SUPPLEMENTARY MATERIAL

Optimizing analytical methods to detect SARS-CoV-2 in wastewater.

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Figure S1. Standard curves for targets N1, N2 and gene E performed with 10-fold dilutions (10^0-10^6 gc/reaction) of the genomic RNA (ATCC VR-1986D) and the synthetic plasmids of genes N (IDT 10006625) and E (IDT 10006896). For each gene and standard material, slope and R^2 are shown.

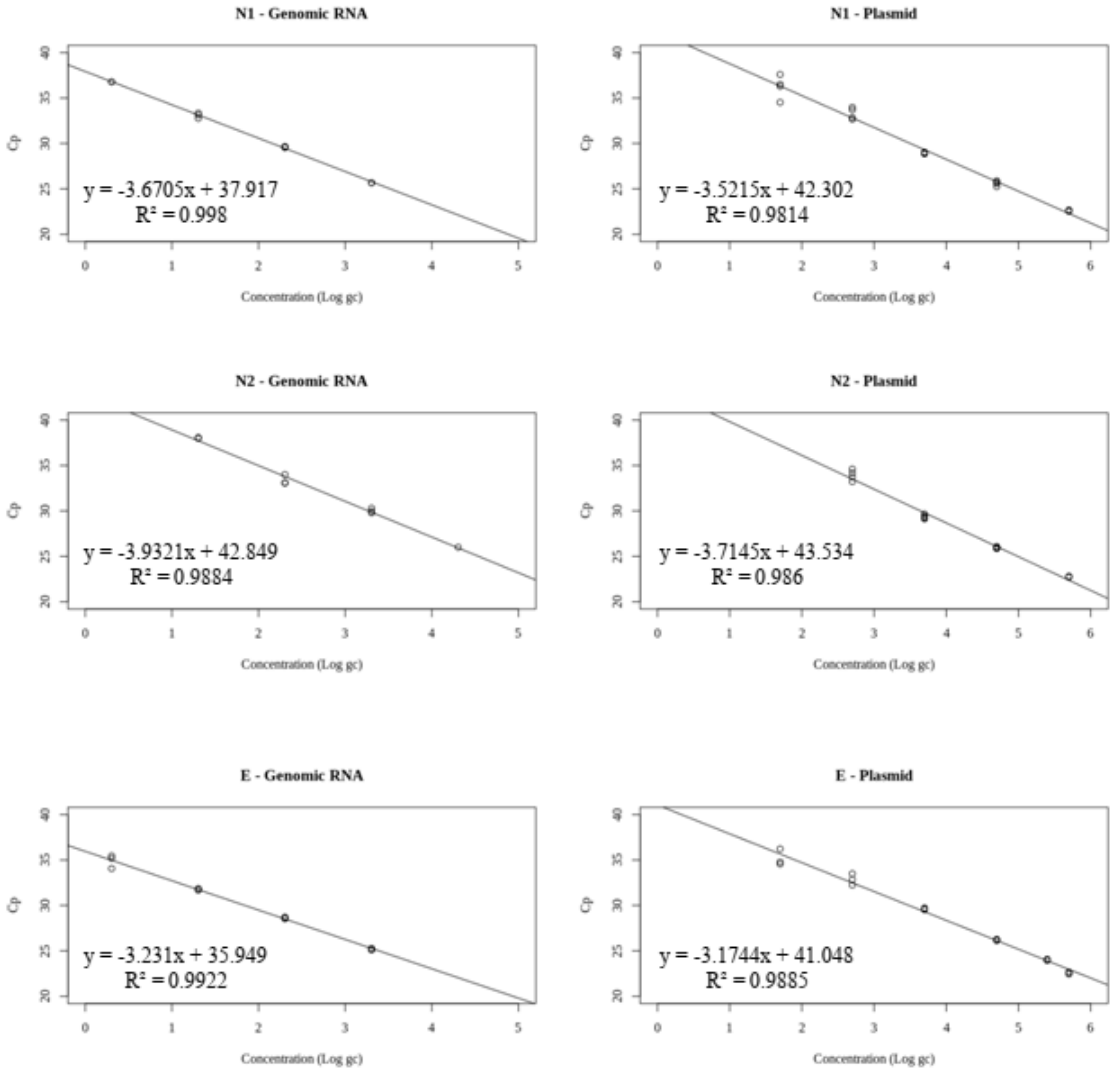

Table S1. Reaction mix volumes (in µl) used for the detection of SARS-CoV-2 by RT-qPCR. Reagents: Buffer, 2X One Step RT-PCR Buffer III; Enzyme 1, Takara Ex Taq HS (5u/ µl); Enzyme 2, PrimeScript RT enzyme Mix II. Total RNA volume of 2.5 µl. Water was added to a final reaction volume of 10 µl.

Target	Buffer	Enzyme 1	Enzyme 2	Forward primer	Reverse primer	Probe
N1, N2	5	0.2	0.2	0.75[a]		
E	5	0.2	0.2	0.5[a]		
IP4	5	0.2	0.2	0.4[b]	0.4[b]	0.2[b]
MgV, PEDV	5	0.2	0.2	0.5[b]	0.5[b]	0.5[b]

[a] Primers at 400nM final concentration; Probes at 200nM final concentration. Primers and probes premixed in commercial kits from IDT Technologies.

[b] From stock with an initial concentration of 10 µM.

Table S2. Thermal amplification conditions for the RT-qPCR used in the study for the detection of SARS-CoV-2, MgV and PEDV.

Target	Retrotranscription	Activation	Denaturation	Annealing	Cycles
N1, N2	50°C, 15 min	95°C, 2 min	95°C, 3 sec	55°C, 30 sec	45
IP2, IP4, E	55°C, 20 min	95°C, 3 min	95°C, 15 sec	58°C, 30 sec	50
MgV, PEDV	55°C, 15 min	95°C, 5 min	95°C, 5 sec	60°C, 10 sec 65°C, 10 sec	45

Table S3. Primers and probes used for the detection of SARS-CoV-2, PEDV and MgV.

Virus	Target	Name	Sequences (5’ – 3’)	Amplicon size (bp)	Reference
SARS-CoV-2	N1	USCDC_N1_F	GACCCCAAAATCAGCGAAAT	72	
		USCDC_N1_P	FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1		
		USCDC_N1_R	TCTGGTTACTGCCAGTTGAATCTG		
	N2	USCDC_N2_F	TTACAAACATTGGCCGCAAA	67	
		USCDC_N2_P	FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1		
		USCDC_N2_R	GCGCGACATTCCGAAGAA		
	E	Charité_E_F	ACAGGTACGTTAATAGTTAATAGCGT	113	
		Charité_E_P	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ		Corman et al., 2020
		Charité_E_R	ATATTGCAGCAGTACGCACACA		
	IP2	Pasteur_IP2_F	ATGAGCTTAGTCCTGTTG	108	
		Pasteur_IP2_P	FAM-AGATGTCTTGTGCTGCCGGTA-BHQ1		
		Pasteur_IP2_R	CTCCCTTTGTTGTGTTGT		
	IP4	Pasteur_IP4_F	GGTAACTGGTATGATTTCG	107	
		Pasteur_IP4_P	FAM-TCATACAAACCACGCCAGG-BHQ1		
		Pasteur_IP4_R	CTGGTCAAGGTTAATATAGG		
PEDV	M gene	PEDV_forward	CAGGACACATTCTTGGTGGTCTT	140	Zhou et al., 2017
		PEDV_probe	FAM-ACGCGCTTCTCACTAC-MGBNFQ		
		PEDV_reverse	CAAGCAATGTACCACTAAGGAGTGTT		
MgV	5’ NCR	Mengo 110	GCGGGTCCTGCCGAAAGT	100	ISO 15216-1:2017
		Mengo probe	FAM-ATCACATTACTGGCCGAAGC-MGBNFQ		
		Mengo 209	GAAGTAACATATAGACAGACGCACAC		

Table S4. Detection by RT-qPCR of SARS-CoV-2 in sewage samples targeting N1 region. Abbreviations: MN, NucleoSpin RNA virus kit (Macherey-Nagel GmbH & Co.); Max, Maxwell RSC (Promega); Ct, RT-qPCR cycle threshold.

Sample	**MN (Ct)**		**Maxwell (Ct)**	
Sample 1	36.50	35.77	34.53	35.18
Sample 2	-	-	-	36.14
Sample 3	-	-	32.34	32.02
Sample 4	34.95	35.54	36.29	35.76
Sample 5	37.05	-	35.78	35.71
Sample 6	-	36.61	34.96	35.96
Sample 7	39.31	39.42	33.99	34.62
Sample 8	40.00	40.00	37.14	38.8
Sample 9	38.32	-	37.04	-
Sample 10	36.93	36.83	36.77	35.7
Sample 11	39.14	-	37.65	36.84

References

Zhou, X.; Zhang, T.; Song, D.; Huang, T.; Peng, Q.; Chen, Y.; Li, A.; Zhang, F.; Wu, Q.; Ye, Y.; Tang, Y., Comparison and evaluation of conventional RT-PCR, SYBR green I and TaqMan real-time RT-PCR assays for the detection of porcine epidemic diarrhea virus. Mol. Cell. Probes 33 (2017) 36–41 10.1016/j.mcp.2017.02.002.