Supplementary Information: Building-level wastewater surveillance using tampon swabs and RT-LAMP for rapid SARS-CoV-2 RNA detection

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Centrifugal Ultrafilter Concentration

A subset of swab sorbate samples were concentrated by passing 15 mL of sorbate through an Amicon Ultra-15 10 kDa Centrifugal Filter Unit (MilliporeSigma, MA, USA) via a 5,000 x g spin for 30 minutes. The retentate was resuspended in 1 mL of PBS/Tween20 solution and 500 µL was transferred into a 2 mL PowerBead tube containing 0.1 mm glass beads (Qiagen, Hilden, Germany) for homogenization prior to extraction. Owing to difficulty passing the entire 15 mL volume through the ultrafilter, this concentration method was abandoned after the first week of sampling.

Swab Sorbate Solids Fractionation

Since enveloped viruses, including SARS-CoV-2, partition favorably to solids in wastewater (1,2), after abandoning ultrafiltration, swab sorbate samples at ND were processed with emphasis on the solids fraction. Each 50 mL sorbate volume was subjected to centrifugation at 10,000 x g for 10 minutes at 4°C. The supernatant was poured off and the pellet was resuspended using 1 mL of PBS/Tween20 solution. A 500 µL aliquot of the resuspension was transferred into a 2 mL PowerBead tube containing 0.1 mm glass beads (Qiagen, Hilden, Germany) for homogenization prior to extraction. For a subset of samples, 15 mL of the resulting supernatant was concentrated via Amicon as described above.

Heat Extraction & No Extraction

A subset of 1 mL swab sorbate samples and 1 mL re-suspended solids samples were subjected to heat extraction by incubation in a heat block at 95°C for 15 minutes. After incubation, the samples, contained in 2 mL microcentrifuge tubes, were centrifuged at 13,000 x g for 2 minutes and 100 uL of supernatant was transferred to a clean 2 mL centrifuge tube for testing by RT-LAMP. A subset of primary influent samples was also tested by RT-LAMP without extraction or pre-treatment.

Heat Extraction Inhibition Rate

After heat extraction, 100% of swab sorbate samples (n=5) were inhibited and remained so even after 1:10 dilution. Among the five solid fraction samples, 100% were inhibited after heat extraction, and 40% remained so even after 1:10 dilution. Given the high rate of inhibition, we abandoned heat extraction as a reliable method for detection in wastewater via RT-LAMP.

No Extraction Inhibition Rate

We attempted extraction-free RT-LAMP on five tampon swab sorbate and four 24-hour composite samples of WWTP influent. The inhibition rate among the five undiluted passive samples was 100%. The inhibition rate for undiluted composite samples was 100% when using 7 µL or 4 µL of input. After 1:10 dilution, no inhibition was observed for 7 µL of input. Given the dilution required to remedy inhibition and the resulting 10x increase in the 95% LOD, we abandoned extraction-free RT-LAMP as a reliable detection method.

Electronegative Membrane Concentration

Primary influent samples and raw sewage composite samples were concentrated using electronegative membrane filtration. Briefly, a 100 mL aliquot of well-mixed sample was filtered through a 0.45 µm mixed-cellulose ester membrane (Pall Corporation, Port Washington, NY, USA) using a vacuum filtration assembly (Sigma-Aldrich, St. Louis, MO, USA). The membrane was then aseptically rolled into a 2 mL Garnet bead tube (Qiagen, Hilden, Germany) and frozen at -80°C until homogenization prior to extraction.

RT-ddPCR

For RT-LAMP validation, SARS-CoV-2 RNA was quantified using the BioRad QX200 Droplet Digital PCR (ddPCR) System and C1000 Touch Thermal Cycler (Hercules, CA, USA) as previously described in detail (Bivins et al. 2021 preprint). Reverse transcription and droplet digital

PCR were performed in a single step using the One-Step RT-ddPCR Advanced Kit for Probes (BioRad, Hercules, CA, USA) using the premixed N1 assay (Liu et al. 2020). RT-ddPCR reactions were prepared in triplicate at a volume of 22 uL consisting of 4 uL sample RNA, 6.45 uL PCR-grade water, 5.25 uL 4X Supermix, 2.1 uL reverse transcriptase, 1.05 uL dithiothreitol, and 3.15 uL of premixed N1 primers and probes (resulting concentrations of 1000 nM and 250 nM, respectively) from Integrated DNA Technologies (Coralville, IA, USA). A 20 uL volume of the reaction mixture, prepared per the BioRad protocol, was pipette mixed and transferred into the droplet generation step. Following thermal cycling (50°C 60 minutes; 95°C 10 minutes; 40 cycles of 95°C 30 seconds and 59°C one minute; 98°C 10 minutes; 4°C hold), droplet fluorescence amplitudes were read, classified as positive or negative, and the N1 copy number calculated using manual thresholding in QuantaSoft Version 1.7.4 (BioRad, Hercules, CA, USA) such that all pertinent negative controls contained no positive droplets.

Table S1 | Characteristics of the 11 WWTPs from which primary influent composite samples were collected for RT-LAMP performance assessment compared to RT-ddPCR. All data were extracted from the Discharge Monitoring Report in the US EPA Enforcement and Compliance History Online database (https://echo.epa.gov/).

WWTP	Population Served	Design Flow (MGD)	Average Flow (MGD)
1	109,294	48	28.63
2	46,557	20	11.41
3	56,227	20	12.14
4	19,054	6	3.48
5	75,138	24	5.91
6	79,652	15	12.57
7	22,627	5.13	5.01
8	6,541	3.90	3.18
9	36,063	10.50	4.91
10	7,304	1.70	1.12
11	8,329	2.80	1.83

Table S2 | SARS-CoV-2 (N2 and E1) and internal control (ACTB) primers used in the NEB SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit (E2019S).

NEB_N2-F3	ACCAGGAACTAATCAGACAAG
NEB_N2-B3	GACTTGATCTTTGAAATTTGGATCT
NEB_N2-FIP	TTCCGAAGAACGCTGAAGCGGAACTGATTACAAACATTGGCC
NEB_N2-BIP	CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA
NEB_N2-LF	GGGGGCAAATTGTGCAATTTG
NEB_N2-LB	CTTCGGGAACGTGGTTGACC
NEB_E1-F3	TGAGTACGAACTTATGTACTCAT
NEB_E1-B3	TTCAGATTTTTAACACGAGAGT
NEB_E1-FIP	ACCACGAAAGCAAGAAAAAGAAGTTCGTTTCGGAAGAGACAG
NEB_E1-BIP	TTGCTAGTTACACTAGCCATCCTTAGGTTTTACAAGACTCACGT
NEB_E1-LB	GCGCTTCGATTGTGTGCGT
NEB_E1-LF	CGCTATTAACTATTAACG
ACTB-F3	AGTACCCCATCGAGCACG
ACTB-B3	AGCCTGGATAGCAACGTACA
ACTB-FIP	GAGCCACACGCAGCTCATTGTATCACCAACTGGGACGACA
ACTB-BIP	CTGAACCCCAAGGCCAACCGGCTGGGGTGTTGAAGGTC
ACTB-LF	TGTGGTGCCAGATTTTCTCCA
ACTB-LB	CGAGAAGATGACCCAGATCATGT

Table S3 | Passive sampling and RT-LAMP methods development and surveillance sample summary table.

Sample Type	Unique WW Sample (n)	Extraction (n)	RT-LAMP	RT-ddPCR
Experimental Purpose: sensitivity, specificity, analytical sensitivity				
primary influent 24-hour composite	42	PowerViral (42)	Y	Y
raw sewage from tampon swab	7	PowerViral (7)	Y	Y
Experimental Purpose: extraction and processing methods				
primary influent 24-hour composite	42	Viral RNA Mini (9) PowerViral (33)	Y	Y
raw sewage from tampon swab	68	Viral RNA Mini (5) PowerViral (63) No extraction (17) Heat extraction (10)	Y	N
Experimental Purpose: Prospective surveillance				
raw sewage	53	PowerViral (53)	Y	N

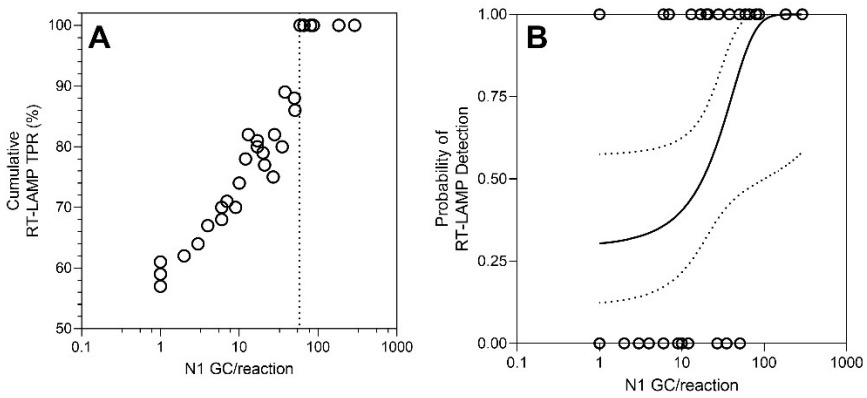

Figure S1 | (A) RT-LAMP cumulative true positive rate (sensitivity; y-axis) among individual reactions along an N1 gene copy (GC) per reaction gradient (x-axis); (B) SARS-CoV-2 RNA probability of detection among RT-LAMP triplicates (y-axis) as estimated by a logistic regression model ($p = 0.0034$; $R^2 = 0.24$) along the same N1 GC gradient (x-axis).

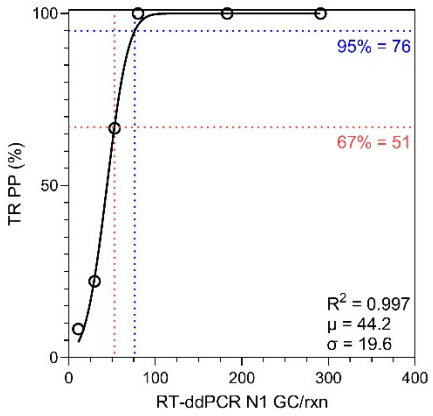

Figure S2 | The 95% and 67% limits of detection (LODs) for a single RT-LAMP reaction as measured along a concentration gradient of N1 gene copies per reaction (rxn) quantified by RT-ddPCR (x-axis). The percentage of technical replicates positive (TR PP; y-axis) was best fit ($R^2 = 0.997$) by a Gaussian distribution with a mean of 44.2 and standard deviation of 19.6.

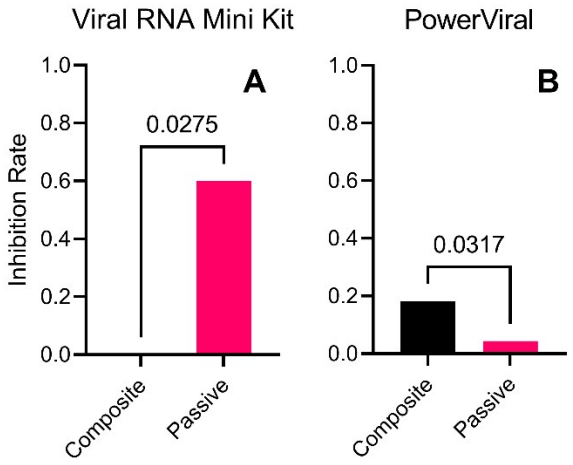

Figure S3 | RT-LAMP inhibition rates among primary influent composite samples (black) and tampon swab sorbate samples (pink) extracted via (A) the Viral RNA Mini Kit and (B) the AllPrep PowerViral DNA/RNA Kit.

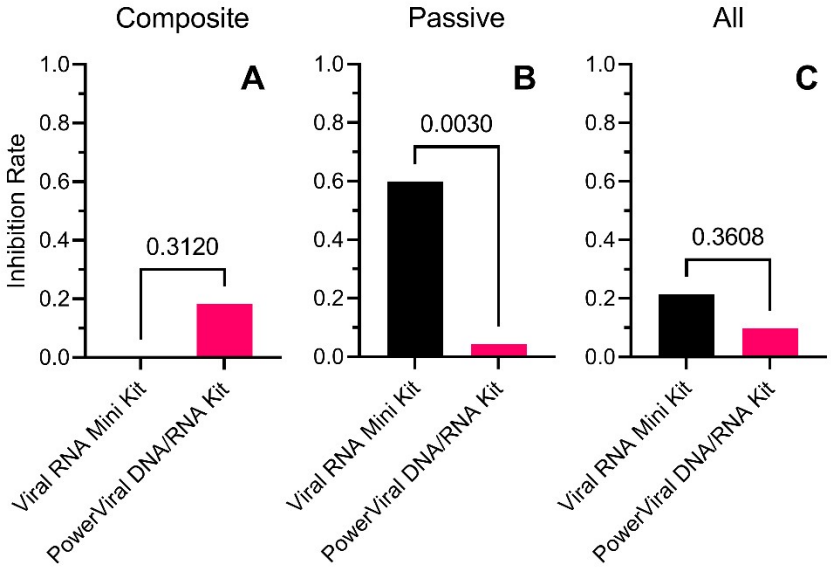

Figure S4 | RT-LAMP inhibition rates among (A) primary influent composite samples, (B) tampon swab sorbate samples, and (C) all wastewater samples extracted via the Viral RNA Mini Kit (black) and the AllPrep PowerViral DNA/RNA Kit (pink).

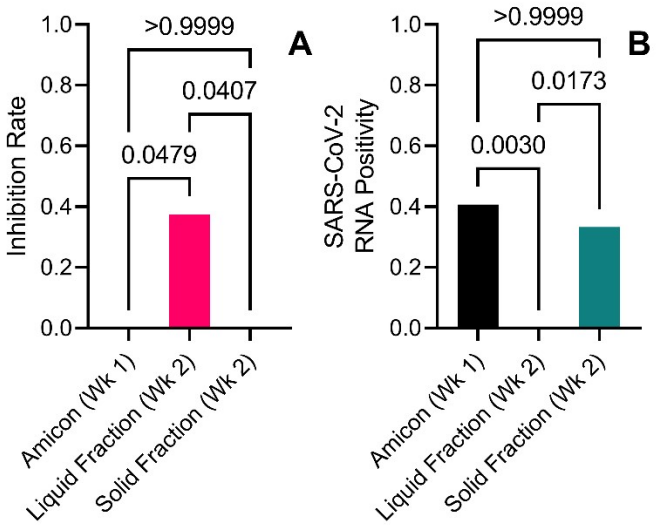

Figure S5 | (A) RT-LAMP inhibition rates among tampon swab sorbate processed by Amicon ultrafiltration, liquid faction only processed by Amicon ultrafiltration, and solid fraction (A), and (B) SARS-CoV-2 RNA positivity by RT-LAMP among the same.

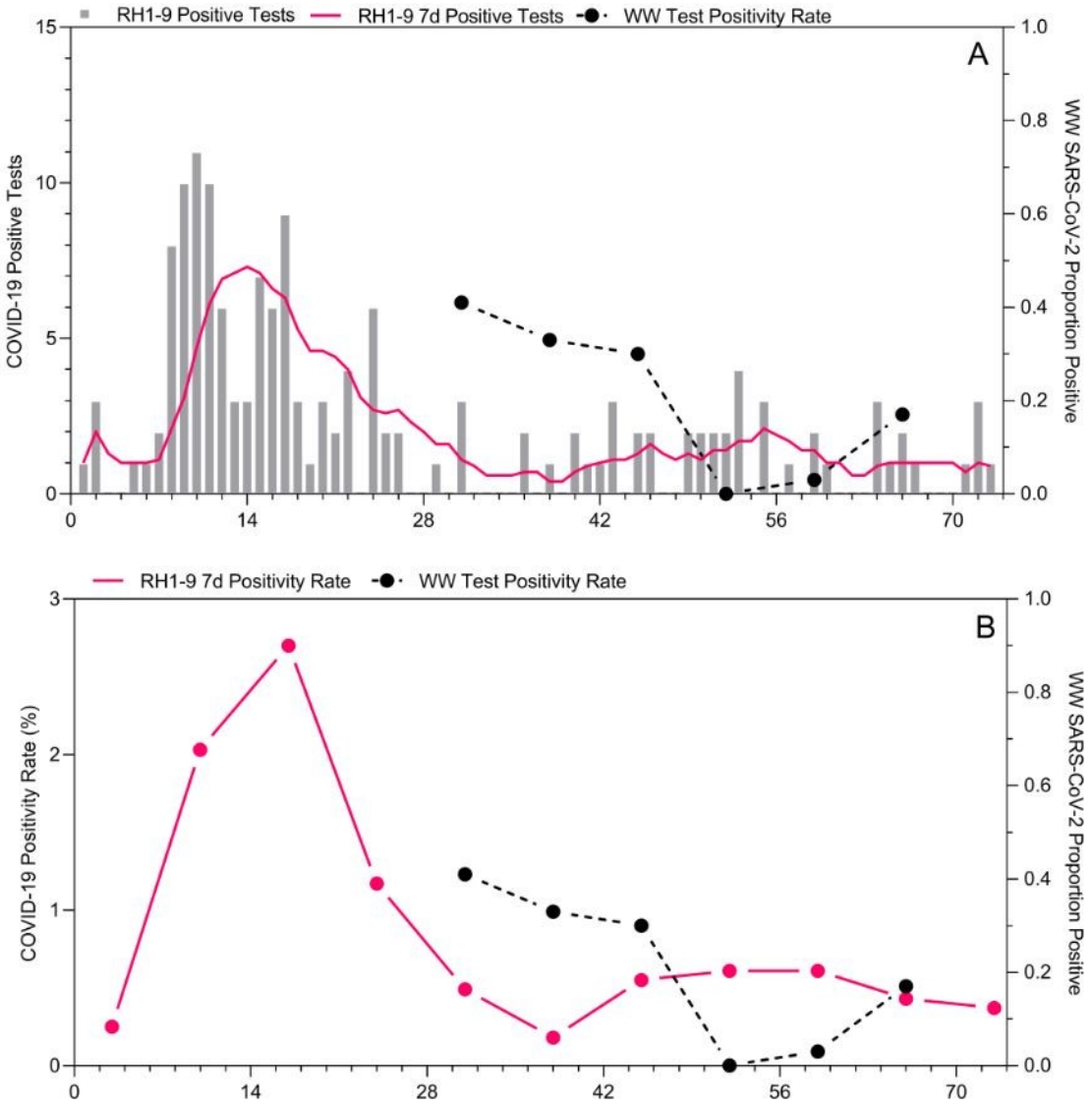

Figure S6 | (A) COVID-19 clinical positives and average 7-day number of positives (left y-axis) and wastewater (WW) SARS-CoV-2 proportion positive (right y-axis) in the nine residence halls included in wastewater monitoring during the study period from 0 to 73 days (x-axis); (B) COVID-19 clinical testing weekly positivity (left y-axis) and wastewater (WW) SARS-CoV-2 proportion positive (right y-axis) in the nine residence halls included in wastewater monitoring during the study period from 0 to 73 days (x-axis).

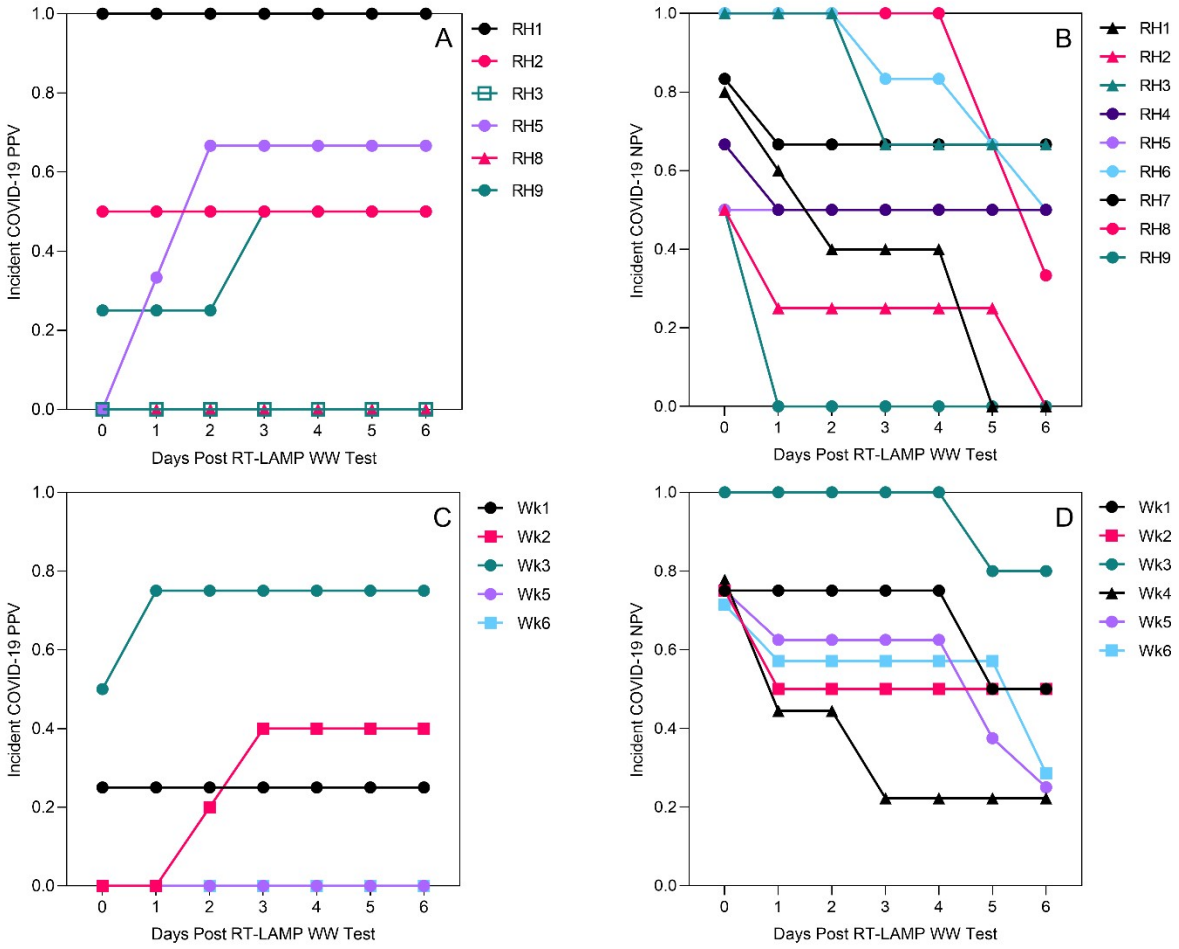

Figure S7 | Positive predictive value (PPV) of RT-LAMP wastewater monitoring (1 of 3 replicates positive) for incident COVID-19 cases from 1 to 7 days post wastewater testing (A) as observed during 6 weeks of monitoring among nine residence halls and (C) as observed among nine residence halls each week for six weeks. Negative predictive value (NPV) of RT-LAMP wastewater monitoring for incident COVID-19 cases from 1 to 7 days post wastewater testing (B) as observed during 6 weeks of monitoring among nine residence halls and (D) as observed among nine residence halls each week for six weeks.

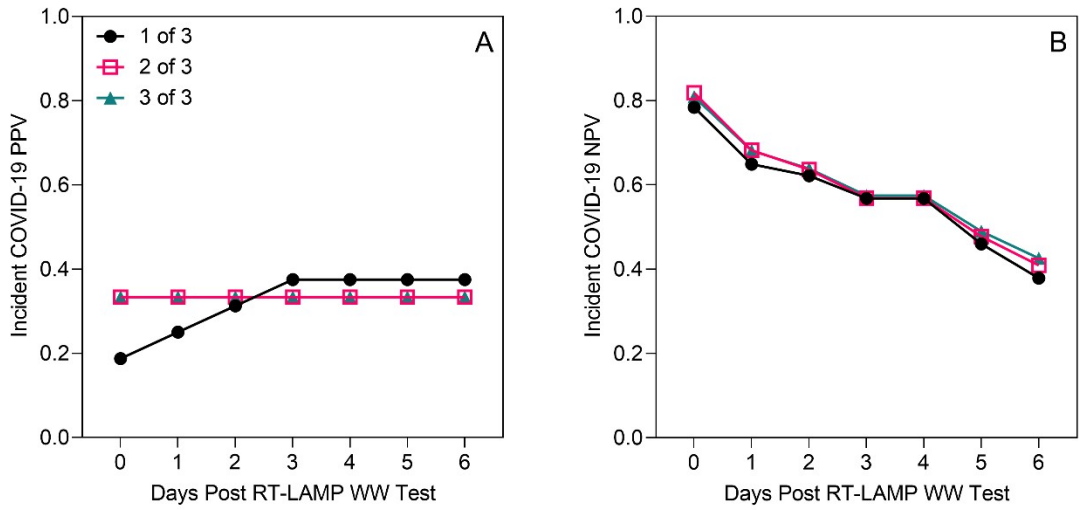

Figure S8 | PPV (A) and NPV (B) in the seven days following wastewater monitoring by tampon swab and RT-LAMP with three different cutoff classifications (1 of 3 reactions, 2 of 3 reactions, 3 of 3 reactions) as observed during monitoring of wastewater from nine residence halls for six weeks.

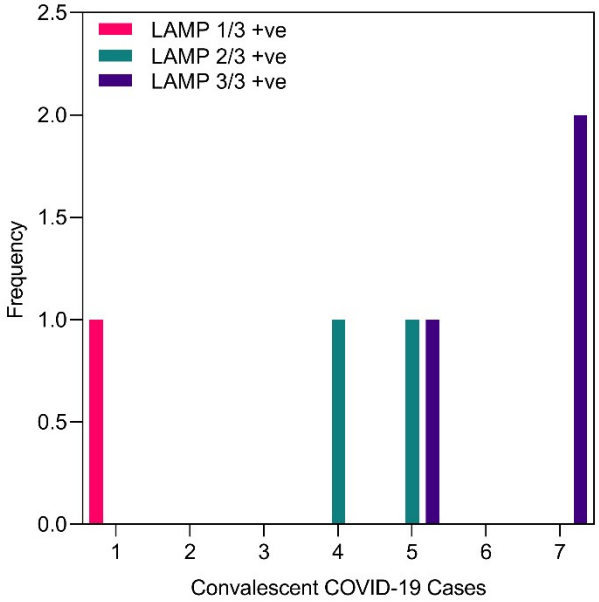

Figure S9 | Cumulative convalescent COVID-19 cases returning from isolation to residence halls in the seven days prior to RT-LAMP wastewater testing when there were no incident COVID-19 cases observed in the seven days following wastewater testing color coded by LAMP positivity cutoffs (1 of 3, 2 of 3, and 3 of 3 reactions positive for SARS-CoV-2 RNA).

References

1. Ye Y, Ellenberg RM, Graham KE, Wigginton KR. Survivability, Partitioning, and Recovery of Enveloped Viruses in Untreated Municipal Wastewater. Environ Sci Technol. 2016;50(10):5077–85.
2. Li B, Di DYW, Saingam P, Jeon MK, Yan T. Fine-Scale Temporal Dynamics of SARS-CoV-2 RNA Abundance in Wastewater during A COVID-19 Lockdown. Water Res [Internet]. 2021 Jun;197:117093. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0043135421002918