

Supplementary Materials

Developing biosensors for SARS-CoV-2 wastewater-based epidemiology: a systematic review of trends, limitations and future perspectives

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SARS-CoV-2 analysis from wastewater.

Sample region	Sampling technique and sample storage	Sample(s) origin	Concentration technique	Extraction method	Detection technique	Target gene	Positive control(s) - Reference virus (surrogate)	Ref
Germany	<u>Sampling:</u> composite, 24h, flow-dependent <u>Transport:</u> on ice <u>Storage:</u> immediate analysis	Raw WwTW influent after sand trap Various WwTW effluents	centrifugal UF	NucleoSpin RNA Virus kit (Macherey Nagel)	Sanger sequencing RT-qPCR	<i>RdRp</i> ^b <i>E</i> <i>M</i> ^c <i>N</i>	endogenous and exogenous bioindicators	[33]
Canada	<u>Sampling:</u> refrigerated composite, 24h, 1 hour intervals <u>Transport:</u> on ice <u>Storage:</u> refrigerated	Raw WRRF influent after grit removal PCS from WRRF	PEG precipitation	RNeasy PowerMicrobiome Kit (Qiagen)	RT-qPCR RT-ddPCR	<i>E</i> N1 N2 N3	human microbiome-specific HF183 Bacteroides 16S ribosomal rRNA eukaryotic 18S rRNA PMMoV	[37]
Sweden and Italy	<u>Sampling:</u> NI <u>Transport:</u> NI <u>Storage:</u> Refrigerated frozen	*Untreated municipal wastewaters	UF double-UF ^a adsorption-extraction, centrifugation-adsorption-extraction	RNeasy PowerMicrobiome Kit (Qiagen) Trizol LS/chloroform	RT-qPCR	N	BCoV PMMoV	[39]
Japan	<u>Sampling:</u> grab composite, generated from defined daily frozen samples <u>Transport:</u> NI <u>Storage:</u> frozen	*Raw WwTW influent	UF EMV PEG precipitation after pre-centrifugation ^a	QIAamp Viral RNA Mini Kit (Qiagen) TRIzol LS/RNeasy PowerMicrobiome Kit (Qiagen)	RT-qPCR	<i>N1</i> <i>N2</i> <i>N3</i>	<i>Pseudomonas</i> phage phi6 MS2 phage	[123]

Australia	<u>Sampling:</u> composite collected refrigerated composite from a submersible in-situ high frequency autosampler <u>Transport:</u> NI <u>Storage:</u> refrigerated	Untreated influent WwTW Pumping station (PS).	UF electronegative vortex	QIAamp Viral RNA Mini Kit (Qiagen) AGPC/ TRIzol (Thermo Fisher Scientific)	Sanger sequencing Amplicon sequencing (Illumina MiSeq) RT-qPCR	N	<i>Oncorhynchus keta</i> (O. <i>keta</i>)	[15]
Japan	<u>Sampling:</u> grab <u>Transport:</u> on ice <u>Storage:</u> immediate analysis	Raw WwTW influent Secondary treated water from WwTW (before chlorination) River samples	EMV ^a membrane adsorption direct RNA	QIAamp Viral RNA Mini Kit (Qiagen)	RT-qPCR Nested PCR	S N1 N2 ORF1a	PMMoV MS2 phage	[124]
Italy	<u>Sampling:</u> grab, 1pm <u>Transport:</u> refrigerated <u>Storage:</u> immediate analysis	Raw WwTW influent WwTW effluent after tertiary disinfection River samples	UF	QIAMP VIRAL RNA mini kit (Qiagen) RNeasy PowerWater Kit (Qiagen)	RT-qPCR Whole genome sequencing	N ORF1ab E	SARS-like coronavirus	[67]
USA	<u>Sampling:</u> composite 24h grab, between 7- 11am <u>Transport:</u> on ice <u>Storage:</u> immediate analysis frozen	Untreated WwTW influent Secondary treated water from WwTW Final effluent after disinfection	UF adsorption–elution method using an electronegative membrane	ZR Viral RNA Kit (ZymoResearch)	RT-qPCR	N1 N2	<i>Pseudomonas</i> phage phi6	[125]
USA	<u>Sampling:</u> grab <u>Transport:</u> NI <u>Storage:</u> refrigerated	Primary WwTW samples Primary sludge samples	UF bag-mediated filtration skimmed milk flocculation ^a PEG precipitation sludge extraction	QIAamp Viral RNAMini Kit (Qiagen) RNeasy PowerSoil Total RNA kit (Qiagen)	RT-qPCR	N1 N2 N3	HCoV OC43	[126]

India	<u>Sampling:</u> grab, 11.30am <u>Transport:</u> on ice <u>Storage:</u> refrigerated	Raw WwTW influent WwTW effluent (no disinfection)	centrifugation filtration/PEG precipitation	NucleoSpin® RNA Virus (Macherey- Nagel)	RT-qPCR	<i>S</i> <i>N</i> <i>ORF1ab</i>	MS2 phage	[127]
Argentina	<u>Sampling:</u> grab <u>Transport:</u> NI <u>Storage:</u> refrigerated	Raw WwTW influent	UF adsorption-elution- PEG precipitation PEG precipitation ^a PAC flocculation ^a skim milk flocculation, adsorption-elution UF	proteinase K treatment / Maxwell®RSC 48 Extraction System (Promega)	RT-qPCR	<i>N1</i> <i>N2</i>	RNase P	[128]
USA	<u>Sampling:</u> grab, 10-11am refrigerated composite, 24h, flow- dependent <u>Transport:</u> NI <u>Storage:</u> NI	WwTW influent WwTW primary effluent River samples Untreated lake surface water Finished drinking water	hollow fiber UF centrifugal filtration PEG precipitation	Purelink Viral RNA/DNA Mini Kit (Thermo Fisher Scientific)	RT-qPCR	<i>E</i> <i>N1</i> <i>N2</i> <i>ORF1a</i>	BCoV PMMoV	[129]
Spain	<u>Sampling:</u> composite, 24h <u>Transport:</u> refrigerated <u>Storage:</u> refrigerated frozen	Raw WwTW influent	centrifugal UF ^a automated concentrating pipette	QIAamp Viral RNA Mini kit (Qiagen)	qPCR RT-qPCR	<i>N1</i>	NI	[130]
Brazil	<u>Sampling:</u> composite, 24h, 25 mins <u>Transport:</u> NI <u>Storage:</u> frozen	Raw sewage from network	glycine/PEG precipitation	QIAamp Viral RNA Mini kit (Qiagen)	RT-qPCR Amplicon sequencing (Illumina DNA Prep kit)	<i>RT- qPCR</i> <i>N1</i> <i>S</i> <i>RdRp</i>	2019-nCoV_N_Positive Control plasmid DNA (IDT)	[32]
Brazil	<u>Sampling:</u> composite, 10h <u>Transport:</u> refrigerated <u>Storage:</u> refrigerated	Raw sewage from network Raw WwTW influent	ultra centrifugation/ glycine	QIAamp® Viral RNA Mini kit (Qiagen)	RT-qPCR Whole genome sequencing (Illumina MiSeq)	<i>N</i>	BRSV/Inforce™ 3 (Zoetis, US) bacteriophage (PP7, ATCC 15692-B2) 2019-nCoV RUO Kit (Integrated DNA Technologies)	[46]

USA	<u>Sampling:</u> refrigerated composite, 24h, flow- dependent <u>Transport:</u> NI <u>Storage:</u> refrigerated	Raw WRRF influent after stone trap	filtration	RNeasy Mini Kit (Qiagen)	RT-qPCR Sanger sequencing Whole genome sequencing (MinION/AR TIC)	N1 N2	control plasmid (IDT#10006625) (PTC)	[45]
USA	<u>Sampling:</u> composite 24h, 15 mins <u>Transport:</u> NI <u>Storage:</u> immediate analysis	Raw sewage from network	UF (viral particles) silica columns or silica milk (total RNA) oligo-capture	AllPrep DNA/RNA mini kit (Qiagen)	RT-qPCR Whole genome sequencing (Illumina NextSeq 550)	N1	synthetic RNA standards of the SARS- CoV-2 genome (Twist Biosciences)	[131]
Cruise ship and Aircraft	<u>Sampling:</u> grab, on arrival <u>Transport:</u> on ice <u>Storage:</u> refrigerated	Influent and effluent of cruise ship membrane bioreactor Aircraft sewage tanks	UF adsorption– extraction with electronegative membrane	RNeasy PowerWater Kit (Qiagen) RNeasy PowerMicrobiome Kit (Qiagen)	RT-qPCR RT-ddPCR Amplicon sequencing (Illumina NextSeq)	N E	NI	[132]

Abbreviations: PEG, polyethylene glycol; PAC, Aluminum polychloride; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; RT-ddPCR, reserve transcription-digital droplet polymerase chain reaction; *RdRp*, RNA dependent RNA polymerase gene; *E*, envelope gene; *M*, membrane gene; *N*, nucleocapsid gene; ORF, open reading frame; BCoV, Bovine coronavirus; HCoV, Human coronavirus; PPMoV, Pepper mild mottle virus; MHV, Murine hepatitis virus-A59; ref, reference. WwTW - wastewater treatment works, WRRF - water resource recovery facility, NI no information given,

Superscript: a, viral concentration technique identified as most suitable; b, viral most specific target; c, viral most sensitive target.

* limited information regarding sampling locations and methods.

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