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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Boehm AB, Hughes B, Duong D, et al. Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: a surveillance study. *Lancet Microbe* 2023; published online March 22. https://doi.org/10.1016/S2666-5247(22)00386-X.

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Supplementary Methods

Validation of newly designed respiratory virus probe-based RT-PCR assays. Primers and probe sequences were screened for specificity *in silico* using NCBI Blast, and then tested *in vitro* against virus panels (NATtrol™ Respiratory Verification Panel NATRVP2-BIO, NATRVP2.1-BIO, NATEVP-C, ZeptoMetrix, Buffalo, NY) that include chemically inactivated intact enteroviruses, influenza viruses, parainfluenza viruses, adenovirus, rhinovirus, metapneumovirus, and coronaviruses, as well as various additional intact respiratory viruses, synthetic viral genomic RNA, or target cDNA sequences (Table S2). RNA was extracted from intact viruses using Chemagic Viral DNA/RNA 300 Kit H96 for Chemagic 360 (PerkinElmer, Waltham, MA). Nucleic acids were used undiluted as a template in digital droplet RT-PCR singleton assays in a single well. The concentration of nucleic acids used in the *in vitro* specificity testing was approximately 500 copies per reaction. The RT-PCR methods described below were used.

We also assessed the specificity of the RT-PCR assays by testing a dilution series of the target nucleic acid in a background of non-target nucleic acids. The background nucleic acids were from one or two other viruses included in the virus panel that the assay for the target virus was multiplexed with (see next section on wastewater solids analysis that provides information on which assays were multiplexed). The nucleic acids used are those listed in Table S1 in the third column and were RNA except in the case of HKU-1 which was cDNA. Table S3 describes the combinations of viruses used in this specificity test. This test was also a test of sensitivity of a subset of the viral assays for which we assayed low concentrations of the virus (<10 copies/reaction) in a background of non-target nucleic acids. The highest concentration of the target nucleic acid dilution series was also run without a background of other nucleic acids. Nucleic acids were used as a template in digital droplet RT-PCR multiplex assays (as described below) in a single well.

Wastewater solids analysis. Immediately upon receipt at the lab, samples were centrifuged to dewater the solids as described elsewhere[1], and the dewatered solids were stored immediately at -80°C until analysis. The time of storage spanned from 4 to 60 weeks. Samples were removed from the freezer and thawed at 4°C overnight. Then, solids were suspended in DNA/RNA Shield (Zymo Research, Irvine, CA) at a concentration of 0.75 mg (wet weight)/ml. This concentration of solids was chosen as it was shown to alleviate inhibition in downstream RT-PCR[2]. An additional aliquot of dewatered solids was dried in an oven[1] to determine its dry weight so that measured concentrations of nucleic acid targets could be normalized to gram dry weight. RNA was extracted from 10 replicate aliquots of dewatered settled solids suspended in the DNA/RNA Shield using the Chemagic Viral DNA/RNA 300 kit H96 for the Perkin Elmer Chemagic 360 (Perkin Elmer, Waltham, MA) followed by PCR inhibitor removal with the Zymo OneStep-96 PCR Inhibitor Removal kit (Zymo Research, Irvine, CA) The pre-analytical methods described here are provided in detail in other publications and on protocols.io [1–5].

Eighteen samples spanning the duration of the time series were selected to measure each HCoV and HPIV, respectively (Table S3). Aliquots of extracted RNA were stored at -80°C for between 1 and 3 months before being thawed to measure concentrations of individual HCoVs and HPIVs.

Droplets were generated using the AutoDG Automated Droplet Generator (Bio-Rad, Hercules, CA). PCR was performed using Mastercycler Pro (Eppendforf, Enfield, CT) with with the following cycling conditions: reverse transcription at 50°C for 60 minutes, enzyme activation at 95°C for 5 minutes, 40 cycles of denaturation at 95°C for 30 seconds and annealing and extension at 59°C for 30 seconds, enzyme deactivation at 98°C for 10 minutes then an indefinite hold at 4°C. The ramp rate for temperature changes were set to 2°C/second and the final hold at 4°C was performed for a minimum of 30 minutes to allow the droplets to stabilize. Droplets were analyzed using the QX200 Droplet Reader (Bio-Rad). A well had to have over 10,000 droplets for inclusion in the analysis. All liquid transfers were performed using the Agilent Bravo (Agilent Technologies, Santa Clara, CA).

The ddRT-PCR methods applied to wastewater solids to measure PMMoV are provided in detail elsewhere[1] and are not repeated here. Here, we provide methods for the respiratory virus assays which have not been reported previously. To obtain the total concentration of seasonal HCoV: the four HCoV assays were multiplexed with HCoV HKU1 and 229E in the FAM channel, and OC43 and NL63 in the HEX channel. To obtain the total concentration of HPIV the five HPIV assays were multiplexed: HPIV1, HPIV3, and HPIV 4A in the FAM channel, and HPIV 4B and HPIV 2 in the HEX channel. IAV (FAM), IBV (HEX), RSV A (FAM/HEX) were multiplexed using the triplex probe mixing approach. HMPV (FAM), HRV (HEX), and RSV B (FAM/HEX) were multiplexed using the triplex

probe mixing approach. Finally, PMMoV (HEX) was measured in singleplex[6]. Using this approach, concentrations of individual genomic targets can be obtained from all assays except those for HCoV and HPIV - for those reactions, all positive droplets were combined to obtain concentrations of all HCoV and HPIV.

The eighteen samples chosen to measure each HCoV and HPIV individually were run as follows. 229E (FAM) and NL63 (HEX), and HKU-1 (FAM) and OC43 (HEX) were multiplexed. HPIV2 (FAM/HEX), HPIV3 (HEX), and HPIV 4A (FAM) were multiplexed , and HPIV1 (HEX) and HPIV 4B (FAM) were multiplexed.

Extracts from each of the 10 replicate nucleic acid extractions from each sample were run as template yielding 10 wells per sample. Each 96-well PCR plate of wastewater samples included PCR positive controls for each target assayed on the plate in 1 well, PCR negative no template controls in two wells, and extraction negative controls (consisting of water and lysis buffer) in two wells. PCR positive controls consisted of viral gRNA or gene blocks (Table S2).

ddRT-PCR was performed on 20 µl samples from a 22 µl reaction volume, prepared using 5.5 µl template, mixed with 5.5 µl of One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad 1863021), 2.2 µl of 200 U/µl Reverse Transcriptase, 1.1 µl of 300 mM dithiothreitol (DDT) and primers and probes mixtures at a final concentration of 900 nM and 250 nM respectively. Primer and probes for assays were purchased from Integrated DNA Technologies (IDT, San Diego, CA) (Table 1).

For the assay specificity testing, the respiratory virus assays were challenged against target and non-target (other respiratory viruses) viral gRNA (Table 1). Each PCR plate contained a negative PCR no template control.

For the dilution series specificity / sensitivity testing, each PCR plate contained a negative PCR no template control.

Thresholding was done using QuantaSoft™ Analysis Pro Software (Bio-Rad, version 1.0.596). In order for a sample to be recorded as positive, it had to have at least 3 positive droplets. Replicate wells were merged for analysis of each sample.

Regional monitoring program SARS-CoV-2 N gene and PMMoV gene concentrations. The wastewater solids samples from the wastewater treatment plant used in this study were collected as part of a larger wastewater monitoring program focused on measuring SARS-CoV-2 RNA in daily samples that were not stored prior to analysis. Detailed methods for that program have been published[1] and some of those results from samples included in this study have been published[1–5]. Herein, we include data on SARS-CoV-2 N gene concentrations in a supplementary manner to provide insight into the progress of the COVID-19 pandemic during this study, and data on PMMoV to assess how storage of the samples potentially affected viral RNA quantification.

Supplementary Results

Additional specificity and sensitivity results. There was no cross-reactivity between the assays and the viruses tested *in silico*. When assays were tested in simplex against the virus panels described in Table S2, there was no cross reactivity observed. There were no positive droplets observed in the reactions. We note that there were some low fluorescence droplets when the human rhinovirus assay was challenged with four coxsackieviruses (A9, A10, A16, B5) from the ZeptoMetrix NATEVP-C panel, but the fluorescence was not high enough for the droplets to be considered positive droplets.

We carried out additional tests of assay specificity by testing viral RNA dilutions in a background of other viral RNA targets in multiplex reactions (Table S3). Concentrations of the viral RNAs were not different when they were run with and without a background of viral RNA. This can be seen in Figure S4 where the measured concentrations of the highest viral RNA concentration of the dilution series is not different when background RNA is and is not present. Even when the target viral RNA is at a lower concentration in a background of other viral RNA, the measured concentration matches well the input concentration. This illustrates no cross-reactivity among the tested assays.

Most of the dilution series described in Table S3 span low concentrations (<3 copies/reaction). When they do, we find that the lowest concentration detectable is 3 or fewer copies/reaction. This is consistent with our use of 3 positive droplets as a cut off for a positive, quantifiable detection of the target and suggests that when used in a digital droplet RT-PCR format, the assays are sensitive.

Additional details for the Environmental Microbiology Minimal Information (EMMI) guidelines. In addition to the details provided in the main text and the appendix, the EMMI guidelines[7] suggest reporting for digital PCR: (1) the volume of each partition (here, a droplet), (2) the average number of partitions (here, droplets) per well and the standard deviation of the number of droplets, (3) the mean number of target nucleic acid copies per partition (here, droplet) and the standard deviation, and (4) an example fluorescence plot from the instrument. The volume of each droplet, on average based on the vendor specification, is 0.795 nanoliters[8]. The average number of droplets per well for our experiments was 18936 droplets and the standard deviation was 1714 droplets. The mean number of target nucleic acid copies per partition for respiratory virus RNA for samples with 3 or more positive droplets was $4.1x10^{-4}$ with a standard deviation of $2.2x10^{-3}$. The mean number of target nucleic acid copies per partition for PMMoV RNA was 0.42 with a standard deviation of 0.16. These averages were calculated using a random subset of 25% of the wells to obtain a reasonable representation of the wells (we ran a total of over 2000 wells for these experiments). The EMMI check list is provided as Figure S2 and example fluorescence plots as Figure S3.

Additional QA/QC for wastewater samples. Each plate run included negative extraction and RT-PCR controls, and positive extraction and PCR controls. In order for a plate to pass QA/QC, the number of positive droplets across merged negative extraction and PCR well could be no larger than 2 for any plate except the PMMoV plate where we allowed up to 13 positive droplets. The larger number of droplets in negative controls were allowed because PMMoV is a high copy number target with typically 71,000 (median across all samples) positive droplets for the samples; therefore addition of up to 13 positive droplets would change the percent of positive droplets by less than 0.02% and have minimal effect on the measured concentration. The number of positive droplets for the positive controls had to be greater than 3 droplets yielding a positive result. Median PMMoV concentrations across the samples were $1.6x10^{9}$ copies/g similar to measurements previously reported for the plant[1]. In addition, levels were stable across all samples (IQR = $0.7x10^{9}$ cp/g) suggesting consistent fecal strength and RNA extraction efficiency. All the tests of associations described in the main text of the paper were repeated with wastewater respiratory viral RNA concentrations normalized by concentrations of PMMoV RNA and the results are unchanged.

The concentration of PMMoV obtained in this study was compared to the concentration of PMMoV measured in the same samples from fresh (not stored) wastewater solids from our routine monitoring program. The median ratio measured concentrations in this study to measured concentrations from fresh samples was 1.1 (interquartile range IQR = 0.7). This suggests that the storage of the solids in this study at -80°C and their subsequent freeze thaw had limited impact on PMMoV RNA concentrations. We therefore assume that the impact was similarly minimal for the other targets.

Results for HCoV for samples collected prior to 2/26/21 (month/day/year) were omitted from the analysis owing to failed positive controls.

Summary of Santa Clara County sentinel lab data (county-aggregated sentinel lab data) and its association with wastewater measurements. The wastewater treatment plant serves approximately 75% (1.5 million of 2 million residents) of the population of Santa Clara County, suggesting that disease incidence data from sentinel laboratories in the county would be a good proxy for disease incidence in the sewershed. However, the county-aggregated surveillance data was sparse, particularly for seasonal HCoV, for which no clinical specimens were assayed. We therefore opted not to present the results from these analysis in the main paper, but include them herein for completeness.

Sentinel laboratories in Santa Clara county tested a median (IQR) of 20 (407) specimens per MMWR week for influenza (IAV and IAB) and RSV, and 7 (6) for HPIV, HMPV, and HRV. No clinical specimen was tested for HCoV. Wastewater viral RNA concentrations were positively associated with the positivity rates for the virus in clinical specimens processed by the county sentinel laboratories for IAV, HPIV, and RSV. Kendall's tau between positivity rates and median wastewater concentrations were 0.54 ($p<10^{-7}$) for IAV, 0.44 ($p=10^{-6}$) for HPIV, and 0.74 ($p<10^{-15}$) for RSV. Clinical specimen testing does not distinguish between RSV A and B, so wastewater RSV B concentration was used as a variable in the test of association as RSV A were mostly non-detect (Table 3). There

was no reported positive clinical specimen for IBV and HCoV so we could not perform a test of association. There was not a correlation between HMPV RNA concentrations in wastewater and HMPV case positivity (tau = 0.067, p = 0.51) or HRV RNA wastewater concentrations and HRV case positivity (tau = 0623, p=0.0023). Given the paucity of county clinical specimens processed for HRV and HMPV, the results should be viewed with caution.

Parameter	Values
Primer size	min 15, optimum 20, max 36
Primer melting temperature	min 50°C, optimal 60°C, max 65°C
GC% content:	min 40%, optimal 50%, high 60%
concentration of divalent cations	3.8 mM
concentration of dNTPs	0.8 mM
Internal oligo size	size min 15, optimal 20, max 30
Internal oligo: Melting temp	min 62°C, optimal 63°C, max 70°C
Internal Oligo: GC%	min 30%, optimal 50%, max 80%

Table S1. Parameters used in Primer3Plus (https://primer3plus.com/, accessed 11/26/22).

Virus(es) (abbreviation used in the paper	Genomic target	Non-target testing (negatives)	Target testing (positives)
Pan Rhinovirus A/B/C (HRV)	Untranscribed region	NATRVP2-BIO, NATEVP-C	Clinical samples sequenced and classified as HRV A, B and C
Human Parainfluenza (HPIV) 1	Hemagglutinin-neuraminidase (HN) gene	NATRVP2-BIO, Intact HPIV 4B virus (ATCC VR-1377), Intact HPIV 4A virus (ATCC VR-1378)	Intact HPIV 1 virus (ATCC VR-94)
HPIV 2	HN gene	NATRVP2-BIO, Intact HPIV 4B virus (ATCC VR-1377), Intact HPIV 4A virus (ATCC VR-1378)	Intact HPIV 2 virus (ATCC VR-92)
HPIV 3	Fusion protein (F) gene	NATRVP2-BIO, Intact HPIV 4B virus (ATCC VR-1377), Intact HPIV 4A virus (ATCC VR-1378)	Intact HPIV 3 virus (ATCC VR-93)
HPIV 4A	Untranscribed region	NATRVP2-BIO, Intact HPIV 4B virus (ATCC VR-1377), Intact HPIV 4A virus (ATCC VR-1378)	Intact HPIV 4A virus (ATCC VR-1378)
HPIV 4B	HN gene	NATRVP2-BIO, Intact HPIV 4A virus (ATCC VR-1378)	Intact HPIA 4B virus (ATCC VR-1377)
Human Coronavirus (HCoV) 229E	Nucleoprotein (N) gene	NATRVP2-BIO	Synthetic HCoV 229E gRNA (TWIST 103011)
HCoV NL63	N gene	NATRVP2-BIO	Synthetic HCoV NL63 gRNA (TWIST 103012)
HCoV OC43	N gene	NATRVP2-BIO	Synthetic HCoV OC43 RNA (TWIST 103013)
HCoV HKU-1	N gene	NATRVP2-BIO	IDT gene Block
Human Metapneumovirus (HMPV)	RNA directed RNA polymerase (L) gene	NATRVP2.1-BIO	Synthetic HMPV RNA (ATCC VR-3250SD) and intact HMPV virus (Zepto NATRVP-IDI)
Influenza B (IBV)	Membrane (M1) gene	NATRVP2.1-BIO	Synthetic IBV gRNA (TWIST 103003) and intact IBV virus (Zepto NATFVP-NNS)
Respiratory syncytial virus (RSV) A	N gene	NATRVP2.1-BIO, Intact RSV B virus (Zepto NATFVP-NNS)	Intact RSV A virus (Zepto NATFVP-NNS)
RSV B	N gene	NATRVP2.1-BIO	Intact RSV B virus (Zepto NATFVP-NNS)

Table S2. List of viruses included in this study, and the name of the gene target RT-PCR probe-based assays targeted. The list of non-target and target controls used to test assay specificity are provided. All non-target controls

are virus panels sold by Zeptomatrix (panels begin with NAT prefix, “Zepto”, Buffalo, NY). The vendor Twist Biosciences (“Twist”) is located in South San Francisco, CA. ATCC is American Type Culture Collection. The viruses in the NATRVP2-BIO or NATRVP2.1-BIO panels include chemically inactivated intact influenza viruses, parainfluenza viruses, adenovirus, rhinovirus, metapneumovirus, and coronaviruses. The viruses in the NATEVP-C panel include chemically inactivated intact coxsackieviruses, echovirus, and parechovirus. The full list of viral species in the panels is available from the vendor.

Target viral RNA	Range of concentrations tested of target viral RNA (lowest concentration detected) units are copies/reaction	Background viral RNA included in reactions (concentrations) units are copies/reaction
IAV	1-50 (3)	IBV (20) and RSV A (100)
IBV	1-20 (3)	IAV (50) and RSB A (100)
RSV A	4-100 (4)	IAV (50) and IBV (20)
RSV B	7-200 (7)	HRV (100) and HMPV (300)
HRV	3-100 (3)	RSV B (200) and HMPV (300)
HMPV	10-300 (10)	HRV (100) and RSV B (200)
OC43	3-100 (3)	HKU-1 (30)
HKU-1	1-30 (2)	OC43 (100)
229E	1-50 (3)	NL63 (90)
NL63	1-20 (3)	OC43 (90)
HPIV 1	3-50 (3)	HPIV 4B (1500)
HPIV 4B	50-1500 (50)	HPIV (50)
HPIV 2	5-100 (5)	HPIV 3 (5000) and HPIV 4A (50)
HPIV 3	150-5000 (150)	HPIV 2 (100) and HPIV 4A (50)
HPIV 4A	2-50 (2)	HPIV 2 (100) and HPIV 3 (5000)

Table S3. Dilution series for specificity and sensitivity testing of assays. A dilution series of target nucleic acids from virus (first column) spanning the concentration range provided in the second column was made in a background of viral nucleic acid targets from one or two other viruses at a fixed concentration (third column). The viruses chosen to include as background are those for which the assay for the target virus in the first column was multiplexed.

Virus group	Samples chosen for analysis of all members of virus group
HCoV	2/26/21, 3/14/21, 4/13/21, 5/20/21, 6/20/21, 7/8/21, 8/17/21, 9/14/21, 10/26/21, 11/25/21, 12/12/21, 1/13/22, 2/15/22, 3/1/22, 4/28/22, 5/10/22, 6/2/22, 6/21/22
HPIV	2/15/21, 3/17/21, 4/17/21, 5/18/21, 6/1/21, 7/11/21, 8/1/21, 9/19/21, 10/17/21, 11/4/21, 12/28/21, 1/16/22, 2/15/22, 3/10/22, 4/7/22, 5/5/22, 6/2/22, 6/21/22

Table S4. Samples chosen for analysis of individual HCoVs and HPIVs. Dates are provided in month/day/year format.

	HMPV	IAV	IBV	HRV	RSV A	RSV B	HPIV	HCoV
IAV	**0.21 $p<10^{-4}$**							
IBV	0.19 p=0.0013	0.10 p=0.11						
HRV	**0.58 $p<10^{-15}$**	0.10 p=0.059	**0.21 p=0.00016**					
RSV A	-0.16 p=0.0067	0.081 p=0.19	0.009 p=0.89	-0.18 p=0.00092				
RSV B	**0.47 $p<10^{-15}$**	**0.23 $p<10^{-4}$**	0.19 p=0.0013	**0.36 $p<10^{-12}$**	-0.16 p=0.0061			
HPIV	**0.52 $p<10^{-15}$**	**0.32 $p<10^{-9}$**	**0.19 p=0.00075**	**0.53 $p<10^{-15}$**	-0.10 p=0.063	**0.43 $p<10^{-15}$**		
HCoV	-0.033 p=0.51	0.070 p=0.18	0.13 p=0.017	0.049 p=0.31	0.056 p=0.31	-0.039 p=0.43	-0.016 p=0.73	
SARS-CoV-2	**0.29 $p<10^{-8}$**	**0.26 $p<10^{-6}$**	0.05 p=0.39	**0.16 p=0.00047**	-0.14 p=0.0091	**0.36 $p<10^{-13}$**	**0.41 $p<10^{-15}$**	-0.10 p=0.026

Table S5. Kendall's test of association between wastewater solids concentrations of viral RNA. Tau and the associated p value are provided. To account for multiple hypothesis testing (53 tests were completed), a p value cut off of 0.0009 (based on Bonferroni's correction) was used to identify correlations statistically different from 0; these are highlighted in bold.

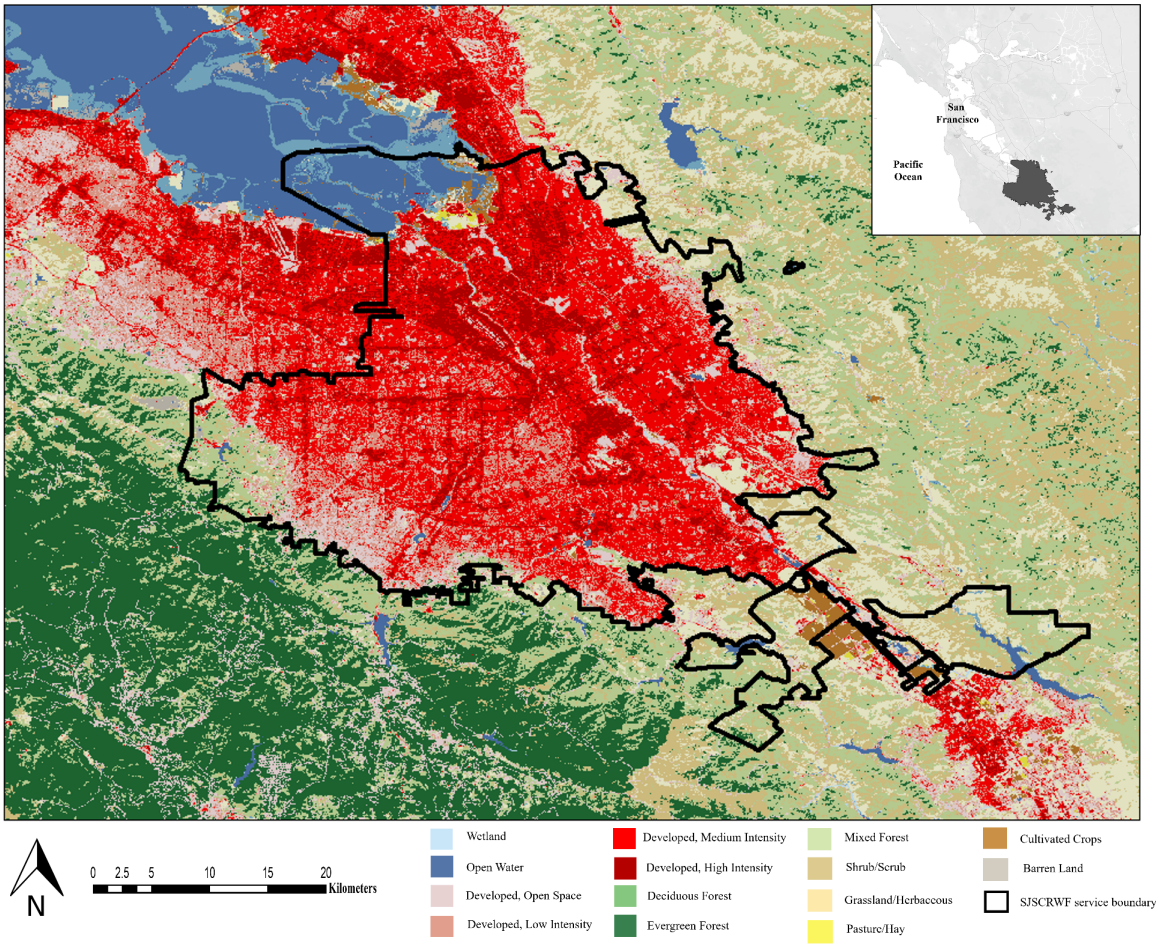

Figure S1. Map of the San José-Santa Clara Regional Wastewater Facility (wastewater treatment plant) sewershed. The black boundary outlines the sewershed. The background is the land use data from USGS National Land Cover Database 2019. The plant serves 1.5 million people living in the region and also serves 17,000 businesses.

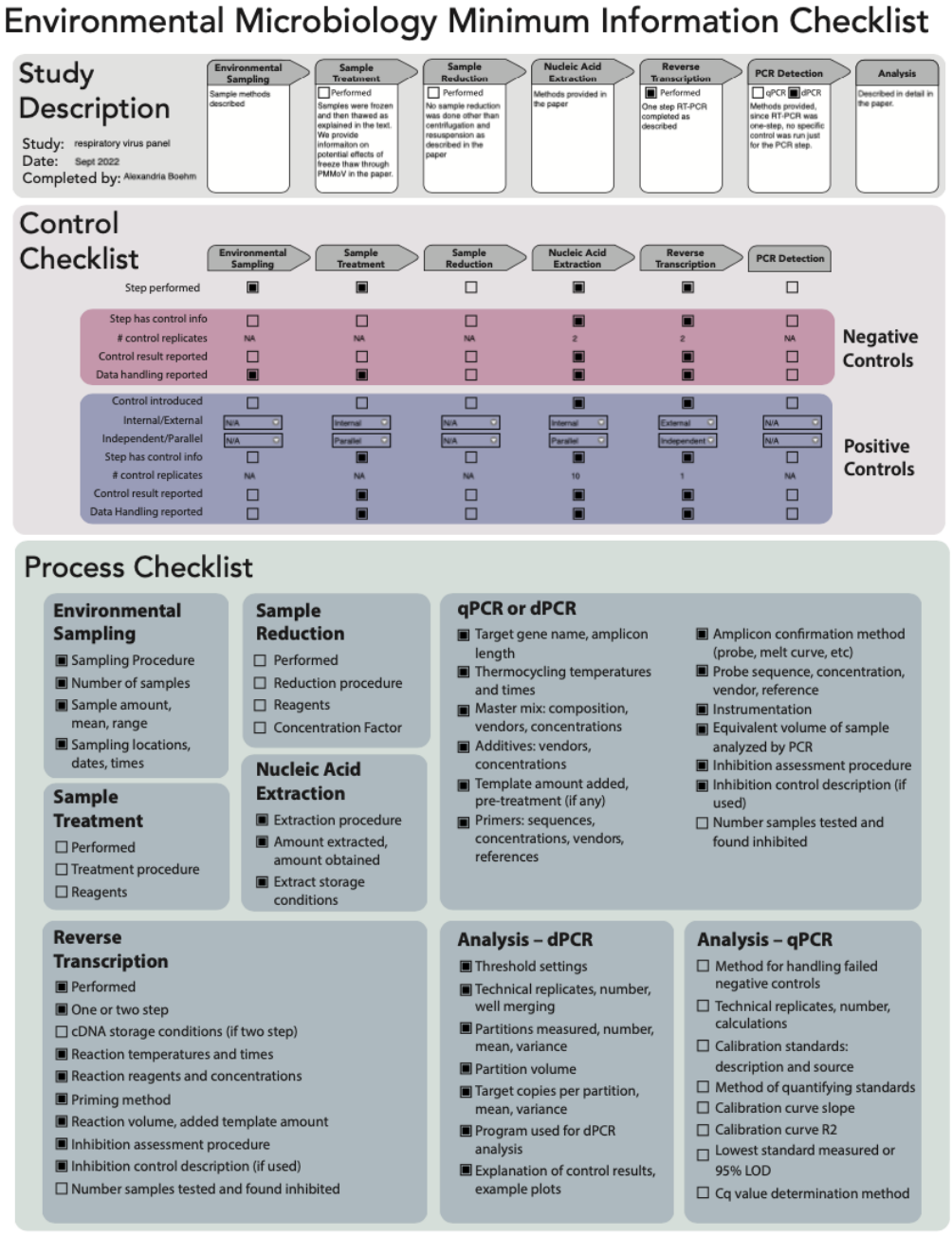

Figure S2. Environmental Microbiology Minimal Information (EMMI) guidelines[7] checklist.

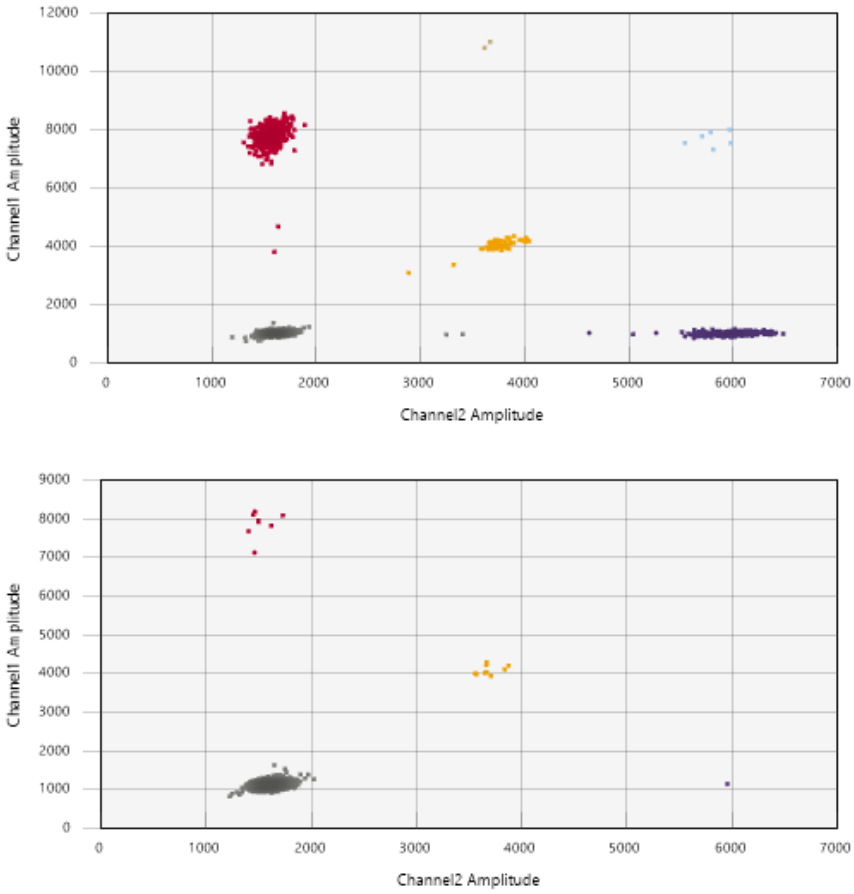

Figure S3. Example fluorescence plots from digital PCR instrument per the EMMI guidelines from samples run for IAV, IBV, and RSV A in multiplex. Top panel shows an example output from a positive control containing a mixture of positive controls for the three multiplexed targets, middle plot is a wastewater sample, and the bottom is a NTC. In these example plots, the wells were assayed for IAV (FAM, measured in channel 1), IBV (HEX, measured in channel 2), and RSV A (FAM/HEX, measured in both channels) . Red dots are droplets positive just for IAV, yellow for just RSV A, and purple for just IBV. Blue dots, only visible in the positive control top panel, are for droplets positive for IAV and IBV. Similarly, tan dots, present only in the positive control top panel (located at approximately 3800 (channel 2), 11000 (channel 1) coordinates are positive for RSV A and IAV. The grey cluster of dots at low fluorescence are negative.

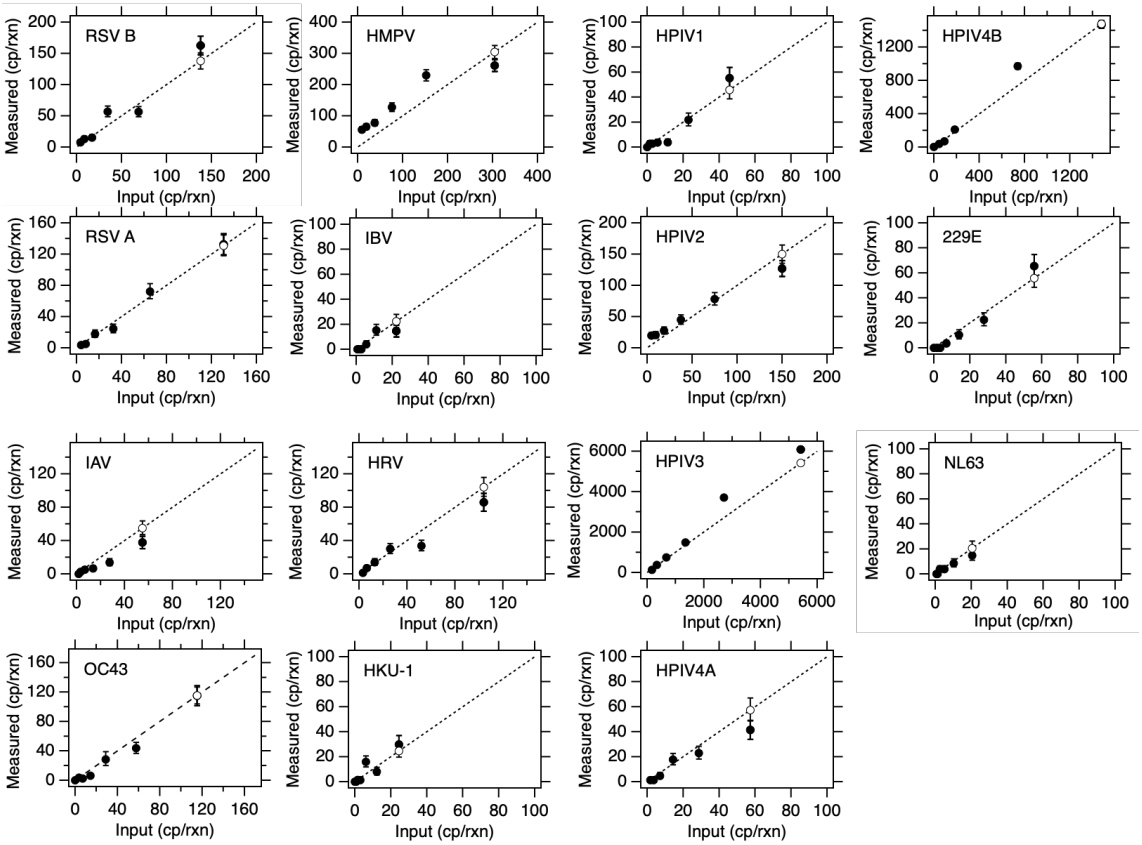

Figure S4. Specificity and sensitivity testing for viral RNA assays in background of other viral RNA targets. Each viral RNA target (upper left corner of each panel) is input as template into a RT-PCR reaction with other viral RNA targets (provided in Table S3) as background. The background viral RNA are chosen to be those with which the target RNA is multiplexed. The input concentration is on the x-axis and the measured concentration on the y-axis. The dotted line shows 1:1 line. Error bars on measurements are standard deviations, if the error bars are not visible, it is because they are smaller than the symbol. The white symbol is the concentration of the target RNA in a reaction with no background RNA from other viruses. For HPIV 4B, the white symbol is under the black symbol and is not visible.

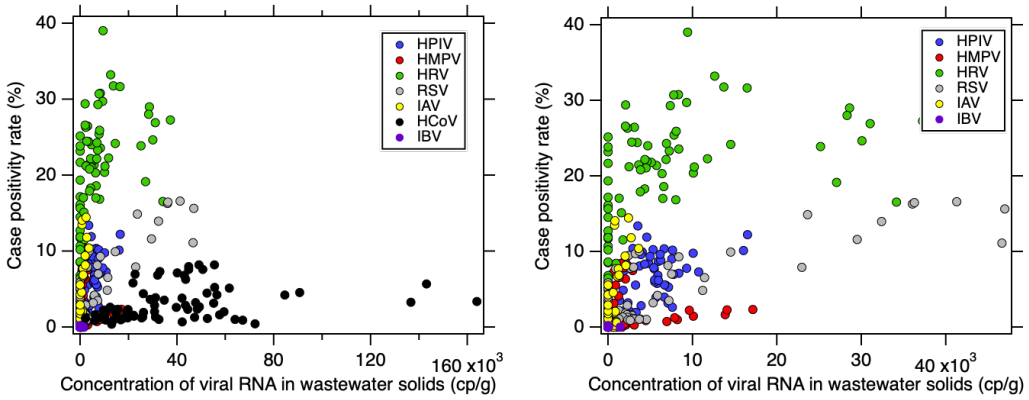

Figure S5. Case positivity from clinical specimens submitted to sentinel laboratories aggregated across the state versus median concentration of viral RNA in wastewater solids for all viral targets for each MMWR week included in the study. RSV B was used to represent RSV in wastewater solids. Left panel shows all viruses, right panel excludes seasonal HCoV data to allow visualization of other data more easily.

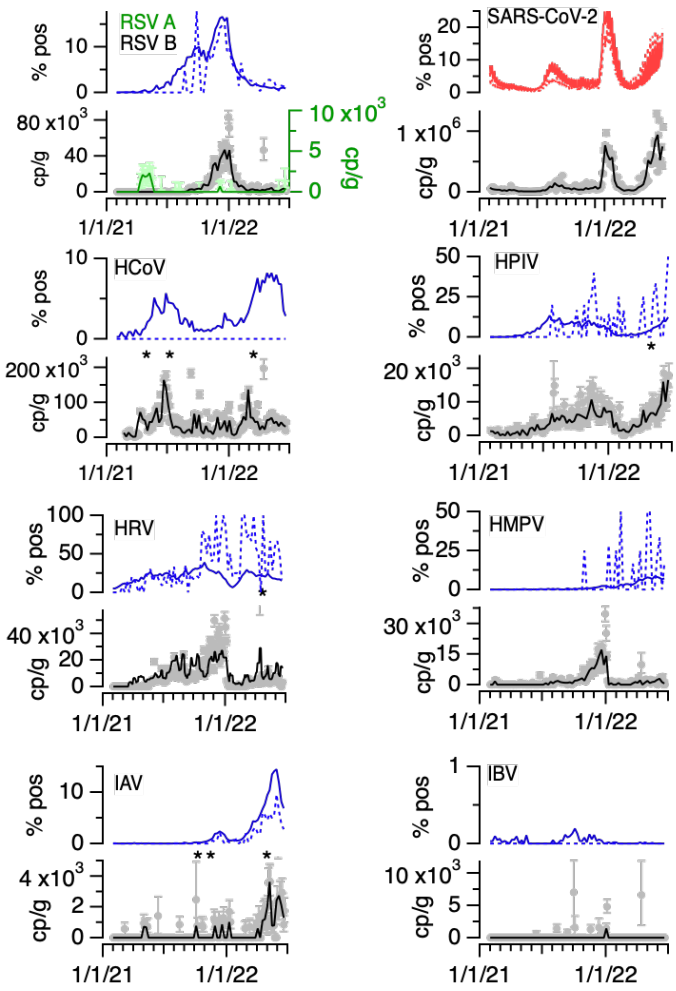

Figure S6. State-aggregated positivity rate (% pos, blue solid line) and county-aggregated positivity rate (% pos, blue dotted line) reported as a percent from sentinel laboratories (except for SARS-CoV-2) (top panel) and viral RNA in wastewater solids for each virus reported in copies per gram dry weight (cp/g) (bottom panel). Upper right panel shows SARS-CoV-2 wastewater results as well as state-aggregated and county aggregated positivity rates from all laboratories in the State (red solid line) and County (red dotted line), respectively (not just sentinel laboratories, shown in red to distinguish from other clinical data) for context. RSV is respiratory syncytial virus (A is shown in green and B in gray/black, RSV A scale is 1/10th that of RSV B and, like the RSV B axis, is scaled by 103), HCoV is the sum of all four seasonal human coronaviruses (OC43, HKU-1, 229E, and NL63), HPIV is the sum of all human parainfluenza viruses (1-4), HRV is human rhinovirus A, B and C, HMPV is human metapneumovirus, and IAV and IBV are influenza A and B viruses, respectively. Gray symbols represent measurements, error bars are standard deviations. The black line is the MMWR weekly median wastewater measurement. For HCoV there are three measurements located off scale ($1.1x10^6$, $3.6x10^5$, and $5.1x10^5$ cp/g on 4/9/21, 6/24/21, and 3/3/22, respectively). For HPIV there is one measurement located off scale ($3.6x10^4$ cp/g on 4/14/22). For HRV there is one measurement located off scale ($7.6x10^4$ cp/g on 4/14/22). For IAV there are 3 measurements located off scale ($2.1x10^4$, $7.5x10^4$, and $1.5x10^4$ cp/g on 9/30/21, 10/31/21, and 4/14/22, respectively). An asterisk (*) is shown on dates where a point is off-scale. Dates are in month/day/year format.

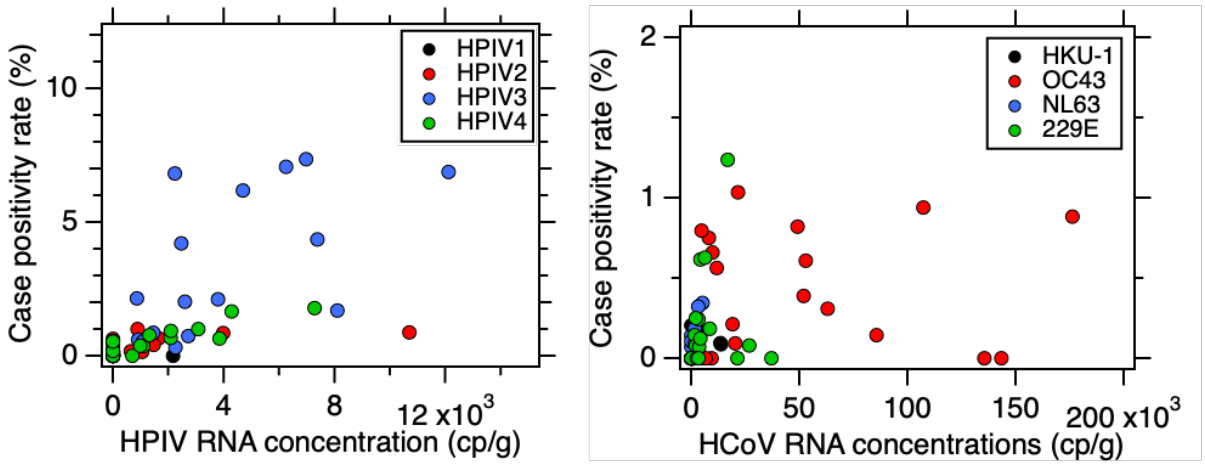

Figure S7. Case positivity rates (as percentages) from clinical specimens submitted to sentinel laboratories aggregated across the state versus median concentration of viral RNA in wastewater solids for individual HPIV (left panel) and HCoV (right panel) each MMWR week included in the study. HPIV 4A and HPIV 4B in wastewater were added to compare to HPIV 4 positivity rates.

References

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