MAO K, ZHANG H & YANG Z. 2020b. Can a Paper-Based Device Trace COVID-19 Sources with Wastewater-Based Epidemiology? Environ Sci Technol 54: 3733-3735.

MAO K, ZHANG K, DU W, ALI W, FENG X & ZHANG H. 2020a. The potential of wastewater-based epidemiology as surveillance and early warning of infectious disease outbreaks. Curr Opin Environ Sci Health 17: 1–7.

MARTIN J, KLAPSA D, WILTON T, ZAMBON M, BENTLEY E, BUJAKI E, FRITZSCHE M, MATE R & MAJUMDAR M. 2020. Tracking SARS-CoV-2 in Sewage: Evidence of Changes in Virus Variant Predominance during COVID-19 Pandemic. Viruses 12: 1144.

MCCALL C, WU H, MIYANI B & XAGORARAKI I. 2020. Identification of multiple potential viral diseases in a large urban center using wastewater surveillance. Water Res 184: 116160.

MEDEMA G, BEEN F, HEIJNEN L & PETTERSON S. 2020b. Implementation of environmental surveillance for SARS-CoV-2 virus to support public health decisions: Opportunities and challenges. Curr Opin Environ Sci Health 17: 49-71.

MEDEMA G, HEIJNEN L, ELSINGA G, ITALIAANDER R & BROUWER A. 2020a. 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 7: 511-516.

MESELSON M. 2020. Droplets and Aerosols in the Transmission of SARS-CoV-2. N Engl J Med 382: 2063-2063.

MESSINA S. 2020. Monitoring Human Waste. Voices in Bioethics Vol 6 (2020).

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.

MIYANI B, FONOLL X, NORTON J, MEHROTRA A & XAGORARAKI I. 2020. SARS-CoV-2 in Detroit Wastewater. J Environ Eng 146: 06020004.

MLEJNKOVA H, SOVOVA K, VASICKOVA P, OCENASKOVA V, JASIKOVA L & JURANOVA E. 2020. Preliminary Study of Sars-Cov-2 Occurrence in Wastewater in the Czech Republic. IJERPH 17: 5508.

MORAWSKA L & CAO J. 2020. Airborne transmission of SARS-CoV-2: The world should face the reality. Environ Int 139: 105730.

MOUCHTOURI VA, KOUREAS M, KYRITSI M, VONTAS A, KOURENTIS L, SAPOUNAS S, RIGAKOS G, PETINAKI E, TSIODRAS S & HADJICHRISTODOULOU C. 2020. Environmental contamination of SARS-CoV-2 on surfaces, air-conditioner and ventilation systems. Nt J Hyg Environ Health 230: 113599.

MUIRHEAD A, ZHU K, BROWN J, BASU M, BRINTON MA, COSTA F, HAYAT MJ & STAUBER CE. 2020. Zika Virus RNA Persistence in Sewage. Environ Sci Technol Lett 7: 659-664.

NABI G, SIDDIQUE R & KHAN S. 2020. Detecting viral outbreaks in future using enhanced environmental surveillance. Environ Res 188: 109731.

NAKAMURA T, HAMASAKI M, YOSHITOMI H, ISHIBASHI T, YOSHIYAMA C, MAEDA E, SERA N & YOSHIDA H. 2015. Environmental surveillance of poliovirus in sewage water around the introduction period for inactivated polio vaccine in Japan. Appl Environ Microbiol 81: 1859-1864.

NEAULT N ET AL. 2020. SARS-CoV-2 Protein in Wastewater Mirrors COVID-19 Prevalence. (preprint). medRxiv 2020.09.01.20185280.

NEMUDRYI A, NEMUDRAIA A, WIEGAND T, SURYA K, BUYUKYORUK M, CICHA C, VANDERWOOD KK, WILKINSON R & WIEDENHEFT B. 2020. Temporal Detection and Phylogenetic Assessment of SARS-CoV-2 in Municipal Wastewater. Cell Reports Medicine 1: 100098.

NIKOLAENKO D. 2020. SARS-CoV-2 and the water environment: discovery of the pathogen in the sample dated March 12, 2019 in Barcelona and its interpretation (preprint). Environ Epidemiol 10.13140/RG.2.2.31298.40649.

ONG SWX, TAN YK, CHIA PY, LEE TH, NG OT, WONG MSY & MARIMUTHU K. 2020. Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient. JAMA 323: 1610.

ONGERTH JE & DANIELSON RE. 2020. RT qLAMP--Direct Detection of SARS-CoV-2 in Raw Sewage (preprint). medRxiv 2020.10.01.20205492.

ORIVE G, LERTXUNDI U & BARCELO D. 2020. Early SARS-CoV-2 outbreak detection by sewage-based epidemiology. Sci Total Environ 732: 139298.

PAOLO C, CARMELO S & MARCELLO M. 2020. Ageusia, gastrointestinal symptoms and marked asthenia in late December. A single case report with positive SARS-Cov2 IgG in Italy. Int J Infect Dis 97: 352-353.

PARASA S ET AL. 2020. Prevalence of Gastrointestinal Symptoms and Fecal Viral Shedding in Patients With Coronavirus Disease 2019: A Systematic Review and Meta-analysis. JAMA Netw Open 3: e2011335.

PARRA GUARDADO AL, SWEENEY CL, HAYES E, TRUEMAN BF, HUANG Y, JAMIESON RC, RAND JL, GAGNON GA & STODDART AK. 2020. Development and optimization of a new method for direct extraction of SARS-CoV-2 RNA from municipal wastewater using magnetic beads (preprint). medRxiv 2020.12.04.20237230.

PATEL J. 2020. Viability of SARS-CoV-2 in faecal bio-aerosols. Colorectal Dis 22: 1022-1022.

PATEL KP, VUNNAM SR, PATEL PA, KRILL KL, KORBITZ PM, GALLAGHER JP, SUH JE & VUNNAM RR. 2020. Transmission of SARS-CoV-2: an update of current literature. Eur J Clin Microbiol Infect Dis 39: 2005-2011.

PECCIA J ET AL. 2020. SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics (preprint). medRxiv 2020.05.19.20105999.

PECSON BM ET AL. 2020. 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 7: 504-520.

PETALA M ET AL. 2020. A physicochemical model for rationalizing SARS-CoV-2 concentration in sewage. Case study: The city of Thessaloniki in Greece. Sci Total Environ 142855.

PICCOLOMINI EL & ZAMA F. 2020. Monitoring Italian COVID-19 spread by an adaptive SEIRD model (preprint). medRxiv 2020.04.03.20049734.

POLO D, QUINTELA-BALUJA M, CORBISHLEY A, JONES DL, SINGER AC, GRAHAM DW & ROMALDE JL. 2020. Making waves: Wastewater-based epidemiology for COVID-19 – approaches and challenges for surveillance and prediction. Water Res 186: 116404.

PONTELLI MC ET AL. 2020. Infection of human lymphomononuclear cells by SARS-CoV-2 (preprint). bioRxiv 2020.07.28.225912.

PRADO T ET AL. 2021. Wastewater-based epidemiology as a useful tool to track SARS-CoV-2 and support public health policies at municipal level in Brazil. Water Res 191: 116810.

QIAN Q, FAN L, LIU W, LI J, YUE J, WANG M, KE X, YIN Y, CHEN Q & JIANG C. 2020. Direct Evidence of Active SARS-CoV-2 Replication in the Intestine. Clin Infect Dis 73: 361-366.

RANDAZZO W, CUEVAS-FERRANDO E, SANJUÁN R, DOMINGO-CALAP P & SÁNCHEZ G. 2020a. Metropolitan wastewater analysis for COVID-19 epidemiological surveillance. Int J Hyg Environ Health 230: 113621.

RANDAZZO W, TRUCHADO P, FERRANDO EC, SIMON P, ALLENDE A & SANCHEZ G. 2020b. SARS-CoV-2 RNA titers in wastewater anticipated COVID-19 occurrence in a low prevalence area (preprint). medRxiv 2020.04.22.20075200.

RANGAN R, ZHELUDEV IN, HAGEY RJ, PHAM EA, WAYMENT-STEELE HK, GLENN JS & DAS R. 2020. RNA genome conservation and secondary structure in SARS-CoV-2 and SARS-related viruses: a first look. RNA 26: 937-959.

RIMOLDI SG ET AL. 2020. Presence and vitality of SARS-CoV-2 virus in wastewaters and rivers. Sci Total Environ 744: 140911.

ROLLEMBERG S, BARROS AN DE & LIMA JPM DE. 2020. Avaliação da contaminação, sobrevivência e remoção do coronavírus em sistemas de tratamento de esgoto sanitário. REVTEC 41.

RUSIÑOL M, MARTÍNEZ-PUCHOL S, FORÉS E, ITARTE M, GIRONES R & BOFILL-MAS S. 2020. Concentration methods for the quantification of coronavirus and other potentially pandemic enveloped virus from wastewater. Curr Opin Environ Sci Health 17: 21-28.

SARAIVA SOARES AF, NUNES BCR, COSTA FCR, SILVA LF DE M, SOUZA & SOUZA LP. 2020. Potencialidades da epidemiologia baseada em esgoto nas ações da Atenção Primária à Saúde em tempos de pandemia pela COVID-19. J Manag Prim Health Care 12: 1-10.

SHARIF S ET AL. 2020. Detection of SARS-Coronavirus-2 in wastewater, using the existing environmental surveillance network: An epidemiological gateway to an early warning for COVID-19 in communities. PLoS ONE 16(6): e0249568.

SHERCHAN SP, SHAHIN S, WARD LM, TANDUKAR S, AW TG, SCHMITZ B, AHMED W & KITAJIMA M. 2020. First detection of SARS-CoV-2 RNA in wastewater in North America: A study in Louisiana, USA. Sci Total Environ 743: 140621.

SHI Y, WANG G, CAI X, DENG J, ZHENG L, ZHU H, ZHENG M, YANG B & CHEN Z. 2020. An overview of COVID-19. J Zhejiang Univ Sci B 21: 343-360.

SHIRATO K ET AL. 2020. Development of Genetic Diagnostic Methods for Detection for Novel Coronavirus 2019(nCoV-2019) in Japan. Jpn J Infect Dis 73: 304-307.

SILVA REIS AA & SANTOS R DA S. 2020. O padrão ouro no diagnóstico molecular na COVID19: O que sabemos sobre a soberania deste método? BJHR 3: 5986-5992.

SIMS N & KASPRZYK-HORDERN B. 2020. Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level. Environ Int 139: 105689.

SODRÉ F, BRANDÃO C, VIZZOTTO C & MALDANER A. 2020. Epidemiologia do esgoto como estratégia para monitoramento comunitário, mapeamento de focos emergentes e elaboração de sistemas de alerta rápido para covid-19. Quím Nova 43: 515-519.

SOUZA LP, SOARES AFS, NUNES BCR, COSTA FCR & SILVA LF DE M. 2020. Presença do novo coronavírus (SARS-CoV-2) nos esgotos sanitários: apontamentos para ações complementares de vigilância à saúde em tempos de pandemia. Visa em Debate 8: 132-138.

STADLER LB ET AL. 2020. Wastewater Analysis of SARS-CoV-2 as a Predictive Metric of Positivity Rate for a Major Metropolis (preprint). medRxiv 2020.11.04.20226191.

SUN P, QIE S, LIU Z, REN J, LI K & XI J. 2020. Clinical characteristics of hospitalized patients with SARS-CoV-2 infection: A single arm meta-analysis. J Med Virol 92: 612-617.

THOMPSON JR, NANCHARAIAH YV, GU X, LEE WL, RAJAL VB, HAINES MB, GIRONES R, NG LC, ALM EJ & WUERTZ S. 2020. Making waves: Wastewater surveillance of SARS-CoV-2

for population-based health management. Water Res 184: 116181.

TIAN H ET AL. 2020. An investigation of transmission control measures during the first 50 days of the COVID-19 epidemic in China. Science 368: 638-642.

TORII S, FURUMAI H & KATAYAMA H. 2021. Applicability of polyethylene glycol precipitation followed by acid guanidinium thiocyanate-phenol-chloroform extraction for the detection of SARS-CoV-2 RNA from municipal wastewater. Sci Total Environ 756: 143067.

TOZE S. 1999. PCR and the detection of microbial pathogens in water and wastewater. Water Res 33: 3545-3556.

TROTTIER J, DARQUES R, AIT MOUHEB N, PARTIOT E, BAKHACHE W, DEFFIEU MS & GAUDIN R. 2020. Post-lockdown detection of SARS-CoV-2 RNA in the wastewater of Montpellier, France. One Health 10: 100157.

TYMM C, ZHOU J, TADIMETY A, BURKLUND A & ZHANG JXJ. 2020. Scalable COVID-19 Detection Enabled by Lab-on-Chip Biosensors. Cel Mol Bioeng 13: 313-329.

VALENTI L ET AL. 2020. SARS-CoV-2 seroprevalence trends in healthy blood donors during the COVID-19 Milan outbreak (preprint). medRxiv 2020.05.11.20098442.

VALLEJO JA ET AL. 2020. Highly predictive regression model of active cases of COVID-19 in a population by screening wastewater viral load (preprint). medRxiv 2020.07.02.20144865.

VAN DOORN AS, MEIJER B, FRAMPTON CMA, BARCLAY ML & DE BOER NKH. 2020. Systematic review with meta-analysis: SARS-CoV-2 stool testing and the potential for faecal-oral transmission. Aliment Pharmacol Ther 52: 1276-1288.

WANG F, PERVAIZ U, TIAN H, MARIAM AOAO, HAMID MAM & WANG D. 2020b. A Novel Primer Probe Set for Detection of SARS-CoV-2 by Sensitive Droplet Digital PCR (preprint). medRxiv 2020.11.03.20224972.

WANG J, FENG H, ZHANG S, NI Z, NI L, CHEN Y, ZHUO L, ZHONG Z & QU T. 2020a. SARS-CoV-2 RNA detection of hospital isolation wards hygiene monitoring during the Coronavirus Disease 2019 outbreak in a Chinese hospital. Int J Infect Dis 94: 103-106.

WEI WE. 2020. Presymptomatic Transmission of SARS-CoV-2 — Singapore, January 23–March 16, 2020. MMWR Morb Mortal Wkly Rep 69.

WESTHAUS S ET AL. 2021. Detection of SARS-CoV-2 in raw and treated wastewater in Germany – Suitability for COVID-19 surveillance and potential transmission risks. Sci Total Environ 751: 141750.

WHITNEY ON ET AL. 2020. Sewage, Salt, Silica and SARS-CoV-2 (4S): An economical kit-free method for direct capture of SARS-CoV-2 RNA from wastewater (preprint). medRxiv 2020.12.01.20242131.

WIGGINTON KR, YE Y & ELLENBERG RM. 2015. Emerging investigators series: the source and fate of pandemic viruses in the urban water cycle. Environ Sci: Water Res Technol 1: 735-746.

WORLD HEALTH ORGANIZATION. 2020a. Water, sanitation, hygiene, and waste management for SARS-CoV-2, the virus that causes COVID-19: interim guidance, 29 July 2020. World Health Organ WHO/2019-nCoV/IPC_WASH/2020.4.

WORLD HEALTH ORGANIZATION. 2020b. WHO Director-General's opening remarks at the media briefing on COVID-19-11 March 2020. Geneva: World Health Organ: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.

WORLD HEALTH ORGANIZATION. 2020c. WHO Coronavirus Disease (COVID-19) Dashboard. World Health Organ https://covid19.who.int/.

WU F ET AL. 2020a. SARS-CoV-2 titers in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases (preprint). medRxiv 2020.06.15.20117747.

WU F ET AL. 2020b. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases (preprint). medRxiv 2020.04.05.20051540.

WURTZER S, MARECHAL V, MOUCHEL J-M, MADAY Y, TEYSSOU R, RICHARD E, ALMAYRAC JL & MOULIN L. 2020. Evaluation of lockdown impact on SARS-CoV-2 dynamics through viral genome quantification in Paris wastewaters (preprint). medRxiv 2020.04.12.20062679.

XIAO F, SUN J, XU Y, LI F, HUANG X, LI H, ZHAO JINGXIAN HUANG J & ZHAO J. 2020. Infectious SARS-CoV-2 in Feces of Patient with Severe COVID-19. Emerg. Infect Dis 26: 1920-1922.

YANG X ET AL. 2020. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 8: 475-481.

YANIV K ET AL. 2020. City-level SARS-CoV-2 sewage surveillance (preprint). medRxiv 2020.10.19.20215244.

YAQUB T ET AL. 2020. A longitudinal survey for genome-based identification of SARS-CoV-2 in sewage water in selected lockdown areas of Lahore city, Pakistan; a potential approach for future smart lockdown strategy (preprint). medRxiv 2020.07.31.20165126.

YE Y, ELLENBERG RM, GRAHAM KE & WIGGINTON KR. 2016. Survivability, Partitioning, and Recovery of Enveloped Viruses in Untreated Municipal Wastewater. Environ Sci Technol 50: 5077-5085.

YEAGER RA, HOLM RH, SAURABH K, FUQUA JL, TALLEY D, BHATNAGAR A & SMITH T. 2020. Wastewater sample site selection to estimate geographically resolved community prevalence of COVID-19: A research protocol (preprint). medRxiv 2020.08.23.20180224.

ZHANG D ET AL. 2020. Potential spreading risks and disinfection challenges of medical wastewater by the presence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral RNA in septic tanks of Fangcang Hospital. Sci Total Environ 741: 140445.

ZHAO L ET AL. 2020. First study on surveillance of SARS-CoV-2 RNA in wastewater systems and related environments in Wuhan: Post-lockdown (preprint). medRxiv 2020.08.19.20172924.

ZHENG Y-Y, MA Y-T, ZHANG J-Y & XIE X. 2020. COVID-19 and the cardiovascular system. Nat Rev Cardiol 17: 259-260.

ZHOU B ET AL. 2020. SARS-CoV-2 spike D614G variant confers enhanced replication and transmissibility (preprint). bioRxiv 2020.10.27.357558.

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