

Supplemental material

Tracing COVID-19 Trails in Wastewater: a systematic review of SARS-CoV-2 Surveillance with Viral Variants

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Table S1. Summary of literature data on the detection of SARS-CoV-2 variants by wastewater surveillance

Country	Wastewater sample/ Virus concentration method	RNA extraction kits Used	Genes targeted	Method (PCR or sequencing)	SARS-CoV-2 variants	Major Findings
USA, (Rothman et al., 2021)	Composite / mixed cellulose ester membranes	BioMérieux NucliSENS extraction kit with magnetic bead capture (bioMérieux, Durham, NC)	^a N1	SARS-CoV-2 RNA quantification: RT- ddPCR; Identification of novel SNVs: metatranscriptomic sequencing	Single-nucleotide variants (SNVs)	-2,558 SNVs (2,002 unique) have been detected. -Although wastewater sequencing is a tool for detecting SNVs across large catchment areas, it is not useful for determining the true prevalence of SNVs.
USA, (Crits-Christoph et al., 2021)	Composite / ultrafiltration	Qiagen AllPrep DNA/RNA minikit	^a N1	SARS-CoV-2 RNA quantification: RT- qPCR; Identification of novel SNVs: metatranscriptomic sequencing	Single-nucleotide variants (SNVs)	-Wastewater detection of SARS-CoV-2 variants was concordant with clinical data.
USA, (Swift et al., 2021)	Composite / centrifugation followed by ultrafiltration	Qiagen AllPrep PowerViral extraction kit	^b S	Sequencing	B.1.526 (Iota), B.1.617.2 (Delta), P.1 (Gamma)	-Wastewater detection of SARS-CoV-2 variants was concordant with clinical data.
USA, (Gregory et al., 2021)	Composite / polyethylene glycol precipitation	QIAamp Viral RNA Mini Kit (Qiagen, Germantown, MD, USA)	^b S	RT-PCR-based Illumina MiSeq sequencing	B.1.1.7 (Alpha), and P.1 (Gamma)	-A novel program called SAM Refiner was developed for detecting novel variants in wastewater and removing chimeric errors generated from PCR. -Reported the emergence of B.1.1.7 (Alpha) and P.1 (Gamma) in wastewater

USA, (Ash et al., 2021)	Grab / filtration followed by ultra-centrifugation	Qiagen QIAamp viral RNA mini kit	^a N1and N3	SARS-CoV-2 RNA quantification: RT-qPCR; Variant analysis: tiling PCR-based Illumina sequencing	B.1.2 and the GH neighbor clade	-B.1.2 variants were circulated in the study area (large college campuses).
USA, (Lee et al., 2021)	Composite / filtration followed by ultra-centrifugation	RNeasy kit (Qiagen, USA)	S genes (69/70 Del, Y144del, and A570D)	One-Step RT-qPCR	B.1.1.7 (Alpha) (69/70 Del, Y144del, and A570D)	-Developed an allele-specific RT-qPCR method to detect and quantify B.1.1.7 SARS-CoV-2 variant and wild type in wastewater. -The sum of concentration by CDC N1 and N2 assays matched the total concentration of B.1.1.7 and wild-type sequences obtained by this newly developed AS RT-qPCR method.
USA, (Rainey et al., 2022)	Composites / filtration through cellulose ester electronegative membrane filter	Zymo Quick-DNA/RNA Viral MagBead kit (Zymo Research, Irvine, CA, USA)	^a N2	SARS-CoV-2 RNA quantification: RT-qPCR' SARS-CoV-2 variants detection: RT-PCR-based Sanger sequencing	B.1.375, B.1	-SARS-CoV-2 was detected in 28.8% (15/52) of wastewater samples tested. -No significant association between SARS-CoV-2 concentrations in wastewater and ambient temperature.
USA, (Boehm et al., 2022)	Composite (wastewater settled solids) / centrifugation	Chemagic Viral DNA/RNA 300 kit	^b LPPA24S: a 9 bp deletion of the BA.2 characteristic mutation; S:477-505: a region of five adjacent SNPs common to BA.1 and BA.2	RT-ddPCR	BA.1 (Omicron), BA.2 (Omicron)	-Wastewater analysis showed regional replacement of Omicron variants BA.1 with BA.2, which was in agreement with clinical sequencing data.

USA, (Li et al., 2021)	Grab / ultrafiltration	AllPrep PowerViral DNA/RNA kit (QIAGEN, Inc., Germantown, MD, USA)	^a N1 and N2	SARS-CoV-2 RNA quantification: RT- qPCR; Identification of variants: RT-PCR based Illumina sequencing	B.1.1.7 (Alpha), B.1.617.2 (Delta), B.1.2, B.1.429 (Epsilon) P.1 (Gamma)	-The concentrations of viral loads in wastewater ranged from 3.00×10^2 to 9.28×10^5 GC/L. -The viral concentration in the wastewater positively correlates with the number of COVID-19 cases. -Alpha (B.1.1.7) and Delta (B.1.617.2) were the dominant variants in both wastewater and clinical samples.
USA, (Vo, et al., 2022a)	Grab, composite / ultrafiltration	N/A	^a orf1a, E_Sarbeco, N1 and N2	SARS-CoV-2 RNA quantification: RT- qPCR; Detection of a variant of concern: Illumina sequencing	B.1.1.7 (Alpha), B.1.429 (Epsilon)	-SARS-CoV-2 RNA loads (ranging from 4.3 log ₁₀ GC/L to 8.7 log ₁₀ GC/L) closely matched COVID-19 incidence. -B.1.1.7 and B.1.429 variants were detected one month earlier than clinical testing.
USA, (Ai et al., 2021)	Composite / electropositive membrane (ViroCap, Scientific Methods, US)	RNeasy Power Microbiome kit (QIAGEN, Germantown, MD, USA)	N1, N2 & E genes	Next- Generation Sequencing (NGS) by hybridization and/or amplicon methods	B.1.427/429 (Epsilon)	-Reported WWS was in concordance with clinical cases.
USA, (Wolfe et al., 2022)	Composite / centrifugation	Chemagic Viral DNA/RNA 300 kit H96 (Perkin Elmer)	N and S genes	RT-ddPCR	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), B.1.621 (Mu), C.37 (Lambda) & B.1.1.529 (Omicron)	-Developed mutation- specific digital RT- PCR assays and quantified those mutations in wastewater-settled solids samples collected from publicly owned treatment

						works. -Normalized concentrations of these mutations by a SARS-CoV-2 N gene correlated with regional estimates of clinical infections caused by each VOC.
USA, (Sutton et al., 2022)	Composite / electronegative filter	MagMAX Viral/Pathogen Nucleic Acid Isolation kit (Thermo Fisher Scientific)	N and S genes	SARS-CoV-2 quantification: dd-RT-PCR; Variant analysis: Amplicon-based sequencing	B.1.351 (Beta)	-Reported the B.1.351 variant in wastewater 12 days before the detection in clinical specimens.
USA, (Yu et al., 2022)	Grab / centrifugation	Chemagic Viral DNA/RNA 300 kit H96	Nucleoprotein (N) gene	Targeted digital RT-PCR mutation assay	B.1.1.7 (Alpha) and B.1.617.2 (Delta)	-Variants in WW measured were strongly correlated with clinically reported cases of each variant $r = 0.82 - 0.97$. - Variants were detected 2 weeks earlier than in clinical cases.
USA, (Oh et al., 2022)	Composite / centrifugation	QIAamp Viral RNA minikit	N1	SARS-CoV-2 RNA quantification: RT-qPCR Variant analysis: RT-qPCR targeting S: A570D (Alpha) and S: P681R (Delta) mutations followed by confirmation via Illumina sequencing	B.1.1.7 (Alpha) and B.1.617.2 (Delta).	-Developed RT-qPCR assay to detect Alpha and Delta variants in wastewater. -The assay was used for monitoring Alpha and Delta variants and further confirmed by sequencing.
USA, (Boehm et al., 2022)	Composite / ultracentrifugation	Chemagic Viral DNA/RNA 300 kit H96	N & S genes	ddRT-PCR	Omicron BA.1 and BA.2	-Reported the VOC with WWS & reported the dynamics between two variants.

USA, (Brumfield et al., 2022)	Composite / NA	QIAamp Viral RNA minikit	Nucleocapsid (N protein)	Targeted SARS-CoV-2 sequencing	B.1.351 (Beta, V2), B.1.1.7 (Alpha, V1)	-Reported important genetic mutations (K417N, D614G, P681H, T716I, S982A, and D1118H).
USA, (Smyth et al., 2022)	Composite / ultracentrifugation	Centrifuged, then the supernatant was filtered and NaCl and PEG were added to the filtrate and centrifuged, and the pellet was resuspended in TRIzol	S genes	Targeted SARS-CoV-2 sequencing	B.1.1.7 (Alpha), B.1.351 (Beta), B.1.427/B.1.429 (Epsilon), B.1.526(Iota), (B.1.617.2(Delta), B.1.617.1 (Kappa), P.1 (Gamma	-Clinical sequences submitted to GISAID from the study area were highly concordant with wastewater analysis samples. -Even new variants were detected in wastewater which were not caught in clinical testing.
USA, (Silva et al., 2022)	Composite / ultracentrifugation	Commercial kits: MagMAX™ Viral/Pathogen Nucleic Acid Isolation kit (Applied Biosystems) and MagMAX™ Microbiome Ultra Nucleic Acid Isolation kit (Applied Biosystems).	TaqMan RT-PCR assays targeted ORF1ab, S-protein, and N-protein	RT-qPCR combine with targeted SARS-CoV-2 sequencing	B.1.1.7 (Alpha) B.1.617.2 (Delta), B.1.1.529 (Omicron)	- SARS-CoV-2 variants in wastewater were in concordance with COVID-19 prevalence. -WWS identified hotspots in neighborhoods and determine circulating variants.
USA, (Layton et al., 2022)	Composite / electronegative membrane filtration and centrifugal ultrafiltration	MagMAX Viral/Pathogen kit	N1 and N2	SARS-CoV-2 RNA: RT-ddPCR Variant analysis: Illumina sequencing	B.1 and B.1.399	- SARS-CoV-2 variants in wastewater were in concordance with COVID-19 prevalence. -WWS identified hotspots in neighborhoods and determine circulating variants.
Canada, (Lin et al., 2021)	N/A Ultrafiltration	N/A	^a N1	Multiplex tiling PCR-based sequencing	B.1.1.7 (Alpha), P.1 (Gamma), B.1.351 (Beta)	-Optimized the primer scheme (400 bp) and sample matrix (influent)

						for detecting the emergence of new variants. -B.1.1.7 and P.1 were the dominant lineages -SARS-CoV-2 variants in wastewater were in concordance with COVID-19 prevalence.
Canada, (Graber et al., 2021)	Composite centrifugation	RNeasy PowerMicrobiome kit (Qiagen, Germantown, MD)	^a N1; ^b D3(a deletion at nt 28271)	Total SARS-CoV-2 N gene quantification: RT-ddPCR. Detection of non-B.1.1.7 alleles (D3) or only the B.1.1.7 allele (D3L): allele-specific RT-qPCR	Quantification of B.1.1.7 (Alpha) versus non-B.1.1.7 allele frequency	-SARS-CoV-2 variants in wastewater were in concordance with COVID-19 prevalence. -This study allowed the quantification of B.1.1.7 versus non-B.1.1.7 allele frequency in wastewater without employing quantitative RT-PCR standard curves.
Canada, (Corchis-Scott et al., 2021)	Grab / ultrafiltration	AllPrep PowerViral DNA/RNA kit (Qiagen, Germantown, MD)	^a N1; ^b N-D3L	RT-qPCR	B.1.1.7 (Alpha)	-Reported B.1.1.7 in wastewater was concordant with clinical diagnosis by targeting the D3L mutation on the N gene.
Canada, (Hubert et al., 2022)	Composite/ ultrafiltration	QIAamp viral RNA minikit (Qiagen)	N1 and N2	RT-PCR and metagenomic sequencing	BA.1 (Omicron), B.1.617.2 (Delta variant by targeting mutations R203K/G204R)	-Omicron and Delta variants were identified across 30 municipalities
Canada, (Lawal et al., 2022)	Composite / nanotrap magnetic particles	QIAamp Viral RNA minikit (Qiagen)	N1 and N2	SARS-CoV-2 RNA: RT-qPCR Variant analysis: Illumina sequencing	Delta sub-lineages AY.25, AY.25.3, B.1.617.2, AY.103, AY.121, AY.4, AY.74, AY.31, AY.25.1) and BA.1.1 (Omicron)	-Multiple VOCs were reported in wastewater.
Canada,	Composite / polyethylene	RNeasy Power	N1, N2, and N200	SARS-CoV-2 RNA:	Delta sub-lineages	-WWS in large cities

(Oloye et al., 2022)	glycol precipitation	Microbiome kit		RT-qPCR Variant analysis: Illumina sequencing	AY.12, AY.25, AY.27, and AY.93, and Omicron sub- lineages BA.1 and BA.2.	detects variants of concern earlier than in small cities. -SARS-CoV-2 load in wastewater during the Omicron wave was higher than in Delta.
Canada, (Peng et al., 2022)	Composite / centrifugation	RNeasy Power Microbiome kit	N1 and N2	SARS-CoV-2 RNA quantification: RT- qPCR; Variant analysis: RT-qPCR targeted mutations D3L (Alpha) and multiplexed assay to detect Omicron, Alpha, Delta, Illumina sequencing, and nested PCR- liquid chromatography- mass spectrometry (nPCR-LC-MS)	B.1.1.7 (Alpha) Omicron sub-lineages BA.1 and BA.2.	- nPCR-LC-MS allowed distinguishing between the presence or deletion D3L mutation compared to allele-specific qPCR, indicating higher selectivity. - nPCR-LC-MS was more sensitive in detecting Omicron than sequencing. - nPCR-LC-MS is an effective tool to measure VOCs for WWS
Canada, (Peterson et al., 2022)	Composite / centrifugation	Magna Pure 96 DNA and Viral NA Large Volume kit (Roche Diagnostics, Laval, QC)	^a N; ^b N501Y, S 69-70 del, N-D3L	RT-qPCR	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	-RT-qPCR assay was developed for detecting new variants -B.1.1.7 (75% of the signal detected) was dominant in wastewater -The sensitivities and specificities were 96.1% and 95.2% for SN501Y, 96.1% and 93.4% for Sdel, and 85.7% and 89.8% for ND3L, respectively, when compared with N1 as the good standard for the qualitative detection of SARS-CoV-2 in

						wastewater.
UK, (Martin et al., 2020)	Composite / filtration- centrifugation	N/A	^a RdRP and E. ^b different regions across the SARS-CoV-2 genome.	SARS-CoV-2 RNA quantification: RT- qPCR. Sequence variation analysis: RT-PCR and nPCR based NGS Sanger sequencing.	G614 (Delta)	-The variants in wastewater were coherent with clinical cases. -Lockdown measures reduced the concentrations of viral RNA in wastewater. -G614 was prevalent in England at the time of the study.
UK, (Wilton et al., 2021)	Composite / filtration- centrifugation	High-Pure Viral RNA kit (Roche)	^b RdRP and ORF8b	RT-PCR and nPCR- based NGS Sanger sequencing	B.1.1.7 (Alpha)	-Reported B.1.1.7 variant for the first time in south-east England wastewater -Reported B.1.1.7 variants in wastewater before the detection in clinical specimens -Prevalence of SARS- CoV-2 alpha variant (B.1.1.7 lineage) in wastewater corresponded with clinical suspicions.
UK, (Mishra et al., 2021)	Composite / filtration followed by centrifugal ultrafiltration	High-Pure Viral RNA kit (Roche)	RdRP and E	SARS-CoV-2 RNA quantification: RT- qPCR Variant analysis: Illumina sequencing	B.1.1.7 (Alpha) and B.1.617.2 (Delta)	-Variant detection in wastewater was concordant with clinical cases. -The shift of Alpha to Delta variants in communities was reflected with WWS.

Spain, (Carcereny et al., 2021)	Grab / aluminum hydroxide adsorption-precipitation	Maxwell RSC PureFood GMO and Authentication kit (Promega Corporation, Madison, US); NucleoSpin RNA Virus kit (Macherey-Nagel GmbH & Co., Düren, Germany),	^a N1, ^b S (Δ HV69/70 deletion)	Duplex RT-qPCR	B.1.1.7 (Alpha)	-SARS-CoV-2 B.1.1.7 variant was reported in 91% of wastewater samples. -Variant detection in wastewater was concordant with clinical cases.
Spain, (Carcereny et al., 2022)	Composite / aluminum hydroxide adsorption-precipitation	Maxwell® RSC PureFood GMO and Authentication kit (Promega)	^a N1, ^b S (Δ HV69/70 deletion)	Duplex RT-qPCR	B.1.1.7 (Alpha)	-A range of one to eight Alpha signature markers were detected in different WWTPs -There was a significant positive correlation between the log10 N1 GC per 100,000 inhabitants in sewage and the total number of clinical cases.
Spain, (Pérez-Cataluña et al., 2022)	Grab / cetyltrimethylammonium bromide and proteinase K solution	Maxwell RSC Pure Food GMO and authentication kit (Promega)	^a N1; ^b ORF1a, S, ORF8, and N	SARS-CoV-2 RNA quantification: RT-qPCR; Variants analysis: multiplex PCR-based Illumina sequencing.	B.1.1.7 (Alpha)	-Variant was detected in wastewater before the detection in clinical specimens. -The highest frequency of mutations was present in nucleocapsid (60% of the samples), then in the ORF8 region (45% of the samples), and the lowest mutations were found in ORF1a.
Spain, (Novoa et al., 2022)	Grab and composite / AlCl ₃ precipitation	QIAamp Viral RNA minikit and Maxwell RSC Pure Food GMO and	N, N1, N2, E	SARS-CoV-2 RNA quantification: RT-qPCR Variant analysis:	20E (EU1), 20A.EU2, B.1.1.7 (Alpha), B.1.351 (Beta), B.1.526 (Iota),	-Variant detection in wastewater was concordant with clinical cases.

		Authentication kit.		Illumina sequencing	P.1 (Gamma), P.2 (Zeta), B.1.427/9 (Epsilon), B.1.525 (Eta),	
France, (Rios et al., 2021)	Composite / filtration followed by ultracentrifugation	AllPrep PowerViral DNA/RNA kit (Qiagen, Hilden, Germany, #28000-50), RNeasy PowerMicrobiome kit (Qiagen, #26000-50)	^a N1;	Total SARS-CoV-2 N gene quantification RT-qPCR; Variant analysis: tiling PCR-based Nanopore sequencing	B.1.160, B.1.177, B.1.367, B.1.474, B.1.221, A.27, B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	-B.1.160 was reported as the most abundant lineage between Oct 2020 and Jan 2021. -The majority of B.1.1.7 variants were found in Jan. and Feb. and subsequently declined in March. -Variant detection in wastewater was concordant with clinical cases.
France, (Wurtz et al., 2021)	Composite / filtration followed by ultracentrifugation	EZ1 Virus Mini kit (Qiagen, Hilden, Germany)	^a N;	SARS-CoV-2 RNA quantification: RT-qPCR; Variants analysis: PCR-based direct Illumina sequencing	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.525 (Eta), B.1.1.138, Marseille-3, Marseille-4, 20A, 20B	-Direct sequencing is more accurate than RT-PCR in the detection of SARS-CoV-2 variants. - Prevalence of the SARS-CoV-2 UK variant was higher than other variants that agreed with the clinical data.
France, a (S Wurtzer et al., 2022)	Composite / filtration followed by ultracentrifugation	QIAasymphony automated extractor (QIAGEN)	^a E; ^b S69-70 del	RT-qPCR	B.1.1.7 (Alpha)	-Variant detection in wastewater was concordant with clinical cases. -The rise of B.1.1.7 could be one of the reasons for the third wave in France.
France, b (Sebastien Wurtzer et al., 2022)	Composite / filtration followed by ultracentrifugation	QIAasymphony automated extractor (QIAGEN) and some samples with extraction with lysis buffer and	^a E; ^b S69-70 del, Alpha_del69-70_F186, BA.1_del69-70_F186_omicron, Delta_L452R_R22979, BA.2_F27367,	digital RT-PCR	B.1.617.2 (Delta) BA.1 (Omicron)	-Variant detection in wastewater was concordant with clinical cases. -The impact of vaccination could be

		purified with phenol/chloroform				the cause of the reduction of delta variant
France, (Barbé et al., 2022)	Composite / ultrafiltration	NucliSens kit & the NucliSens miniMAG purification system (bioMérieux, Marcy L'Etoile, France)	WGS	WGS with ARTIC Network and a MinION Mk1C sequencer primers and protocol (Oxford Nanopore Tec., UK)	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta)	-Detected multiple SNVs and assessed the circulation of VOC Alpha, Beta, Gamma, and Delta.
Netherlands, (Heijnen et al., 2021)	Composite / ultrafiltration	Biomerieux Nuclisens kit (Biomerieux, Amersfoort, the Netherlands) in combination with the semi-automated KingFisher mL (Thermo Scientific, Bleiswijk, Netherlands)	^a N2; ^b S - N501Y mutation	SARS-CoV-2 RNA quantification; RT-qPCR. N501Y mutation quantification: RT-ddPCR	B.1.351 (Beta)	-RT-ddPCR assay determined the proportion of lineage B.1.351 in samples with mixtures of wild type. -A linear relationship between the values measured with RT-qPCR and RT-ddPCR was found. -SARS-CoV-2 concentrations obtained by RT-qPCR are consistently higher (61%) than SARS-CoV-2 concentrations obtained by RT-ddPCR. -Variant detection in wastewater was concordant with clinical cases.
Germany, (Ho et al., 2022)	Composite / polyethylene glycol precipitation	innuPrep Virus DNA/RNA 121 kit – IPC16 (Analytik Jena GmbH)	^a N, E, ORF1ab, RdRP2 and RdRP1; ^b N501Y	RT-ddPCR	B.1.1.7 (Alpha)	-The detected N501Y mutations in wastewater were similar to those in the individual clinical tests. -SARS-CoV-2 RNA showed its stability

						under anaerobic conditions for several days. -The copy numbers of SARS-CoV-2 reduced quickly in dissolved oxygen.
Germany, (Rubio-Acero et al., 2021)	Grab / centrifugation	RNeasy PowerMicrobiome kit (Qiagen)	^a N1	SARS-CoV-2 RNA quantification: RT-PCR and ddPCR; Variants analysis: Illumina sequencing	B.1.1.7 (Alpha)	-Variant detection in wastewater was concordant with clinical cases. -B.1.1.7 was the dominant variant in the wastewater samples.
Germany, (Schumann et al., 2022)	Composite / filtration through two glass fibers followed by centrifugation	Direct-zol RNA miniprep kit (Zymo cat# R2052)	^b Whole genome	Illumina sequencing	B.1.1.7 (Alpha), B.1.617.2 (Delta), B.1.351 (Beta), P.1 (Gamma)	-A significant association between SARS-CoV-2 mutation load in wastewater and incidence rate was found. - The increase of viral RNA in wastewater was shown two weeks before the increase in detected COVID-19 cases. -B.1.1.7 was the dominant viral strain throughout the study.
Germany, (Agrawal, Orschler, Tavazzi, et al., 2022)	Composite / polyethylene glycol precipitation	MagMAX Microbiome Ultra Nucleic Acid isolation kit (Thermo Fisher Scientific)	Genome sequencing	Used 237 primer pairs, resulting in 125–275 bp long amplicons, capturing nearly full SARS-CoV-2 genome.	B.1.1.529 (Omicron)	-Major mutations in Omicron were reported.
Italy, a (G. La Rosa et al., 2020)	NA/ polyethylene glycol precipitation	MiniMAG extraction system (bioMerieux)	S	Nested RT-PCR followed by Sanger sequencing and Nanopore sequencing	B.1.1.7 (Alpha), P.1 (Gamma), B.1.525 (Eta)	- B.1.1.7 variant detection in wastewater was concordant with clinical cases. - P.1 was absent in wastewater samples in

						April, which agreed with clinical data. -RT-qPCR reduces errors in RT-PCR false-negative in WWS.
Italy, b (Giuseppina La Rosa et al., 2021)	Composite, grab / polyethylene glycol-dextran separation	Nu- cliSens extraction system (bioMerieux, Marcy-l'Etoile, France).	S	Nested RT-PCR followed by Sanger sequencing	B.1.1.7 (Alpha), P.1 (Gamma)	-The first detection of SARS-CoV-2 B.1.1.7 variant (20I/501Y.V1) and P.1 variant (20J/501Y.V3) in wastewater were recorded. -Compared with whole-genome sequencing, RT-PCR followed by Sanger sequencing can be a more sensitive and economic option.
Italy, (G. La Rosa et al., 2022)	Composite/ NA	N/A	N/A	Nested RT-PCR and Sanger Sanger Sequencing	B.1.1.529 (Omicron)	-Detection rate of the SARS-CoV-2 variant (Omicron) from wastewater was simultaneous with clinical samples confirmed by the real-time RT-PCR and Sanger sequencing.
Italy, (Lee et al., 2022)	Composite / polyethylene glycol precipitation	Scientific KingFisher Flex automated instrument (ThermoFisher Scientific) and the MagMAX CORE Nucleic Acid Purification kit (ThermoFisher)	Spike gene 493-498	RT-qPCR (Bio-Rad CFX96)	B.1.1.529 (Omicron)	-Developed an allele-specific RT-qPCR assay to simultaneously detect the stretch of mutations from Q493R to Q498R of the Omicron variant. -This assay was successfully applied for WWS -Variant detection in wastewater was concordant with clinical

						cases.
Italy, (D'Agostino et al., 2022)	Composite / polyethylene glycol (PEG) precipitation method (10% PEG and 2.25% NaCl)	SensiFAST cDNA Synthesis kit (Bioline)	N, ORF1ab	qPCR & Whole Genome Sequencing	B.1.617.2 (Delta)	-PCR-based rapid method was reported to be sensitive and able to determine allelic discrimination assay. -Variant detection in wastewater was concordant with clinical cases. SARS-CoV-2 whole-genome sequencing is used to confirm results in up to 10 viral genome positions.
Italy, (Cutrupi et al., 2022)	Composite / polyethylene glycol precipitation	Magnetic silica beads	<i>orf1b</i> for SARS-CoV-2; Mutations H655Y, N679K, and P681H (ISS assay), and 8 mutations (JRC assay)	SARS-CoV-2 RNA quantification; RT-qPCR Variant analysis: mutation-specific q-PCR followed by Sanger sequencing	Omicron (B.1.1.529)	-SARS-CoV-2 concentration in wastewater during the Omicron wave was 4–20 times higher than in Alpha and Delta waves. -Wastewater surveillance allows an understanding of the burden of virus circulation despite a low number of clinical cases due to mass vaccination.
Hungary, (Róka et al., 2022)	Composite, grab / ultrafiltration	QIAamp Viral RNA Mini kit (Qiagen, Germany)	^a N1; ^b Spike mutations N501Y and del H69/V70	SARS-CoV-2 RNA quantification; RT-qPCR, Mutation detection: RT-ddPCR	B.1.1.7 (Alpha)	-Variant detection in wastewater was concordant with clinical cases. -Mutations H69/V70 and N501Y of B.1.1.7 lineage were detected in equal concentrations.

Greece, (Avgeris et al., 2021)	Composite / polyethylene glycol precipitation	Water DNA/RNA Magnetic Bead kit (IDEXX Laboratories Inc., Westbrook, Maine, USA)	S, ORF3a, ORF1ab/RdRP, N	Nested PCR/real-time PCR-based amplicon sequencing	B.1.1.7 (Alpha)	-SARS-CoV-2 RNA was detected in 56.7% (17/30) of wastewater samples. -Variant detection in wastewater was concordant with clinical cases. -The agreement of nested PCR assay with real-time PCR results was 96.7%.
Greece, (Galani et al., 2022)	NA/ polyethylene glycol precipitation	Magnetic-bead extraction	^a N1 and N2; ^b Whole-genome	SARS-CoV-2 RNA quantification and stability evaluation: RT-qPCR; Variants analysis: Emulsion PCR-based NGS sequencing	B.1.1.7 (Alpha)	-B.1.1.7 lineage was found in 96.3%, whereas B.1.351 and P.1 were absent
Greece, (Pechlivanis et al., 2022)	Composite / centrifugation followed by filtration through electronegative membranes	Phenol–chloroform-method combined with magnetic bead binding.	^a N2; ^b Whole genome	SARS-CoV-2 RNA quantification: RT-qPCR; Variant analysis: RT-PCR based Illumina sequencing	B.1.1.7 (Alpha), B.1.351 (Beta),	-A novel method called “lineagespot” was validated for detecting and quantifying differences in the mutational load of SARS-CoV-2.
Greece, (Chassalevris et al., 2022)	Composite / mixed cellulose esters (MCE) membrane filter	Phenol–chloroform-method combined with magnetic bead binding.	N2, Spike Δ69-70 deletion	SARS-CoV-2 RNA quantification; RT-qPCR Variant analysis: mutation-specific q-PCR	BA.1 (Omicron)	-Developed an RT-qPCR assay that targets S: Δ69-70 deletion indicating Omicron BA.1. -The same assay enabled the detection of Omicron in wastewater nine days before detection by clinical surveillance.
Switzerland, (Jahn et al., 2022)	Composite, grab / filtration followed by concentration	QiaAmp Viral RNA MiniKit (Qiagen, USA)	Whole genome	Illumina sequencing	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	-Several signature mutations of both B.1.1.7 and 501.V2 lineages have been

						detected in wastewater.
Switzerland, (Caduff et al., 2022)	Composite / ultrafiltration	QiaAmp Viral RNA MiniKit (Qiagen, USA)	Spike Δ 69-70 and ORF1a Δ 3675-3677	Drop-Off RT-dPCR	B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma)	-RT-dPCR assay was adapted to quantitative detection of B.1.1.7, P.1, and B.1.351 in wastewater. -The proportion of 1.1.7, B.1.351, and P.1 in wastewater increased earlier than in clinical samples.
54 European municipalities from 20 countries, (Agrawal, Orschler, Schubert, et al., 2022)	Composite / filtration through electronegative membrane; ultrafiltration	Fast RNA Blue kit (MP Biomedicals), Ultra Microbiome kit (ThermoFisher Scientific)	^a N2	SARS-CoV-2 RNA quantification: RT- qPCR; Variants analysis: sequencing	B.1.1.7 (Alpha), P.1 (Gamma), B.1.351 (Beta) B.1.617.2 (Delta)	-Variant B.1.1.7 was dominant in all countries except Cyprus. -Variant B.1.351 seemed to be more prevalent in Finland than other countries. -ORF8 mutations (i.e. Q27stop, R52I) were widely detected in the circulated SARS-COV- 2 variants in the sampled regions
Austria, (Radu et al., 2022)	Composite/ polyethylene glycol precipitation	Maxwell® RSC AS4500 (Promega)	^a N;	SARS-CoV-2 RNA quantification: RT- qPCR; Variants analysis: Illumina sequencing	B.1.1.7 (Alpha)	- SARS-CoV-2 RNA concentration in wastewater was influenced commonly by the emergence of Alpha lineage. -The alpha variant and N501Y mutation percentages matched in both wastewater and clinical samples for the same study period.

Austria, (Amman et al., 2022)	Composite / polyethylene glycol precipitation	Monarch™ total RNA Miniprep kit	^a N1	SARS-CoV-2 RNA quantification: RT-qPCR; Variant analysis: Illumina Whole Genome Sequencing	B.1.160, B.1.351 (Beta), B.1.1.7 (Alpha), B.1.617.2 (Delta)	-Reported that a robust and error-tolerant method was developed successfully as a quantitative detection assay for different viral variants from wastewater. -Alpha, Delta, and Omicrons were the dominant variants. -Spatial variation of different variants in the nation was reported. -Highly correlated with clinical cases, even a single case in a catchment area was captured by WWS analysis of a variant.
Austria, (Markt et al., 2022)	Composite / polyethylene glycol precipitation	Monarch total RNA Miniprep kit (New England Biolabs, Ipswich, USA)	^a N1	SARS-CoV-2 RNA quantification: RT- qPCR; Variant analysis: Illumina sequencing	B.1.1.7 (Alpha)	-Variant detection in wastewater was concordant with clinical cases. - B.1.1.7 lineage was detected in wastewater 14 days before individual testing.
Luxembourg, (Herold et al., 2021)	Composite / centrifugation followed by filtration	QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany)	^a E, RdRP, N, and S	SARS-CoV-2 RNA quantification: Multiplex RT- qPCR; Variant analysis: Illumina sequencing	B.1.1.7 (Alpha), B.1.1.420, B.1.160, B.1.177, B.1.221, B.1.258, B.1.351 (Beta), B.1.474	-B.1.1.7 was the dominant variant during the time of the study. - Mutations were more enriched in the S gene than in other tested genes in the study. -Frequencies of mutations found in wastewater corresponded to those detected in the

						population. Wastewater-based variant surveillance and novel SARS-CoV-2 variants detection
Belgium, (Boogaerts et al., 2022)	Composite / centrifugal filter	Automated Maxwell PureFood GMO	^a E; ^b SΔ69/70 deletion, N501Y, SΔ241 and SΔ157	Multiplex dPCR	B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta)	-Multiplex dPCR assay was developed and validated to detect B.1.1.7, B.1.351, and B.1.617.2 strains in influent wastewater. -B.1.351 strain was not detected in wastewater.
Ireland, (Reynolds et al., 2022)	Composite / centricon Plus- 70 filters	RNeasy PowerMicrobiome kit (Qiagen, Germany)	N1,	RT-qPCR/ ddPCR, alpha (B.1.1.7) variant, and the delta (B.1.617.2) variant	B.1.1.7 (Alpha), B.1.617.2 (Delta)	-Variant detection in wastewater was concordant with clinical cases. -Alpha-variant in WW was detected before clinical cases.
Denmark, (Rasmussen et al., 2022)	Composite / not mentioned	Not mentioned	Not mentioned	SARS-CoV-2 RNA quantification: RT- qPCR Variant analysis: Initial screening by qPCR targeting K417N mutation followed by Nanopore sequencing	B.1.1.529 (Omicron)	-Combination of mutation-specific qPCR and nanopore sequencing for WWS can identify variants even at low SARS- CoV-2 loads.
South Africa, (Johnson et al., 2022)	Grab / centrifugation	RNeasy PowerSoil kit (Qiagen, Germany)	^a N1 and N2; ^b S	SARS-CoV-2 RNA quantification: RT-qPCR; Variants analysis: genotyping PCR and sequencing	B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta)	-SARS-CoV-2 nucleic acid fragments shed from numerous infected people were mixed in wastewater. -Beta variant was the predominant variant during the study.

Brazil, (Barbosa et al., 2022)	Grab / ultracentrifugation and glycine elution	QIAamp® Viral RNA Mini kit (Qiagen, Germany)	^a N1 and N2; ^b Whole viral genome	SARS-CoV-2 RNA quantification; RT-qPCR; Variants analysis: RT-PCR based Illumina sequencing	P.1 (Gamma)	Lineage P.1 was the most prevalent variant in sewage and clinical data
Argentina, (Masachessi et al., 2022)	Grab / polyethylene glycol precipitation	MagNA Pure 96 DNA and Viral NA Large Volume kit	N and Orflab gene	SARS-CoV-2 RNA quantification: RT-qPCR Variant analysis: RT-qPCR targeting N501Y, E484K, delH69V70, K417T, delL242_244L, L452R and P681R	B.1.1.7 (Alpha), P.1 (Gamma), B.1.617.2 (Delta)	-WWS of SARS-CoV-2 allows tracking of variants in studied communities.
Australia, (Ahmed, et al., 2022a)	Composite / concentrating Pipette Select	QiaAmp Viral RNA MiniKit (Qiagen, USA)	N and S (H69-V70 del) genes	SARS-CoV-2 and H69-V70del quantification: RT-PCR; Variant analysis: ARTIC Network V3 scheme with Nanopore and ATOPlex amplicon sequencing	B.1.1.529 (Omicron)	Detected the Omicron variant in wastewater samples collected from an aircraft.
Nepal, (Napit et al., 2021)	Composite, grab / polyethylene glycol precipitation	Automated DNA and RNA extraction system (abGenixAIT 159 Biotech, Singapore)	^a E, N, and ORF1	SARS-CoV-2 RNA quantification: RT-PCR; Variants analysis: Tiled amplicon sequencing (Nanopore)	B.1, B.1.36, B.1.1	-SARS-CoV-2 RNA was detected in 16 of 20 different sampled sites. - The circulated lineages of SARS-CoV-2 in wastewater were B.1.1 (50.0%), B.1.36 (33.3%), and B.1 (16.7%).
Philippines, (Otero et al., 2021)	Grab / polyethylene glycol precipitation	MagMax 175 Viral/Pathogen Nucleic Acid Isolation kit (Thermo Fisher Scientific Inc., USA)	^a N, RdRP, E	SARS-CoV-2 RNA detection: RT-PCR; Variants analysis: Nanopore sequencing	B.1, B.1.1, B.6	-SARS-CoV-2 RNA was detected in the wastewater samples at 91.7% (22/24). - RdRP was the most abundant in samples (87.5%), followed by N

						(83.3%), then E (62.5%). -The RNA levels concentrated in wastewater correlated with SARS-CoV-2 RNA Ct values.
Japan, (Malla et al., 2022)	Grab / modified PEG precipitation	QiaAmp Viral RNA MiniKit (Qiagen, USA)	N, S, and pathogenic virus genes.	High-throughput RT-qPCR performed with the Biomark HD system (Fluidigm; currently Standard BioTools)	B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta) B.1.617.1 (Kappa)	-Simultaneously detected 22 targets, including SARS-CoV-2, variants, and pathogenic viruses in a single run. -Alpha and Delta variants detection in wastewater coincided with the first clinical cases. -The chance of SARS-CoV-2 detection increased when multiple SARS-CoV-2 detection assays were used.
Israel, (Yaniv et al., 2022)	Composite / centrifugation and filtration	QiaAmp 96 Viral RNA kit (Qiagen, Germany)	S gene	One Step PrimeScript III RT-qPCR mix RR600 TAKARA, Japan) using Applied Biosystems Thermocycler (Thermo Scientific)	B.1.617.2 (Delta), B.1.1.529 (Omicron)	-Developed Omicron primers-probe set was highly specific and sensitive, with a LOD of 1.4 copies per μ L reaction. -Noted a special pattern between the outbreak of Delta -Omicron dynamics.
Israel, (Bar-Or et al., 2022)	Composite / electronegative MCE membranes	NucliSENS easyMAG system (Biomérieux, Marcy-l'Etoile, France)	E, N, and S	Combination of inclusive and selective quantitative PCR assays	A19/A20 or B.1.1.7 (Alpha)	-Dynamics between two variants were reported, where B.1.1.7 replaced the A19 during the study period.

Israel, (Erster et al., 2022)	Composite / centrifugation, MgCl ₂ and filtration	MagNA Pure 96 system (Roche) or the PPS MagLEAD system	E, N, S, and ORF8	SARS-CoV-2 RNA quantification: RT- qPCR. Sequence variation analysis: RT-PCR- and nPCR-based NGS Sanger sequencing.	B.1.1.7 (Alpha) and B.1.617.2 (Delta)	-New PCR-based method is rapid and cost-effective at targeting different mutations of SARS- CoV-2.
Israel, (Bar-Or et al., 2021)	Composite / centrifugation and filtration	NucliSENS easyMAG system (bioMérieux, Marcyl'Etoile, France)	^a E and RdRp; ^b Whole- genome	Multiplex RT-qPCR (Bio-Rad CFX96). Sanger sequencing of culture-derived and clinical samples.	B.1.1.7 (Alpha), and B.1.617.2 (Delta), P.1 (Gamma) B.1.429 (Epsilon) B.1.526 (Iota), A.23.1 B.1.525 (Eta)	-WWS of SARS-CoV- 2 allows tracking of variants in studied communities. -WWS worked as early warning tool -Wastewater detection of SARS-CoV-2 variants was concordant with clinical data.
India, (Joshi et al., 2022)	Grab / polyethylene glycol precipitation	NucleoSpin® RNA Virus isolation kit (Macherey- Nagel GmbH & Co. KG, Germany)	^a E and RdRp; ^b Whole- genome	Ion AmpliSeq Community SARS- CoV-2 research panel and Ion AmpliSeq library kit Plus (Invitrogen/ Thermo Fisher Scientific) Illumina sequencing. SARS-CoV-2 RNA quantification; RT- qPCR. Variants analysis: RT-PCR- based direct Illumina sequencing	B.1.617.2 (Delta)	-35 mutations in the spike protein were reported across four samples and were categorized into 23 types. -Spike protein mutations related to the B.1.617.2 lineage. -B.1.617.2 appeared in wastewater about a month before the first clinically diagnostic case of the same variant.
India, (Nag et al., 2022)	Grab / centrifugation	MagMAX Viral/Pathogen Nucleic Acid Isolation kit (Applied Biosystems,	^a E, RdRP, N, and ORF1ab; ^b Single nucleotide polymorphisms (SNPs)	SARS-CoV-2 RNA quantification; RT- qPCR; SNPs analysis: RT- PCR-based Illumina sequencing	B.1, B.1.617.2 (Delta)	-E, N, RdRP gene regions were detected in all wastewater influent. -B.1.617.2 was most frequent and detected in all samples, except

		Waltham, MA USA)				for two samples where B.1 was detected.
India, (Dharmadhikari et al., 2022)	Grab / mixed cellulose ester filter	RNeasy PowerWater kit (Qiagen; 14700-50- NF)	^a E and N; ^b 39 types of mutations	SARS-CoV-2 RNA quantification; RT- qPCR; Variants analysis: Nanopore sequencing	B.1.617.2 (Delta)	-Five novel mutations were detected in variants monitored in wastewater that were not reported from clinical sequence data in India. -Prevalence of B.617.2 percentage over wild type in wastewater was similar to the infection cases associated with B.617.2.

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