Supporting Information for

Adsorption of Respiratory Syncytial Virus (RSV), Rhinovirus, SARS-CoV-2, and F+ bacteriophage MS2 RNA onto wastewater solids from raw wastewater

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Linear and Langmuir models

The Linear and Langmuir isotherm equations are defined as

$$\text{Linear::} \qquad K_d = q_e/C_e \qquad (1)$$

$$\text{Langmuir:} \qquad q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \qquad (2)$$

where q_e is the equilibrium concentration of viral genomes in solids (cp/g), C_e is the equilibrium concentration of viral genomes in the liquid fraction of wastewater, q_{max} is the maximum adsorption capacity (cp/g), K_L is the Langmuir constant, and K_d is the distribution coefficient.

Additional details related to EMMI[1] guidelines

The average number of droplets per two merged wells was 40,476 (standard deviation = 3,184). The machine vendor reports the droplet size as 0.00085 µL. Average number of copies per partition (λ) (standard deviation) for SARS-CoV-2 N gene was 0.027 (0.0008) and for BCoV was 0.238 (0.004). λ for other human viruses was similar to SARS-CoV-2.

Table S1: Primers and probes for RT-ddPCR assays

Target	Sequence	Reference
SARS-CoV-2 (N gene)	Forward primer: 5′-CATTACGTTTGGTGGACCCT-3' Reverse primer: 5′-CCTTGCCATGTTGAGTGAGA-3' Probe: CGCGATCAAAACAACGTCGG (5' FAM/ZEN/3' IBFQ)	[2]
RSV (N gene)	Forward primer: 5′- CTCCAGAATAYAGGCATGAYTCTCC-3' Reverse primer: 5′- GCYCTYCTAATYACWGCTGTAAGAC-3' Probe: TAACCAAATTAGCAGCAGGAGATAGATCAG (5' HEX/ZEN/3' IBFQ)	[3]
RV	Forward primer: 5′-GCCYGCGTGGCKGCC-3' Reverse primer: 5′-GAAACACGGACACCCAAAG-3' Probe: TCCTCCGGCCCCTGAATG (5' FAM/ZEN/3' IBFQ)	[3]
MS2	Forward: 5'-GTCCATACCTTAGATGCGTTAGC-3' Reverse: 5'-CCGTTAGCGAAGTTGCTTGG-3' Probe: ACGTCGCCAGTTCCGCCATTGTCG (5' FAM/ZEN/3' IBFQ)	[4,5]
BCoV (Extraction control)	Forward primer: 5'-CTGGAAGTTGGTGGAGTT-3' Reverse primer: 5'-ATTATCGGCCTAACATACATC-3' Probe: CCTTCATATCTATACACATCAAGTTGTT (5' FAM/ZEN/3' IBFQ)	[6]

All probes contained fluorescent molecules and quenchers (5′ FAM and or HEX/ZEN/3′ IBFQ); FAM, 6-fluorescein amidite; HEX, hexachloro-fluorescein; ZEN, a proprietary internal quencher from Integrated DNA Technologies (Coralville, IA, USA); and IBFQ, Iowa Black FQ.

Table S2: Thermal cycling conditions for SARS-CoV-2, RSV, RV, MS2, and BCoV

Cycling Step	Temperature °C	Time	Number of Cycles
Reverse transcription	50	60 min	1
Enzyme activation	95	10 min	1
Denaturation	95	30 sec	40
Annealing/extension	SARS-CoV-2 and RSV: 59 RV: 61 MS2: 60 BCoV: 56	1 min*	40
Enzyme deactivation	98	10 min	1
Hold	4	Infinite	1

*Ramp rate set to 2°C/sec

Table S3: Served population and annual average daily flow (MGD) of wastewater treatment plants

Plant	Location	Approximate number of people served in the sewershed[†]	Annual Average daily flow (MGD)	Min and Max TSS reported in 2022 (mg/L)	Min and Max pH reported in 2022 [-]
Oceanside Water Pollution Control Plant (OS)	San Francisco	250,000	21.7	33–1,630	*
Southeast Water Pollution Control Plant (SE)	San Francisco	580,000	56.9	26–727	7.0–7.9
Silicon Valley Clean Water Wastewater Treatment Plant (SV)	Redwood City	199,000	12.4	160–400	7.0–7.6
Sunnyvale Water Pollution Control Plant (SU	Sunnyvale	161,021	12.6	164–376	6.4–7.2
San Jose-Santa Clara Regional Wastewater Facility (SJ)	San Jose	1,419,393	90.7	250–400	7.5–7.9
South County Regional Wastewater Treatment Plant (GI)	Gilroy	105,394	8.5	152–552	6.5–7.3

Notes:

[†] Based on the most recent National Pollution Discharge Elimination System (NPDES) permit and U.S. Census Bureau, 2020 American Community Survey block data

* Wastewater treatment plant does not measure pH in raw influent

Table S4: Langmuir and Linear isotherm parameters for SARS-CoV-2, RSV-A, RV-B, and MS2 into wastewater solids and their respective average relative error (ARE).

Virus	Temp. (°C)	Langmuir model			Linear model	
		K_L ($g \cdot ml^{-1}$)	q_{max} ($cp \cdot g^{-1}$)	ARE	K_d ($g \cdot ml^{-1}$)	ARE
SARS-CoV-2	4	$4.5x10^{-4}$	$2.6x10^{7}$	0.52	7,460	0.45
	22	$1.0x10^{-3}$	$9.5x10^{7}$	0.94	46,044	3.83
RSV-A	4	$6.2x10^{-4}$	$2.6x10^{8}$	3.72	136,809	2.97
	22	$2.6x10^{-3}$	$2.5x10^{8}$	3.76	425,372	4.49
RV-B	4	$3.3x10^{-4}$	$2.3x10^{7}$	0.15	3,396	0.41
	22	$4.0x10^{-6}$	$1.6x10^{7}$	0.73	1,583	0.99
MS2	4	$4.1x10^{-5}$	$6.1x10^{7}$	0.65	1,260	0.42
	22	$3.3x10^{-5}$	$1.1x10^{7}$	0.68	148	0.93

Table S5: Background concentration of endogenous of SARS-CoV-2, RSV, RV, and F+ coliphage/MS2 in wastewater influent samples stored at 4°C and 22°C

Virus	4°C experiment		22°C experiment	
	Conc. in liquid fraction (cp/ml)	Conc. in solid fraction (cp/g)	Conc. in liquid fraction (cp/ml)	Conc. in solid fraction (cp/g)
SARS-CoV-2	0.50	13,451	0.33	93,752
RSV	0.67	4,208	0.97	22,190
RV	0.88	90,729	0.87	22,580
F+ coliphage/ MS2	0.22	28,731	0.25	4,121

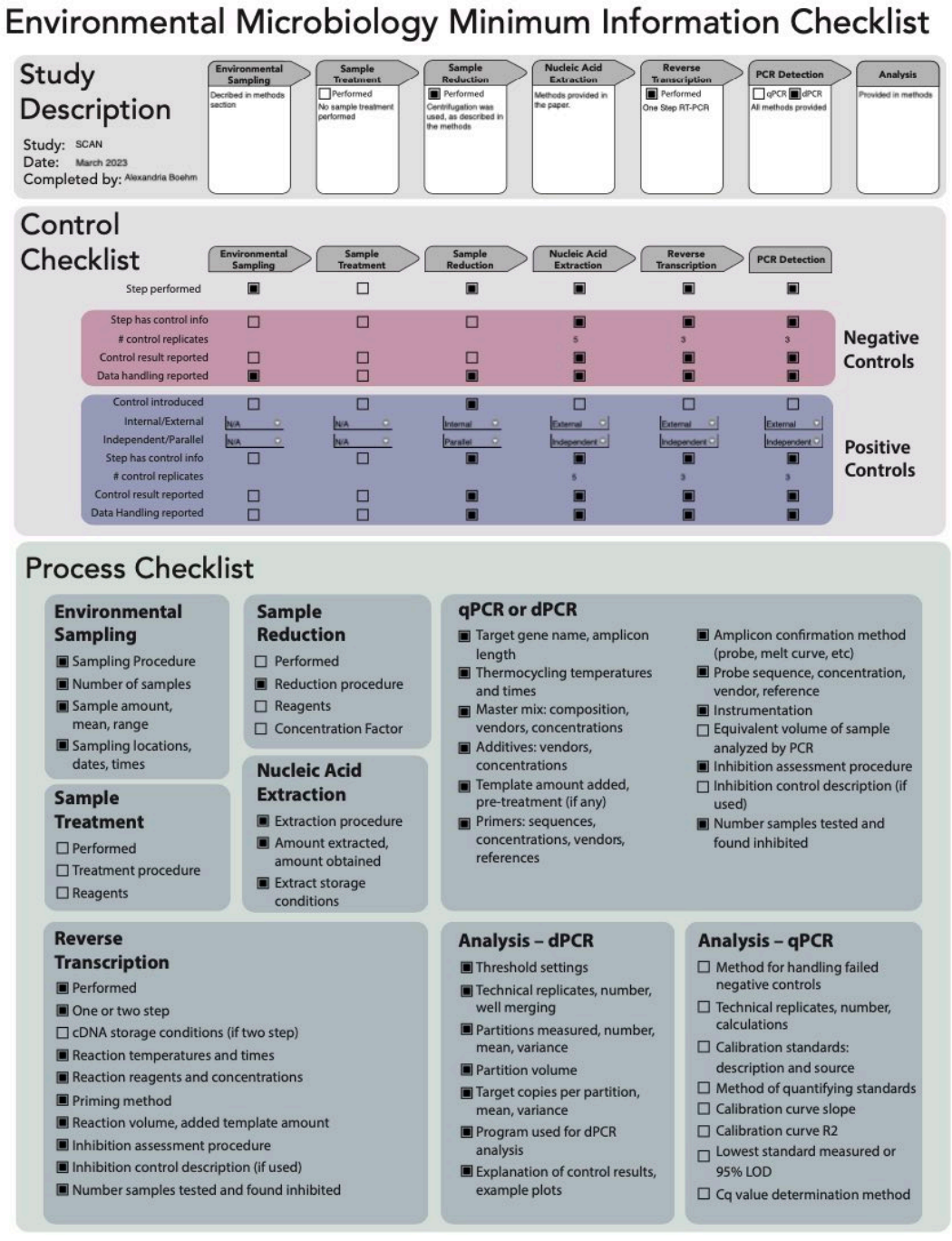

Figure S1. EMMI[1] checklist for reporting.

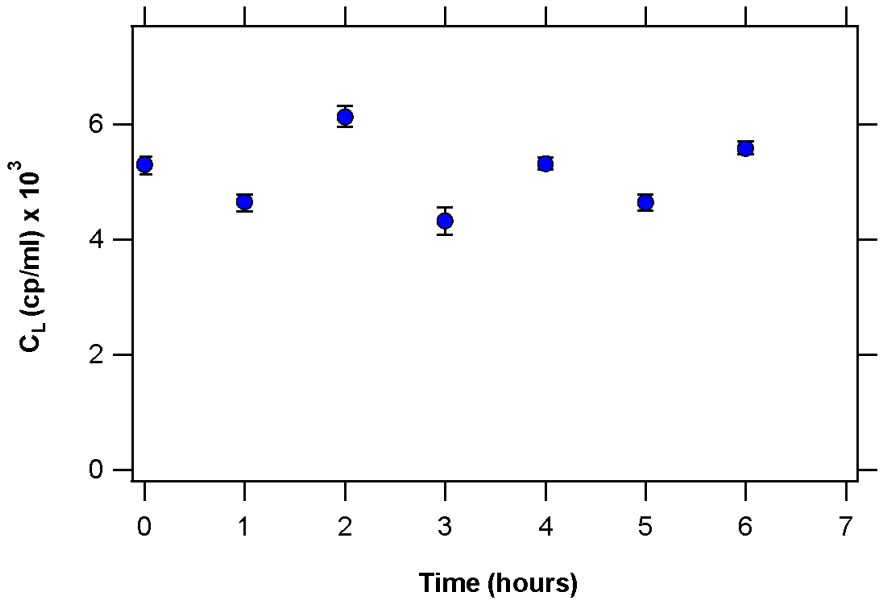

Figure S2: MS2 RNA concentration in liquid fraction at t=0,1,2,3,4,5, and 6 hours. Error bars represent the 68% confidence interval from the ddPCR. Background concentration: ND

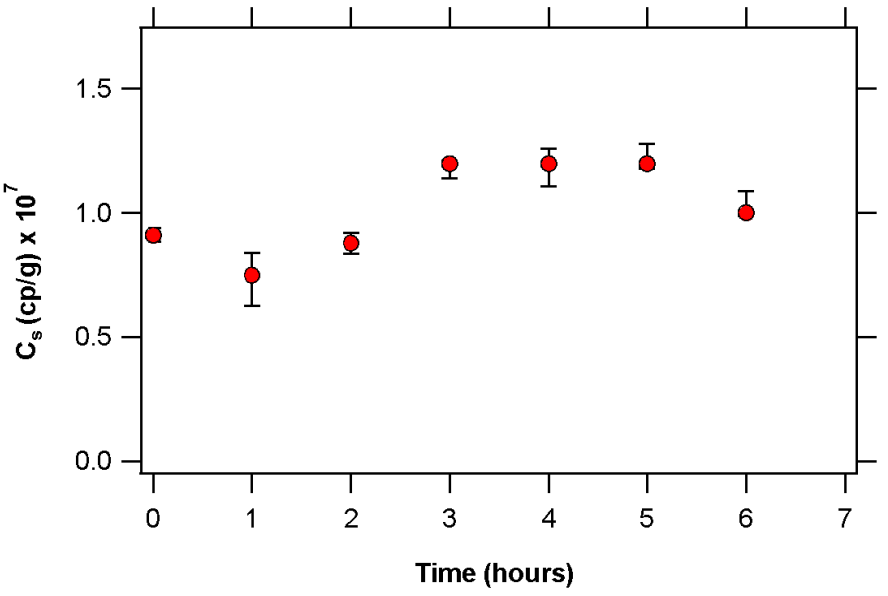

Figure S3: MS2 RNA concentration in solid fraction at t=0,1,2,3,4,5, and 6 hours. Error bars represent the 68% confidence interval from the ddPCR. Background concentration ~ $3.6x10^4$ gc/g dry.

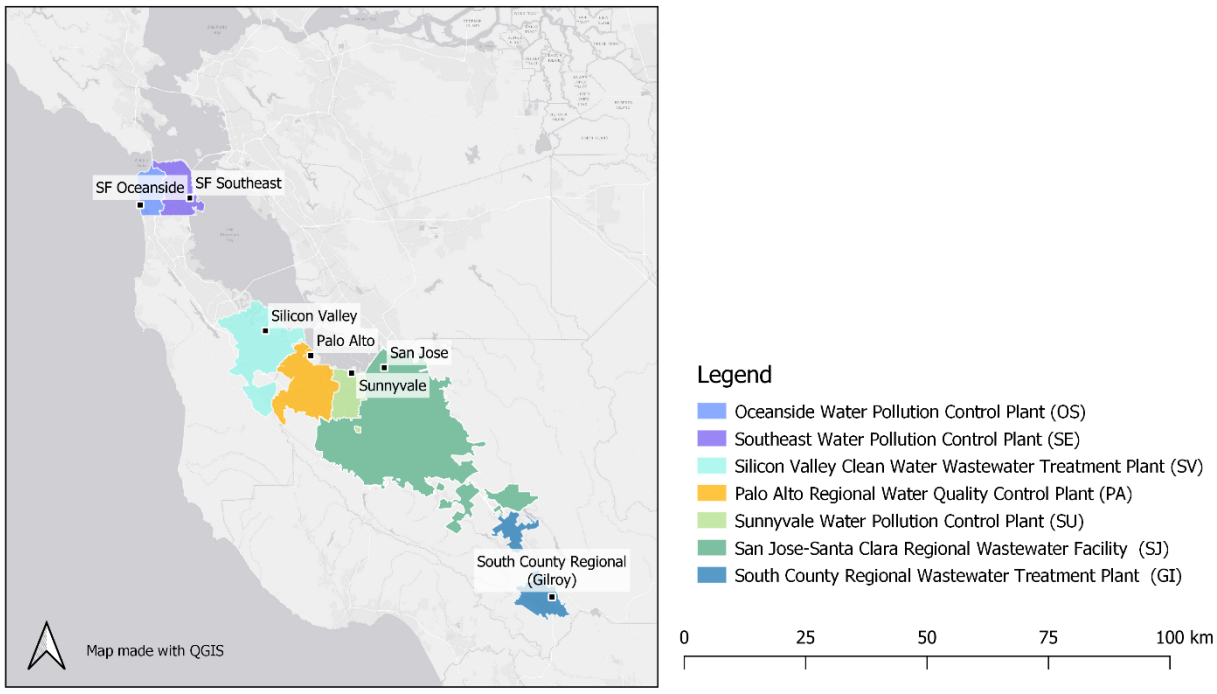

Figure S4: Outline of wastewater treatment plant service area (sewersheds)

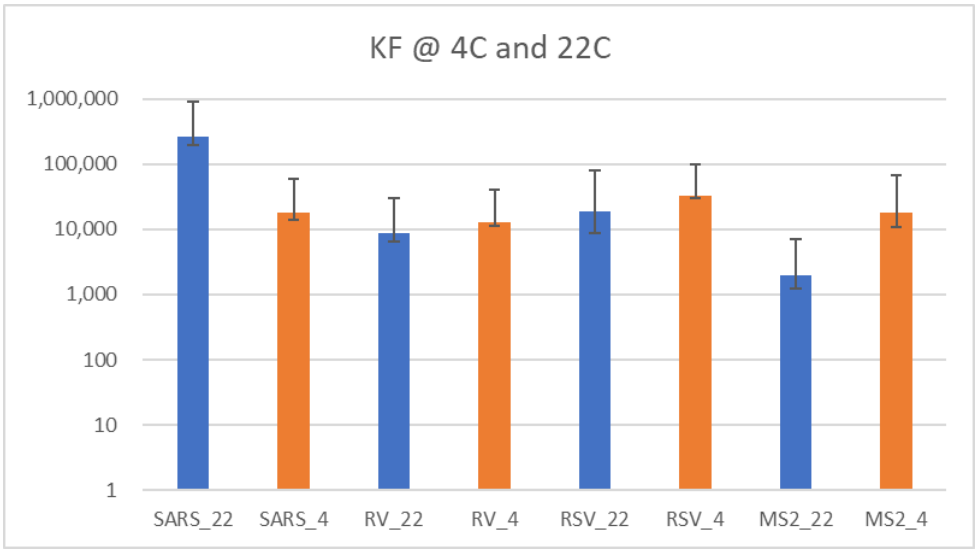

Figure S5: Partition coefficient (K_F) for SARS-CoV-2, RV, RV, and MS2 in wastewater influent samples stored at 4°C and 22°C. Standard errors (SE) were obtained from the linear regression of the Freundlich model in R (summary function).

References

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