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An opinion on Wastewater-Based Epidemiological Monitoring (WBEM) with Clinical Diagnostic Test (CDT) for detecting high-prevalence areas of community COVID-19 infections

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Abstract

Wastewater-Based Epidemiological Monitoring (WBEM) is an efficient surveillance tool during the COVID-19 pandemic as it meets all requirements of a complete monitoring system including early warning, tracking the current trend, prevalence of the disease, detection of genetic diversity as well as the upsurging SARS-CoV-2 new variants with mutations from the wastewater samples. Subsequently, Clinical Diagnostic Test (CDT) is widely acknowledged as the global gold standard method for disease monitoring, despite several drawbacks such as high diagnosis cost, reporting bias, and the difficulty of tracking asymptomatic patients (silent spreaders of the COVID-19 infection who manifest no symptoms of the disease). In this current review and opinion-based study, we first propose a combined approach for detecting COVID-19 infection in communities using wastewater and clinical sample testing, which may be feasible and effective as an emerging public health tool for the long-term nationwide surveillance system. The viral concentrations in wastewater samples can be used as indicators to monitor ongoing SARS-CoV-2 trends, predict asymptomatic carriers, and detect COVID-19 hotspot areas, while clinical samples help in detecting mostly symptomatic individuals for isolating positive cases in communities and validate WBEM protocol for mass vaccination including booster doses for COVID-19.

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Keywords
SARS-CoV-2, Wastewater-based surveillance, Wastewater-based epidemiological monitoring, Clinical diagnostic test, Genetic diversity, New variants and mutations, Mass vaccination, Booster doses, Wastewater and clinical samples.

Abbreviations
WBEM, Wastewater-Based Epidemiological Monitoring; WBE, Wastewater-Based Epidemiology; WBS, Wastewater-Based Surveillance; CDT, Clinical Diagnostic Test; WS, Wastewater Sample; CS, Clinical Sample; CT, Cycle Threshold; WWTP/WTP, Wastewater Treatment Plants.

Introduction

The current ongoing coronavirus disease-2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has wreaked havoc on global economy, businesses, communities, and public health due to widespread infection, with 43.7 million confirmed cases and 1.17 million deaths in 218 countries as of March 31, 2022 (WHO, COVID-19 Dashboard). Two major obstacles in managing SARS-CoV-2 rapid infections are related to the difficulties in identifying infected people who are asymptomatic for preventing the spread of viral infections in the communities [1,2]. Over the past 18 months, the SARS-CoV-2 has diversified through multiple new mutations and various genetic variants such as Alpha (B.1.1.7) with seven, Beta (B.1.351) with nine, Gamma (P.1) with 12, Delta (B.1.6, B.1.6.2) with 17 new mutations in spike protein gene. The latest Omicron (B.1.1.529) and Neocov variants have been discovered and propagated into different parts of the world as variants of interest and concern [3–5]. These altered genetic factors increased transmissibility, virulence, disease severity, and mortalities while they also decreased the effectiveness of current therapeutics and vaccines [6–8].

Since the beginning of the COVID-19 pandemic, the standard clinical diagnostic testing (CDT) has been a recognized, valid system for monitoring infectious diseases like COVID-19 with some negative sides [9], which relies on the patient's signs and symptoms. As an alternative to individual tracking, wastewater-based epidemiology (WBE) has been used on a wide scale across the world [10–12] to monitor the prevalence of COVID-19 patients. Despite the simplicity of wastewater sampling and transportation on time, viral RNA concentration and extraction are very difficult for low RNA quantities [13,14]. Hence, it is very crucial to establish a unified system incorporating CDT and WBEM to identify infected individuals with COVID-19 hotspots while discovering new variants and mutations, monitoring the current pandemic scenario, followed by anticipating future waves [15]. CDT and WBEM can be used synergistically to track local COVID-19 epidemics where clinical samples will be used to identify the SARS-CoV-2 symptomatic patients and WBEM will be optimized as a validated method for further analyses of wastewater released into communal drains from individual household drains and public places (e.g. bus and rail stands, airports, rivers, and market) [16–20]. As a result, adapting the collective approach combining WBEM and CDT could relieve burdens on the public health system, while it assists in making informed decisions for better and proper timely treatments, receiving vaccines, or booster doses of vaccines for COVID-19.

Based on our experience, it is worth mentioning that continuing WBEM without proper sanitation systems is strenuous, especially for low-middle income countries or non-WASH (Water, Sanitation, and Hygiene) countries [1,21,22]. However, adopting a combined approach can be the best model for both the developing and developed world [25].

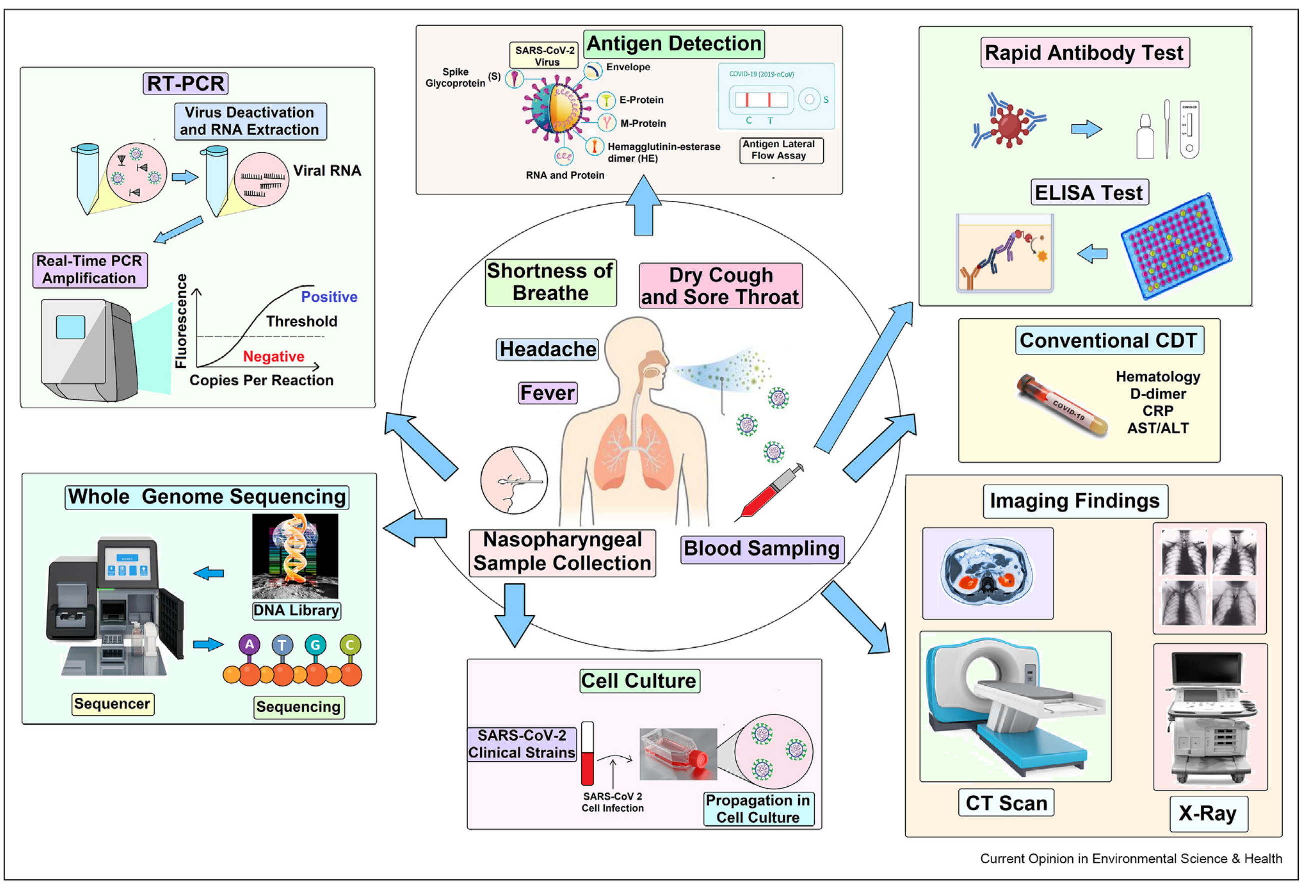
In developing countries, WBEM of COVID-19 is more challenging without CDT, as the majority of households are not connected to sewerage systems. The CDT for SARS-CoV-2 can detect the viral genetic markers of the viral RNA in-between 7–14 days following the exposure and are unable to detect asymptomatic individuals (silent spreaders of COVID-19) within the communities [26,27]. WBEM is an approach for tracking the pandemic through the identification of severely infected areas (COVID-19 hotspot zone) and monitoring of the infection trends [24,27]. However, the recovery of the genetic biomarkers of the SARS-CoV-2 viral RNA in wastewater is very challenging due to differential stability in sewage streams, various environmental factors such as rainfall and temperature, as well as the presence of inhibitory substances (Ribonuclease Enzyme-RNase) [29,30]. As a result, performing a well-structured surveillance combining both CDT and WBEM for symptomatic, asymptomatic, and paucisymptomatic carriers would allow early detection of new variants, and be of potential help for advancing the process of vaccine development. From our recently completed 30 days follow-up on the quantitative analyses of SARS-CoV-2 genetic materials in wastewater from the residence of a positive patient family [118], the number of SARS-CoV-2 positive patients were lowest when the CT value was high (lowest gene copy number) in wastewater samples. On the other hand, when the number of positive patients increased the corresponding CT value was low (the highest copy number) in sewage samples as detected in the same study. In addition, increased signals of the SARS-CoV-2 genetic biomarkers were noticed earlier in WS compared to the viral load in clinical samples of the positive patients.

There are limited studies that link the concentration of SARS-CoV-2 viral biomarkers in wastewater with the identification of clinical cases in a specific residential area lacking wastewater treatment plants in developing countries [31–33]. The combined CDT and WBEM follow-up study was performed in our laboratory to determine the relationship between the positive cases of SARS-CoV-2 infections and their discharged wastewater viral loads from one single house enrolling the entire family members' clinical sample in a developing country without having a proper sewage system. The research findings demonstrated that a wastewater sample monitoring system tailored to a specific location could be established as a tool to identify SARS-CoV-2 infection and complement the clinical testing. This review emphasizes that the combined monitoring of SARS-CoV-2 using CDT and WBE systems can guide the way forward for effective surveillance of the prevalence of infectious disease such as COVID-19.

Clinical diagnostic test (CDT) and Wastewater-based Epidemiologic Monitoring (WBEM)

The accurate and rapid clinical diagnostic tests are essential for identifying the SARS-CoV-2 positive cases, contact tracing, and making public health decisions [9]. Clinical testing is a conventional method for monitoring the status of COVID-19. The clinical signs and

Figure 1



Clinical diagnostic tests for COVID-19 patients include general clinical signs and symptoms, diagnostic imaging, and laboratory markers [38]. In vitro, diagnostic, and clinical laboratory tests include molecular techniques e.g., viral antigen detection, antibody tests; nucleic acid amplification tests (NAAT); real-time polymerase chain reaction test (RT-PCR); next-generation sequencing (NGS); cell culture; enzyme-linked immunosorbent assay (ELISA); and traditional clinical tests: neutrophil-lymphocyte ratio (NLR); c-reactive protein test (CRP); erythrocyte sedimentation rate test (ESR); IL-6/interleukin-6; lactate dehydrogenase test (LDH); aspartate aminotransferase test (AST); alanine aminotransferase test (ALT); imaging method**:** computed tomography (CT); ultrasound sonography (USG), X-ray.

symptoms of a patient such as fever, dry cough, headache, and shortness of breath usually develop 2–14 days after the exposure to SARS-CoV-2 [5,25] (Supplementary Table ST 1). The CDT is recommended for the diagnosis of any diseases based on the patient's specific signs and symptoms [35] (Figure 1, Supplementary Figure SF1). However, the maximum COVID-19 positive individuals are asymptomatic [36,37], and clinical data may be limited due to testing capacity, reagent cost, laboratory facilities with proper instruments, expert hands, and availability issues [81,120,121].

Community wastewater can be used to identify and observe COVID-19 infection scenarios in the same area, in the same manner, that was previously used for the eradication of poliovirus, and this is recognized as the first application of wastewater-based epidemiological (WBE) investigations [39]. The SARS-CoV-2 genetic markers have been identified in feces from pre-symptomatic persons even 1–5 days before the positive clinical test [29,30] and in people with mild signs and symptoms [41]. Recent investigations of a few WBE studies have detected COVID-19 patients before the onset of clinical symptoms from feces samples, and 48–67% of diseased people had SARS-CoV-2 viral RNA in their stool, which survives in wastewater and can last up to >33 days [41–44]. Previous findings showed an association between wastewater viral concentration and COVID-19 confirmed cases where SARS-CoV-2 viral RNA were between 2.0 and 6.0 $\log_{10}$ gc/L (genomic copies per liter), which is similar to our recent research findings [36,38,41]. In addition, the accuracy of WBE was found to be reasonably good in many studies [35–38]. Betancourt et al. [46] found that WBEM had a sensitivity of 76.0%, specificity of 90.7%, positive and negative predictive value of 79.8%, and 88.6%, respectively, when findings of wastewater samples were compared with clinical samples [46]. Furthermore, according to previous studies, the prevalence of SARS-CoV-2 RNA biomarkers in stool was higher (48.1%) as compared to the swab samples of the patients detected with gastrointestinal symptoms (17%) [42]. Although WBEM is capable to detect SARS-CoV-2 RNA genetic biomarkers for monitoring the pandemic, there is an ongoing debate over how wastewater data should be used and to what extent the approaches are useful to public health decisions [45,47,118].

As the COVID-19 pandemic continues, individual clinical diagnostic testing (CDT) did not represent itself as a holistic approach to community health status determination. One major concern with the COVID-19 pandemic is that in most cases in the United States, patients were generally asymptomatic and pre-symptomatic, allowing infected people to spread the virus as healthy carriers [47]. Moreover, a significant percentage of COVID-19 survivors might still be carrying and shedding the virus [48]. Hence, in addition to the clinical test, wastewater surveillance should be used together with clinical data to infer the average virus-shedding patterns at a population level [49,50]. Figure 2 depicts a high-level overview of the WBEM system from sample collection to interpretation of results. The selection of WS collection points plays an important role in representing a particular catchment area (wastewater treatment plants, sewer drains, primary networking system or communal watershed, river course, bus stand, and airport) [22]. Heat treatment (60 °C, 30 min), filtration (to remove large particles), or chemical treatment with NaOCl can be used for sample processing and disintegrating the viruses [19]. Several methods are already used in various studies for concentrating viral biomarkers like polyethylene glycol (PEG), ultrafiltration, ultracentrifugation, centrifugation, or skim milk procedure [51]. Viral nucleic acid can be extracted in the laboratory manually using TRIzol reagent or commercially available Qiagen, Thermo Fisher kits [52]. For calculating viral copy number maximum studies have used the equations 1 and 2 [51, 53].

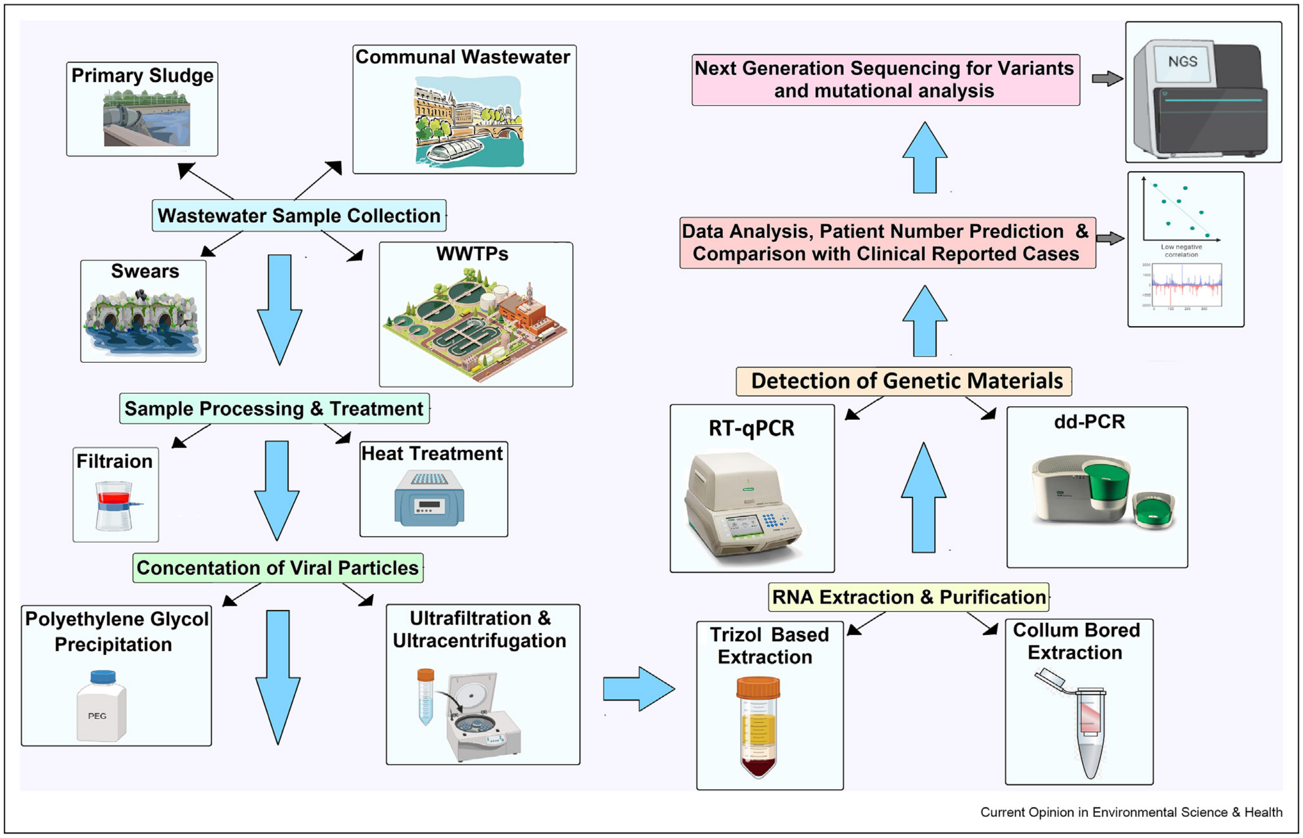
$$\text{Number of infected individuals} = \frac{\frac{\text{RNA Copies}}{\text{Water}\,(L)} \times \frac{\text{Water}\,(L)}{\text{day}}}{\frac{\text{feces}\,(g)}{\text{day}} \times \frac{\text{RNA Copies}}{\text{feces}\,(g)}} \tag{1}$$

$$\text{Number of infected individuals} = \frac{\text{No. of RNA Copies per L}}{\text{Contribution of RNA Copies per person to total sewage water (L)}} \tag{2}$$

Positive, negative, no-template, and extraction controls should be used with standard curve calculation as well as PCR inhibitors should be checked according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) rules [54]. Major roles and drawbacks of both WBEM and CDT are given in Table 1. Considering the previous research outcomes based on WBEM and CDT results with various validated methods and comparisons of the two approaches, we suggest the following for disease burden correlation: i) analysis of the trends in WBEM data and comparison with the clinical diagnostic test data; ii) observing the effectiveness of the interventions with the declining number of patients; and iii) detection of hotspot areas with COVID-19 cases for vaccination and booster doses [55].

One previous study in Massachusetts between March and May 2020 found similar trends of the abundance of

Figure 2



Overview of WBEM procedure. Wastewater sample collection and processing, viral RNA concentration, nucleic acid/RNA extraction from the concentrated samples, interpretation of RT-PCR results, and monitoring the trend of disease outbreak compared with CDT and Whole Genome Sequencing (WGS) for new variants and mutations.

SARS-CoV-2 RNA biomarkers in wastewater with the number of affected patients [56]. Another study in Utah used 9-week wastewater sampling and found a link between a community outbreak and an increase in SARS-CoV-2 RNA [57]. The SARS-CoV-2 virus concentrations in wastewater samples in Ottawa, Canada surged by more than 400% just 48 h after a 300% or greater rise in detected cases [58], and in Utah showed a strong link between community outbreaks and an increase in SARS-CoV-2 RNA in wastewater [57]. Environmental parameters are also linked to SARS-CoV-2 genetic materials, as evidenced by an increase in wastewater temperature resulting from a decrease in viral gene copy numbers [2].

Examples of combined wastewater-based monitoring with clinical diagnostic tests

The previous WBS studies have found a direct correlation between CDT-confirmed COVID-19 cases and wastewater SARS-CoV-2 viral concentration [68,69]. Various findings reflected how SARS-CoV-2 WBEM provided early warnings in the population analyzed, and detected viral RNA in WS before CDT [65–67]. Viral RNA was found in wastewater samples in Milan, Italy few days after the first confirmed COVID-19 patient by clinical test [70], in Australia (Brisbane), when there were hundreds of clinical cases [71]; in Japan (Yamanashi Prefecture), when clinical test results were at their peak [72]; and in Spain (Murcia), when the COVID-19 cases were the least in the Iberian Peninsula [73] (Table 2). The detection of SARS-CoV-2 RNA in the wastewater treatment plant was also reported initially in Louisiana, USA [61], Gujarat, India [74], Dubai [75], Gothenburg, Sweden [76], and in the Southeast England of the United Kingdom [77]. Medema et al. (2021) [78] successfully detected SARS-CoV-2 viral RNA from the city wastewater in the Netherlands six days before the first confirmed clinical case [79], and

Table 1

A comparison of the major advantages and drawbacks of CDT and WBEM.

Parameters	Advantages	Disadvantages	Reference
CDT	• High sensitivity and specificity. • Rapid testing method. • Determination of new variants. • Simple, safe, and cost-effective. • Antigen and nucleic acid amplification methods are more suitable for the early and accurate detection of acute infection with COVID-19. • Antibody detection methods play an important role in seroprevalence analysis, allowing countries to estimate the rate of exposure and take. • Precautionary measures to handle waves of the pandemic which are useful for epidemiologic purposes.	• Not applicable for asymptomatic patients. • Test kits are costly. • Cross-reactivity and false positive results. • The sensitivity of antibody tests for the detection of active infection is highly variable and not suitable for the early detection of COVID-19. • Limitation in handling pandemic situations using RT-PCR technique as the standard test. • Expensive equipment, well-trained staff, and equipped laboratories are required.	[59–63]
WBEM	• Cost-effective. • Useful to track COVID-19 hotspots, an estimate of the number of patients, and disease trends in communities. • Serve as an early warning tool, signaling the presence of infected people in a certain community or within a specified wastewater treatment plant (WWTP) catchment region. • Can be used to back-calculate chemical exposure or usage, as well as the prevalence of infection, with the use of appropriate models.	• Quantifying different viruses and viral nucleic acid biomarkers in wastewater is notoriously challenging. • Concentration of viral RNA biomarkers in WS is influenced by rainfall, industrial inputs, substances that could degrade viruses (detergent, pH, salt), and the amount of feces. • RT-PCR inhibitors present in WS and chemicals used for concentration might inhibit the detection system. • Without a proper sewer facility, it is difficult to implement this tool.	[23,64–67]

another group of researchers from northeastern of the United States reported that viral titers in WS indicated COVID-19 infections were higher than clinical reports [80].

Padilla-Reyes et al. (2022a) found that the concentration of genomic copies of SARS-CoV-2 viral biomarkers (103 and 106 gc/L) were compatible with the reported clinical cases of COVID-19 in three out of four wastewater treatment plants (WTP). The study also revealed that WBEM was capable of giving a signal 2–7 days in advance as an early warning, which might be helpful in low-income countries. Another study performed in Mexico found an increasing number of SARS-CoV-2 viral genes in WS two weeks before the clinical cases were raised [82]. According to Hillary et al. (2021a), Giraud-Billoud et al. (2021), and Peccia et al. (2020), wastewater viral RNA detection precedes clinical reports by two to five days, three to six days, and six to eight days respectively [65,84,85,88]. In another study, Zhang et al. (2020) [86] claimed that the SARS-CoV-2 viral concentration in wastewater was well correlated with COVID-19 clinical cases when samples were collected on day-to-day basis for monitoring the pandemic. According to Nemudryi et al. (2020), the SARS-CoV-2 viral RNA concentration in wastewater samples correlated with dates from sample collection to RT-PCR detection, where viral genes are detectable in the wastewater samples 5–8 days after collection [68]. Zhang et al. (2020) claimed that, SARS-CoV-2 in the stool specimen was found significantly elevated than in the serum/blood specimen or nasal swab samples [86].

A recent WBE study conducted in over 40 US cities found that a weekly incidence might not be sufficient to support the interpretation of viral concentration in wastewater [88]. Wu et al. [88] recommended that at least two wastewater samples in a week are necessary to ascertain the accuracy while analyzing the COVID-19 trends. In another study, Petala et al. [89] suggested that a weekly-based sampling method for viral quantification with fixed sampling time could be scheduled to understand the day-to-day deviation. In addition, they strongly proposed that wastewater sample test results should be validated with clinical data. WBES could be followed for other

Table 2

Salient examples of integration of wastewater based epidemiologic monitoring with clinical testing data.

Country	Area/population/time	Sampling site, type	Concentration method	RNA/nucleic acid extraction kit name	RT-PCR kit and covered gene	Viral load range/ CT value	Clinical cases/ range	Main findings (correlation of waste water result with clinical data)	Reference
Australia	Brisbane 934,000 November,2020	WWTP Composite	Filtration	RNeasy Mini Kit	iTaq™ Universal ProbesOne-Step Reaction Mix N1, N2, E	135 to 11,992 gc/100 mL	0–40	No correlation	[100]
Bangladesh	Noalkahli NF 22nd October to 30th November, 2021	Household wastewater	PEG	QIAamp Viral RNA Mini kit	Sansure SARS-CoV-2 RT-PCR kit, Designed primer	~7450 gc/L	2–6	Correlated	[118]
Brazil	Belo Horizonte ~2,000,000 August 2020	WWTP Influent Composite	Adsorption	RT-qPCR PrepPowerViral DNA/ RNA,	iTaq™ Universal probes One Step reaction mix N1	5.6× 10^1 –2.1×10^5 gc/L	0–1200	A similar trend with hospital cases affected by COVID-19	[105]
Brazil	Florianopolis ~5000 March 2020	Raw sewage	Adsorption	QIAamp Viral RNA Mini kit	One-Step qPCR Quantinovakit, Seegene Allplex™2019-nCoV N1,S,RdRp	Avg 3.1× 10^5–4.8× 10^6 gc/L	NR	WS detected prior to CDT	[108]
France	Nancy 250,000 April 2020	raw WWTP	NucliSENS® lysis buffer	One-Step RT-ddPCR™ Kit for Probes	Two concentration procedures, based on ultrafiltration and on PEG 6000 precipitation, r	2.1 × 10^7 ± 1.1 × 10^7 gc/L and 1.6 × 10^7 ± 1.4 × 10^7 gc/L uncon and conc	100–1000	Decrease viral load during lockdown with decreasing patient's number of COVID-19.	[96]
France	Montpellier ≈ 470,000 July 2020	WWTP raw composite	.Filtration, Centrifugation	Nucleo Spin RNA Virus kit (Macherey-Nagel)	Primer, Probe based detection N1, N3	100–10,000 gc/100mL	0–75	WS detected SARS-CoV-2 genes before CS	[115]
Germany	North Rhine-Westphalia 3,725,633 August 2020	WWTP	Filtration	RNA Blue Kit	N gene, S gene, and ORF1ab gene	6.16 × 10^{14}	1–10000	WS could be used as an early warning tool.	[91]
India	Ahmedabad NF September 2020	WWTP Grab Influent wastewater	PEG	NucleoSpin® RNA Virus isolation kit	TaqPathTM Covid-19 RT-PCR Kit ORF1ab, N, and S	(~10,729 gc/L) > September (~3047 gc/L) > October (~454 gc/L)	50–650	Identification of COVID-19 hotspots	[94]
India	Chennai 9.6 million January 2021	WWTP, Composite wastewater	UV, Pasteurization, Filtration Corning Spin X-ultrafiltration	Manually (TRIzol for RNA extraction)	2019-nCoV CDC EUA Kit	1.41×10^4– 1.99×10^4 gc/L	3983–5523	Higher viral load than CS	[101]
Italy	Milan, Turin Bologna 4,998,600 Feb,2020	WWTP Composite raw sewage		Nucli SEN Smini MAG	Super Fi Green PCR Master Mix RdRp, ORF1ab	2.9× 10^2 – 5.6× 10^4 gc/L	3095 in Latium Region and 2186 in the provinceof Rome	WS dtected before first case by CDT	[109]
Japan	Ishikawa and Toyama 465,243 April 2020	Influent wastewater WWTP grab	PEG and NaCl	QIAamp Viral RNA Mini Kit	Prime Script™ N2, N3	1×10^0– 3.5 × 10^4 gc/L	5–30	Higher than clinical data	[112]

(*continued on next page*)

Table 2. (*continued*)

Country	Area/population/time	Sampling site, type	Concentration method	RNA/nucleic acid extraction kit name	RT-PCR kit and covered gene	Viral load range/ CT value	Clinical cases/ range	Main findings (correlation of waste water result with clinical data)	Reference
Japan	Yamanashi Prefecture NF May 2020	WWTP Grab	Adsorption-elution, Electronegative filtration	RNeasy Power Water Kit	Probe qPCR Mix with UNG N1, N2	1.4×10^2– 2.5×10^3 gc/L	NF	Similar to clinical data	[113]
Mexico	3.8 million	WWTP Grab	NF	RNeasy Mini Kit	QuantiNova® SYBR® Green PCR Kits (Qiagen, USA) N1, N2	1.9×10^3 – 3.5×10^6 gc/L	74 to 82,690	WS detected SARS-CoV-2 2–7 days earlierthan clinical reports.	[93]
Netherlands	Amsterdam, DenHaag, Utrect, Apeldoorn, Amersfoot, Tilburg and Schiphol 2,802,800 July 2020	WWTP Composite	Ultrafiltration	Biomerieux Nuclisens kit	Neasy PowerMicrobiome Kit (Qiagen, Hilden, Germany)	2.6–30 gc/mL	20–140	WS detected RNA prior to CS.	[98]
Qatar	Doha 2,503,457 August 2020	Influent WWTP Composite	PEG	RT-qPCRSARS-CoV-2(2019-nCoV)kit N1,N2	Quick-RNA Viral Kits (Zymo Research, Irvine CA, USA Cat. No. R1041)	7.8×10^3– 5.4×10^5 gc/L	500–2500	Higher than CS	[107]
Qatar	Doha 2.8 million January 2021	WWTP Influent Raw wastewater composite	PEG	Quick-RNA Viral Kit	Bio-Radi Taq Universal Probes One-Step Kit	7.8×10^3 – 5.4×10^5 gc/L	31,181 to 542,313	A similar trend as both WS and CS were decreasing at the same time	[90]
Spain	Murcia 716,388 April 2020	Influent, WWTPs Composite	Aluminum hydroxide adsorption–precipitation method with 3% beef extract	Nucleo Spin RNA virus kit	TaqMan one-step master mix (N1, N2, and E)	1×10^5 – 3.4×10^5 gc/L	12–622	Detected RNA in low prevalence area	[110]
Spain	Barcelona 2.7 million July 2020	WWTP Composite and grab raw samples	Polyethylene glycol-6000	NucliSENSminiMAG extraction system	NF RdRp, IP2, IP4, E, N1	10^3-10^5 gc/L	2000–8000	WS detectiom prior to CDT	[116]
South Africa	NF Durban April 2021	WWTP composite and Grab Raw samples	Ultrafiltration, Adsorption	RNeasey Power Soil Total RNA Kit,	Primer, Probe	1.55×10^5– 7.32×10^6 gc/L	95,000 to 2.3 million	WS viral load similar with CS	[99]
Sweden	Gothenburg 755,940 July 2020	WWTP Influent, Composite & grab	Adsorption, Filtration	DNeasy Blood and Tissue kit	qPCR Reaction Mix (Invitrogen)	6.7×10^3– 1.8×10^6 gc/L	0–90	WS trend peaked when hospitalized COVID-19 increased	[106]
UAE	June,2020	Raw sewage WWT Composite	Ultrafiltration columns, PEG/TRIzol	ABIO pure Viral DNA/ RNA Extraction kits	GENESIG COVID-19 kits RdRP	7.5×10^2– 3.4×10^4 gc/L	0–800	Decrease of WS load related to CS decline	[114]
UK	Gwynedd, Cardiff, Liverpool, Manchester, Wirral, Wrexham ~3 million July 2020	Untreated WWTP Influent; Composite and Grab samples	Centrifugation, Ultrafiltration	NucliSEN SeasyMag	Ultrasense Reaction Mix, Enzyme Mix; N1 and E	<1.2×10^3– 1.5×10^4 gc/100mL	5000–20,000	Both positive and negative correlation	[83]
UK	South East England 4 million April 2020	Raw WWTP influent; Composite	Filtration	High Pure Viral RNA kit	Invitrogen SuperScript III One-Step RT-PCR System	3.5-5.27 log10 gc/L 3.1×10^3– 6.0×10^5 gc/L	0–10,000	Able to detect prevalence variant by WS sequencing	[97]

USA	New Orleans 290,321 June 2020	WWTP composite and grab samples	Ultrafiltration and adsorption-eluting using electronegative membrane	ZR Viral RNA Kit	NF	3.1×10^3–7.5×10^3 gc/L	NF	Detection of viral RNA in WS after first caseby clinical diagnostic test	[61]
USA	Boston(Massachusetts) NR June, 2021	WWTP raw sewage composite	Pasteurization, Filtration	RNeasey PowerSoil Total RNA Kit,	Amicon Ultra-15 centrifugal ultrafiltration units (Millipore UFC903096)	NF	NF N1, N2	WS detected SARS-CoV-2 RNA before CS in the first wave.	[95]
USA	Utah 1597460 July 2021	Influent, activated and anaerobic ally digested sludge, composite	Filtration Centrifugation	AllPrep Power Viral DNA/RNA kit	TaqPathTM 1-Step RT-qPCR Master Mix N1, N2	1.0×10^5 – 1.0×10^6 gc/L.	NF	Primary sludge can be used to predict disease prevalence	[92]
USA	Virginia 1,700,000 March 2020	Raw wastewater WWTP Grab samples NI	Innova Prep Concentrating method	Nucli SEN Seasy Mag	RT-ddPCR, One-Step RT-ddPCR Advanced Kit, N1, N2, N3	10^2–10^5gc/L		Trend correlated with clinical data	[101]
USA	Indiana NF November 2020	University Sewer Manhole, Raw sewage, Grab	Filtration, Centrifugation	QIAmp Viral Mini Kit	RT-PCR master mix N1, N2	1.0×10^4–1.0×10^6gc/L	0–120	Early detected than clinical	[102]
USA	Las Vegas 1,060,000 March 2020	WWTP Grab samples	Raw influent wastewater and primary effluent composite and grab sample	RNeasy Mini Kit	i Script™ Select Kit, E,N1,N2,ORF1a	10^4–10^6gc/L	20 and 200	Correlation with clinical data	[103]
USA	Charlotte >2550 November,2020	University plumbing cleanouts & manhole, composite Raw	Electronegative filtration	QIAamp viral mini kit	iTaq™ Universal ProbesOne-Step Reaction Mix N1,	394–2,990,271gc/L	400–13,00	Asymptomatic patients detection	[104]
USA	Virginia, 143,000 September 2020	WWTP, Hospitals, Dormitoriecomposite, and Grab Influent wastewater	Filtration, PEG	QiaAmp viral RNA mini kit and NucleoSpin RNA Plus kit	NF N1, N2, RP	Ct value (30.6–41.9)	NR	Consistency with clinical data	[111]
USA	Boston(Massachusetts) NF July 2020	WTP composite and Grab samples	PEG+NaCL	TRIzol-chloroform	PCR is used by New England Biolab Master Mix, N1, N2, and N3	0-500 gc/L	NF	SARS-CoV-2 WS titer higher than CS	[117]

emerging and re-emerging viruses to detect hotspots together with the help of CDT [28,34,87,119,120].

Conclusion

WBEM has the potentiality to detect hotspots, identify the prevalence, and predict early warning for various disease. On the other hand, CDT can be used to diagnose positive patients, undertake mass vaccination, and quarantine measures to limit direct, indirect, or close contact. In the context of making the CDT method more cost-effective and efficient, it is important to improve it in terms the of rapidness, sensitivity, and portability of the analyses to demonstrate it as a functional diagnostic tool for detecting cases of positivity. It is also noteworthy that, the presence of SARS-CoV-2 in the community will be detected earlier by the WBEM than by the CDT. Hence, the dual monitoring of COVID-19 by using WBEM and CDT will immensely help control the spread and threat of the COVID-19 global pandemic.

Ethical statement

No ethical statement is required for this review article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.coesh.2022.100396.

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