**Supplemental Files: Building-level wastewater monitoring using passive samplers and RT-LAMP for SARS-CoV-2 RNA detection**

Aaron Bivins1,2, Megan Lott3, Marlee Shaffer1, Zhenyu Wu1, Devin North1, Erin K. Lipp3, Kyle Bibby1,2\*

1 Department of Civil & Environmental Engineering & Earth Sciences, University of Notre Dame, 156 Fitzpatrick Hall, Notre Dame, IN 46556

2 Environmental Change Initiative, University of Notre Dame, 721 Flanner Hall, Notre Dame, IN 46556

3 Department of Environmental Health Science, University of Georgia, 150 East Green Street, Athens, GA 30602

\*[kbibby@nd.edu](mailto:kbibby@nd.edu)

Table S1 RT-LAMP Primers

Figure S1 RT-LAMP 95% and 67% LODs

Figure S2 RT-LAMP true positive rate

Figure S3 Composite versus passive inhibition rates by extraction kit

Figure S4 Viral RNA Mini versus Power Viral inhibition rates by sample type

Figure S5 Inhibition rate and positivity of tampon swab sorbate fractions

Figure S6 WW proportion positive by RT-LAMP and campus-wide COVID-19 clinical metrics

Figure S7 WW proportion positive by RT-LAMP and COVID-19 clinical metrics aggregated for all residence halls included in wastewater monitoring

Figure S8 Hourly domestic water use in three RHs

Figure S9 RT-LAMP COVID-19 case PPV and NPV stratified by residence halls and weeks of monitoring

Figure S10 RT-LAMP results frequency distribution for convalescent COVID-19 cases returning to residence halls from quarantine

Figure S11 RT-LAMP COVID-19 case PPV and NPV after correction for detection of convalescent COVID-19 cases returning to residence halls from quarantine

Table S1 | 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  NEB\_N2-B3  NEB\_N2-FIP  NEB\_N2-BIP  NEB\_N2-LF  NEB\_N2-LB | ACCAGGAACTAATCAGACAAG  GACTTGATCTTTGAAATTTGGATCT  TTCCGAAGAACGCTGAAGCGGAACTGATTACAAACATTGGCC  CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA  GGGGGCAAATTGTGCAATTTG  CTTCGGGAACGTGGTTGACC |
| NEB\_E1-F3  NEB\_E1-B3  NEB\_E1-FIP  NEB\_E1-BIP  NEB\_E1-LB  NEB\_E1-LF | TGAGTACGAACTTATGTACTCAT  TTCAGATTTTTAACACGAGAGT  ACCACGAAAGCAAGAAAAAGAAGTTCGTTTCGGAAGAGACAG  TTGCTAGTTACACTAGCCATCCTTAGGTTTTACAAGACTCACGT  GCGCTTCGATTGTGTGCGT  CGCTATTAACTATTAACG |
| ACTB-F3  ACTB-B3  ACTB-FIP  ACTB-BIP  ACTB-LF  ACTB-LB | AGTACCCCATCGAGCACG  AGCCTGGATAGCAACGTACA  GAGCCACACGCAGCTCATTGTATCACCAACTGGGACGACA  CTGAACCCCAAGGCCAACCGGCTGGGGTGTTGAAGGTC  TGTGGTGCCAGATTTTCTCCA  CGAGAAGATGACCCAGATCATGT |

Chart, line chart

Description automatically generated

Figure S1 | 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 (R2 = 0.997) by a Gaussian distribution with a mean of 44.2 and standard deviation of 19.6.

Chart

Description automatically generated

Figure S2 | (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; R2 = 0.24) along the same N1 GC gradient (x-axis); (C) the receiver operating characteristic curve for RT-LAMP performed in triplicate compared to RT-ddPCR detection of SARS-CoV-2 RNA (area under curve = 0.81 95%CI: 0.65 - 0.97; p = 0.0047).

Chart

Description automatically generated

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.

Chart, waterfall chart

Description automatically generated

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).

Chart

Description automatically generated

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.

Chart, histogram

Description automatically generated

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

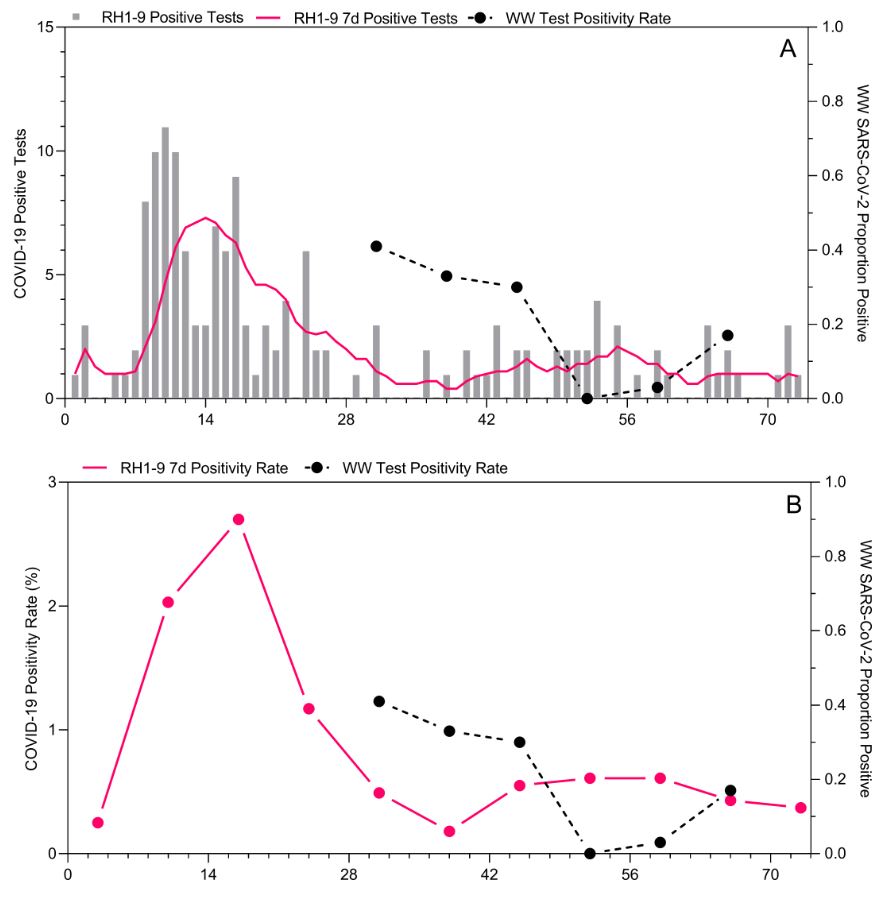


Figure S7 | (A) COVID-19 clinical positives and average 7-day number of positives (left y-axis) and wastewater (WW) SARS-CoV-2 proportion positive (right y-axis) in the nine residence halls included in wastewater monitoring during the study period from 0 to 73 days (x-axis); (B) COVID-19 clinical testing 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).

Chart, histogram, scatter chart

Description automatically generated

Figure S8 | Hourly domestic water use (y-axis) in RH2, 6, and 7 during weeks 3 (black) and 4 (pink) of wastewater monitoring.

A picture containing graphical user interface

Description automatically generated

Figure S9 | Positive predictive value (PPV) of RT-LAMP wastewater monitoring 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.

Chart, histogram

Description automatically generated

Figure S10 | 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).

Chart, line chart

Description automatically generated

Figure S11 | Negative predictive value (NPV) and positive predictive value (PPV) of tampon swab and RT-LAMP wastewater monitoring with correction for false positives attributable to the detection of convalescent COVID-19 cases.