Table S1: Overview of sample handling, concentration and extraction workflows, physicochemical parameters, and downstream analytical methods. In table A, you can find an overview of sample characteristics, physicochemical parameters and downstream analysis across concentration and extraction methods. For each time point, 50 mL of wastewater was concentrated using different concentration methods (INNOV = InnovaPrep; NANO = Nanotrap Magnetic Virus Particles; Prec = PEG/NaCl precipitation; UltraC = ultracentrifugation; Zymo = ZymoPURE Water DNA/RNA Kit) and extracted using either the QIAamp RNeasy PowerFecal Pro Kit (RN), the QIAamp Viral RNA Mini Kit (QIA), or the ZymoPURE kit (Zymo). Eluates were stored at −80 °C prior to downstream analysis. Physicochemical parameters (pH and conductivity) were measured at the time of sampling. The table indicates which downstream applications were performed for each method and time point, including (RT-)ddPCR, Illumina-based SARS-CoV-2 targeted sequencing, and Oxford Nanopore Technologies (ONT) metagenomic sequencing. Table B summarizes key methodological metadata recommended by the Environmental Microbiology Minimum Information (EMMI) guidelines, including sample type, processed volume, storage conditions, sampling time points, physicochemical parameters (pH and conductivity), concentration and extraction methods, and downstream analytical applications.

A

	Sample volume processed	Storage temperature eluate	Time Points	pH	Conductivity (µS/cm)	Concentration method	Extraction Method	(RT-)ddPCR	Illumina sequencing	ONT metagenomics	Inhibition assessment
Raw + Spiked wastewater at WWTP A	50 mL	-80°C	T1	7.14	979.3	INNOV	RN	Yes	No	No	No
						NANO	RN	Yes	No	No	No
						Prec	QIA	Yes	No	No	No
						Prec	RN	Yes	No	No	No
						UltraC	RN	Yes	No	No	No
						Zymo	Zymo	Yes	No	No	No
			T2	7.62	954.00	INNOV	RN	Yes	Yes	No	No
						NANO	RN	Yes	Yes	Yes	No
						Prec	QIA	Yes	Yes	No	No
						Prec	RN	Yes	Yes	Yes	No
						UltraC	RN	Yes	Yes	No	No
						Zymo	Zymo	Yes	Yes	Yes	No
			T3	7.59	1615.00	INNOV	RN	Yes	No	No	Yes
						NANO	RN	Yes	No	No	Yes
						Prec	QIA	Yes	No	No	Yes

Sample	Volume	Temp	T	pH	Value	Method	Kit				
						Prec	RN	Yes	No	No	Yes
						UltraC	RN	Yes	No	No	Yes
						Zymo	Zymo	Yes	No	No	Yes
			T4	7.63	972.60	INNOV	RN	Yes	No	No	No
						NANO	RN	Yes	No	Yes	No
						Prec	QIA	Yes	No	No	No
						Prec	RN	Yes	No	Yes	No
						UltraC	RN	Yes	No	No	No
						Zymo	Zymo	Yes	No	Yes	No
			T5	7.83	1174.00	INNOV	RN	Yes	Yes	No	No
						NANO	RN	Yes	Yes	Yes	No
						Prec	QIA	Yes	Yes	No	No
						Prec	RN	Yes	Yes	Yes	No
						UltraC	RN	Yes	Yes	No	No
						Zymo	Zymo	Yes	Yes	Yes	No
Raw + Spiked wastewater at WWTP B	50 mL	-80°C	T1	7.47	782.30	INNOV	RN	Yes	No	No	No
						NANO	RN	Yes	No	No	No
						Prec	QIA	Yes	No	No	No
						Prec	RN	Yes	No	No	No
						UltraC	RN	Yes	No	No	No
						Zymo	Zymo	Yes	No	No	No
			T2	7.39	934.60	INNOV	RN	Yes	No	No	No
						NANO	RN	Yes	No	No	No
						Prec	QIA	Yes	No	No	No
						Prec	RN	Yes	No	No	No
						UltraC	RN	Yes	No	No	No
						Zymo	Zymo	Yes	No	No	No
			T3	7.43	888.70	INNOV	RN	Yes	No	No	Yes

				NANO	RN	Yes	No	No	Yes
				Prec	QIA	Yes	No	No	Yes
				Prec	RN	Yes	No	No	Yes
				UltraC	RN	Yes	No	No	Yes
				Zymo	Zymo	Yes	No	No	Yes
		T4	7.74	1344.00	INNOV	RN	Yes	No	No	No
					NANO	RN	Yes	No	No	No
					Prec	QIA	Yes	No	No	No
					Prec	RN	Yes	No	No	No
					UltraC	RN	Yes	No	No	No
					Zymo	Zymo	Yes	No	No	No
		T5	7.70	1126.00	INNOV	RN	Yes	No	No	No
					NANO	RN	Yes	No	No	No
					Prec	QIA	Yes	No	No	No
					Prec	RN	Yes	No	No	No
					UltraC	RN	Yes	No	No	No
					Zymo	Zymo	Yes	No	No	No

B

EMMI Category	Parameter	Description / Reporting in this study
Sampling	Sampling location	Influent wastewater collected from two Belgian wastewater treatment plants
	Sampling type	24-hour composite samples
	Sampling dates	Five time points every two weeks in the spring of 2024
	Sample volume processed	50 mL
Sample handling and storage	Time processing	Samples were processed with 72 hours of collection
	Storage conditions	Samples stored at 4°C prior to processing; aliquots of extracted sample stored at -80°C
	Freeze-thaw cycles	Minimized, no freeze-thaw cycles prior to nucleic acide extraction
Physicochemical metadata	pH	Measured for all samples
	Conductivity	Measured for all samples
	Additional parameters (TSS, COD,…)	Not measured
Concentration methods	Methods evaluated	PEG/NaCl precipitation; Nanotrap Magnetic Virus Particles; ultrafiltration (InnovaPrep); ultracentrifugation ; ZymoPURE Water DNA/RNA Kit
	Fraction analysed	Liquid fraction of wastewater except for Nanotrap
Extraction methods	Kits evaluated	QIAamp RNeasy PowerFecal Pro Kit; QIAamp Viral RNA Mini Kit; ZymoPURE Water DNA/RNA Kit
	Elution volume	100 µL for QIAamp RNeasy PowerFecal Pro Kit and 60 µL for QIAamp Viral RNA Mini Kit and ZymoPURE Water DNA/RNA Kit
	Inhibitor removal	Included in PowerFecal Pro and ZymoPURE workflows; not included in Viral RNA Mini Kit
Controls	Positive controls	Spiked wastewater samples with SARS-CoV-2, RSV, and influenza viruses
	Negative controls	No-template controls included for ddPCR assays
	Process controls	No external process controls used for absolute recovery calculation
Quantification assays	PCR platform	Digital droplet PCR (ddPCR) and RT-ddPCR
	Targets (RNA)	SARS-CoV-2 (ORF1a, RdRP), RSV A/B, influenza A/B, norovirus GI/GII
	Targets (DNA)	AMR genes blaCTX-M and tetM
Sequencing (targeted)	Platform	Illumina
	Application	SARS-CoV-2 amplicon sequencing
	Input selection	See Table A
Sequencing (metagenomics)	Platform	Oxford Nanopore
	Library type	Shotgun metagenomics
	Input selection	See Table A