Supporting Information

Detection and infectivity of SARS-CoV-2 in Korean municipal wastewater facilities and characterization of environmental factors influencing wastewater-bound SARS-CoV-2

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SI Table S1.

Primers and probes for RT-qPCR PEDV quantification, cycling conditions, and equations for calculating the recovery rate.

Forward primer	**Reverse primer**	**Probe**	**Cycling conditions**	**Recovery rate calculation**
5′-CGC AAA GAC TGA ACC CAC TAA TTT-3′	5′-TTG CCT CTG TTG TTA CTT GGA GAT-3′	5′-HEX-TGT TGC CAT TGC CAC GAC TCC TGC-BHQ2-3′	50℃ for 20 min, 95℃ for 10 min, 5 cycles of 95℃ for 10s and 60℃ for 30s, and 40 cycles of 95℃ for 10s and 60℃ for 30s.	Recovery rate = $\frac{Concentration\ of\ quantified\ PEDV\ in\ wastewater\ after\ sample\ processing\ (gene\ copies/L)}{Concentration\ of\ seeded\ PEDV\ in\ wastewater\ before\ sample\ processing\ (gene\ copies/L)}$

SI Table S2.

Information of SARS-CoV-2 RT-qPCR detection kits.

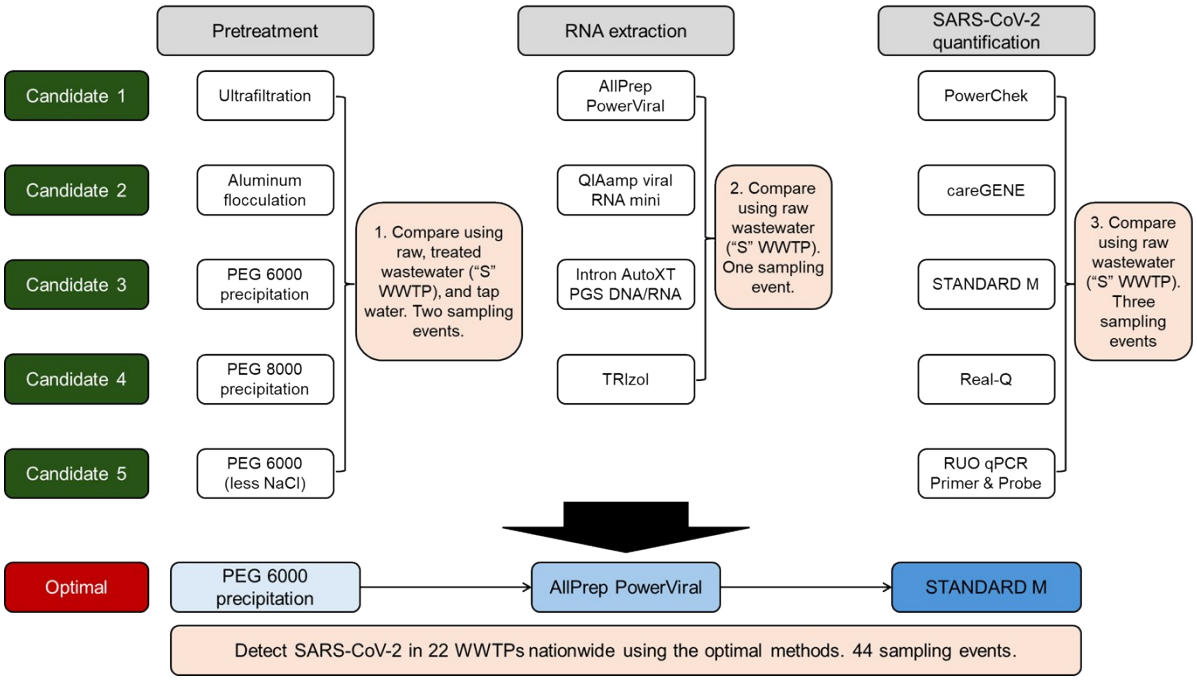
Kit	Target gene	Primers and probe (5′-3′)	Cycling conditions	Limit of detection	Detection criteria for positive
PowerChek™ 2019-nCoV Real-time PCR	RdRp and E gene	RdRp Forward: GTG ARA TGG TCA TGT GTG GCG G RdRp Reverse: CAR ATG TTA AAS ACA CTA TTA GCA TA RdRp Probe: FAM-CAG GTG GAA CCT CAT CAG GAG ATG C-BHQ	50 ℃ for 30 min, 95 ℃ for 10 min, and 40 cycles of 95 ℃ for 15 s and 60 ℃ for 1 min.	18 gene copies /reaction	RdRp $C_t \leq 37$, E gene $C_t \leq 37$, and positive control $C_t \leq 28$
careGENE™ N-CoV RT-PCR			25 ℃ for 2 min, 55 ℃ for 10 min, 94 ℃ for 3 min, and 45 cycles of 94 ℃ for 15 s and 60 ℃ for 30 s.	50 gene copies /reaction	RdRp, E gene, and positive control $C_t \leq 45$
STANDARD™ M nCoV Real-time Detection			50 ℃ for 15 min, 95 ℃ for 3 min, 5 cycles of 95 ℃ for 5 s and 60 ℃ for 40 s, and 40 cycles of 95 ℃ for 5 s and 60 ℃ for 40 s.	5 gene copies /reaction	RdRp $C_t \leq 36$, E gene $C_t \leq 36$, and positive control $C_t \leq 32$
Real-Q 2019-nCoV Detection		E gene Forward: ACA GGT ACG TTA ATA GTT AAT AGC GT E gene Reverse: ATA TTG CAG CAG TAC GCA CAC A E gene Probe: FAM (HEX)-ACA CTA GCC ATC CTT ACT GCG CTT CG-BHQ	50 °C for 30 min, 95 °C for 15 min and 40 cycles at 95 °C for 15 s, and 62 °C for 45 s	31.25 gene copies /reaction	RdRp $C_t \leq 38$, E gene $C_t \leq 38$, and positive control $C_t \leq 28 \pm 5$
SARS-CoV-2 Research Use Only qPCR Primer & Probe Kit	RdRp		45 °C for 20 min, 95 °C for 2 min and 45 cycles at 95 °C for 10 s, and 55 °C for 30 s	35 gene copies/reaction (Barra etal.,2020; Kaya et al., 2022)	$C_t \leq 35$ and positive control $C_t \leq 35$

SI Table S3.

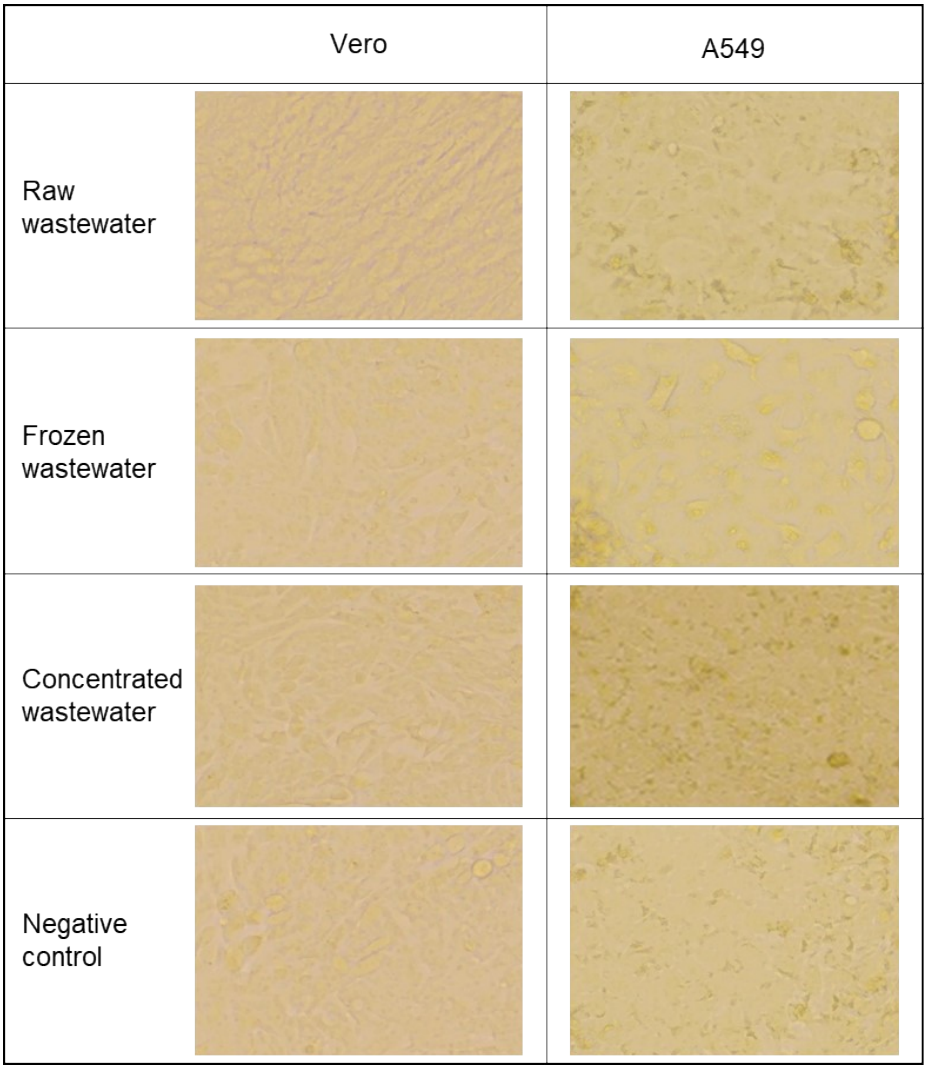
Cycle threshold results of RT-qPCR for SARS-CoV-2 detection in cell lysates from wastewater (WW) for infectivity testing.

Sample	Passage 1		Passage 2		Passage 3		Detection
	RdRp	E gene	RdRp	E gene	RdRp	E gene	
Raw WW (Vero)	> 40	> 40	> 40	> 40	> 40	> 40	Negative
Frozen WW (Vero)	> 40	> 40	> 40	> 40	> 40	> 40	Negative
Concentrated WW (Vero)	> 40	> 40	> 40	> 40	> 40	> 40	Negative
Raw WW (A549)	> 40	> 40	> 40	> 40	> 40	> 40	Negative
Frozen WW (A549)	> 40	> 40	> 40	> 40	> 40	> 40	Negative
Concentrated WW (A549)	> 40	> 40	> 40	> 40	> 40	> 40	Negative
Negative control	> 40	> 40	> 40	> 40	> 40	> 40	Negative
Positive control[1]	24.18	26.57	24.05	27.78	26.12	27.10	Positive

[1]Positive material corresponding to 100 gene copies/reaction was used for RT-qPCR.



SI Fig. S1. Summary of candidate virus-detection methods (pretreatment, RNA extraction, and SARS-CoV-2 quantification methods; see Section 2.2 for details) and an optimal method based on a comparative study.



SI Fig. S2 Infectivity results of wastewater-bound SARS-CoV-2 in four different samples tested on two different cell types. No cytopathic effects were observed in any of the samples.

References

Barra, G. B., Santa Rita, T. H., Mesquita, P. G., Jácomo, R. H., & Nery, L. F. A. (2020). Analytical Sensitivity and Specificity of Two RT-qPCR Protocols for SARS-CoV-2 Detection Performed in an Automated Workflow. Genes, 11, 1183. https://doi.org/10.3390/genes11101183

Kaya, D., Niemeier, D., Ahmed, W., & Kjellerup, B. V. (2022). Evaluation of multiple analytical methods for SARS-CoV-2 surveillance in wastewater samples. Science of The Total Environment, 808, 152033. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.152033