Supplementary Information

Membrane-Based in-Gel Loop-Mediated Isothermal Amplification (mgLAMP) System for SARS-CoV-2 Quantification in Environmental Waters

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Text S1. Principles for theoretical screening of QUASR quenching probe designs

Our principles for designing the quenching probes include: 1) the melting temperature ($T_m$) of the probe-primer hybrid should be well below reaction temperature of $65^\circ C$ so that the quenching probe can be released from the primer to allow proper primer annealing and efficient amplification; 2) this $T_m$ should also be well above room temperature, with a highly negative $\Delta G_{25\,^\circ C}$ to allow stable binding of the quenching probe and unused FluoroPs for a higher signal-to-noise ratio; 3) the hybridization between the quenching probe and other primers should also have the $T_m$ well below room temperature and $\Delta G_{25\,^\circ C}$ not highly negative to reduce background fluorescence; 4) the formation of quenching probe self-dimers should be avoided by having $\Delta G_{25\,^\circ C}$ close to or above zero. In this work, although primer set 10 was one of the most efficient primers, it was not further considered for QUASR probe design because both of its FIP and BIP start with a guanine (G) at 5’-end, which would quench the fluorophore attached to it due to photoinduced electron transfer\textsuperscript{1}.

Text S2. Comparison between QUASR and molecular beacon probing strategies

We note that the results reported about QUASR was for primer set 11, the primer set we have been using since an aerosol contamination accident occurred while using primer set 3. Before the primer switch, molecular beacon was also tried for primer set 3 and was compared with its QUASR designs (Figure S5). For molecular beacons, loop primer LB or LF is modified by adding 5-6 nt to the 5’-end so that these nucleotides would self-hybridize into a hairpin structure for signal quenching.\textsuperscript{2} The 5’-end is labeled with a fluorophore, and the 3’-end is labeled with a dye quencher molecule. It was observed that QUASR probes generally yielded higher fluorescence intensity for target-spiked samples and had a higher S/N. The systematically better performance of QUASR
probes was likely due to the quencher-fluorophore interaction upon target binding with the fluorophore-labeled primer. The QUASR quenching probe is completely released, whereas the quencher is still in vicinity of the fluorophore in molecular beacon which merely opens the hairpin structure.

**Text S3. Optimization of mgLAMP incubation time**

To optimize the incubation time for mgLAMP using primer set 11, mgLAMP images with the same spiked SARS-CoV-2 concentration were compared for 30 min, 35 min, and 40 min (Figure S7a-c). The NTC was clear of amplicon dot after 40 min of incubation (Figure S7d), indicating no false positive results within 40 min. With 30 min of incubation, in the porous hydrogel network, LAMP incubation time could affect the target quantification output in terms of amplicon dot count and dot size\(^3\). However, we observed no increase in amplicon dot count and no discernible size growth with longer incubation time, indicating maximal amplification was achieved within 30 min. The halted amplification could be due to the pH drop in the chamber when large amount of amplification products has already been generated.\(^4\) Therefore, in our finalized protocol using primer set 11, mgLAMP reactions were incubated at 65 °C for 30 minutes.

**Text S4. Performance of mgLAMP vs. RT-qPCR in spiked Milli-Q water**

mgLAMP amplicon dot count had a linear correlation with concentration spiked SARS-CoV-2 particles \((R^2 = 0.9976)\), indicating that the dynamic range of mgLAMP covers 0.96 to 9600 copies/mL. We note that higher concentrations were spiked into 1 mL of Milli-Q water, whereas the lower concentrations of 0.96 and 9.6 copies/mL was detected from 100 mL and 10 mL, respectively. The lower count observed in mgLAMP compared to the spiked concentration (slope = 0.0345) was likely attributed to the limited RT-LAMP amplification efficiency of this primer set, the preliminary LOD of which was already among the highest in literature\(^5-9\). The higher end
of the dynamic range was limited by the total amount of dots held in one slide. Huang et al.\textsuperscript{3} reported that 1000 dots per slide as most could be well captured by a smartphone camera for dot counting. The dot count of 336 ± 41 from highest concentration tested (9600 copies/mL) was at the order of magnitude that the dots were still countable. The golden-standard RT-qPCR had a good amplification efficiency of 103\%, but the preliminary LOD was much higher at around 1920 copies/mL due to the small volume (2 μL) spiked in assay. The dynamic range of RT-qPCR spanned at least 4 orders of magnitudes towards higher end of SARS-CoV-2 concentration (around 1920 to above 1.92 × 10\textsuperscript{7} copies/mL). Compared to RT-qPCR, mgLAMP demonstrated its advantage of simplified sample processing, minimal instrument requirement, capability of absolute quantification, and a much lower detectable target concentration owing to the integrated viral concentration. These traits manifest its distinctive suitability for environmental applications where low concentrations of target virus are present.

Text S5. Environmental sample preparation

The environmental samples were subject to a brief pretreatment for virus dislodging and a modified membrane filtration step involving 3-tier filtration, as shown in Figure S2. The protocol for the virus dislodging step was slightly modified from literature\textsuperscript{10-11}. Sodium pyrophosphate decahydrate (Na\textsubscript{4}P\textsubscript{2}O\textsubscript{7}·10H\textsubscript{2}O, Sigma Aldrich, St. Louis, MO) was prepared into a 100 mM stock by dissolving in Milli-Q water, and was added to environmental samples at a final concentration of 5 mM. The samples were incubated at room temperature for 10 min, and then sonicated (46kHz, 30W, Branson\textsuperscript{TM} Ultrasonic Cleaner Model B200, Branson Ultrasonics, Brookfield, CT) in an ice-bath for 3 min. During the 3 min of sonication, the samples were taken out and gently shaken by hand for 30 seconds after every minute of sonication. Immediately after the virus dislodging pretreatment, the samples were loaded into a 5-mL syringe for 3-tier filtration. For the 1st tier, a 13
mm PCTE membrane with 1 μm pores was used. The 2nd tier filter employed a 25 mm PCTE membrane with 0.2 μm pores. The filtration membranes were not supported by any mesh spacer for the first two tiers. The 3rd tier, resembling regular mgLAMP setup, contained a 13 mm 0.08 μm-pore PCTE membrane supported by a 13 mm PETE drain disc mesh spacer. The membranes were loaded into 13 mm or 25 mm Swinnex filter holder (MilliporeSigma, Burlington, MA). The filter holders were then connected and attached to the LuerLock of the syringe. A syringe pump was used to drive the sample flowing through the 3-tier filtration at a flow rate of 0.5 mL/min. The membrane from the 3rd tier was retrieved for mgLAMP analysis. For surface water mgLAMP, the 2nd tier of filtration was omitted.

Text S6. Adaption of mgLAMP for bacterial detection

Milli-Q water spiked with E. coli (ATCC 10798) or S. Typhi (CVD 909) was filtered through the PCTE membrane with pores of 0.2 μm rather than 80 nm to adapt for bacterial capture. Customized reaction system reported by Lin et al. was deployed and modified for bacterial detection in mgLAMP assays. Each 30-μL reaction contained 3 μL 10× Isothermal Amplification Buffer (NEB, Ipswich, MA), 4 mM MgSO4 (NEB, Ipswich, MA), 1.4 mM dNTP (Deoxynucleotide (dNTP) Solution Mix, NEB, Ipswich, MA), 640 U/mL Bst 2.0 WarmStart® DNA Polymerase (NEB, Ipswich, MA), 1.5 mg/mL bovine serum albumin (BSA, Molecular Biology Grade, NEB, Ipswich, MA), 2 mM NaF (sodium fluoride, Sigma Aldrich, St. Louis, MO), 0.1 mg/mL Lysozyme Solution (Thermo Scientific, Waltham, MA), 1.6 μM FIB/BIP, 0.2 μM F3/B3, 0.8 μM LF/LB, 0.4 μM molecular beacon, nuclease-free water, and 10% (w/v) hydrogel. Primer sequences for E. coli and S. Typhi were listed in Table S8. Molecular beacon sequences for E. coli and S. Typhi were respectively (6-FAM)-5’-CACCTTATCAATCTCGATATGGAAGGTG-(3IABkFQ) and
(6-FAM)-5’-AGGAACCTGGATGGCTTCGTTCCT-3’-(3IABkFQ). All primers and molecular beacons were ordered from IDT.
## Supplementary Tables

### Table S1

| No. | Primer | Sequence (5' to 3') | Target gene | Reference |
|-----|--------|---------------------|-------------|-----------|
| 1   | F3     | TGGACCCCAAAATCAGCG  | N gene      | Gonzalez- Gonzalez et al. 6 |
|     | B3     | GCGTTGTCCTCGAGGGAAT |             |           |
|     | FIP    | CCACTGCGTTCTCCATTTCTTGGTAAATGCACCCGCATTACG |             |           |
|     | BIP    | CGCGATCAAAAGCACTGCGCCCTTTGCATGTTGAGTGAAGA |             |           |
|     | LF     | TGAAATCTGAGGGTCCTACCAA |             |           |
|     | LB     | TTACCACAAATATCGCTCTTGGT |             |           |
| 2   | F3     | CCAAGATGGAAGAAGAGG  | N gene      |           |
|     | B3     | CCGTCACCACCCAGAATTT |             |           |
|     | FIP    | AGAGGCAGACACAGGATGGAGCTGCTGATTGGAAGAAGAAGAG |             |           |
|     | BIP    | TCAACTGGAAGAAGAAGAAGCAAGTATGTGCTCCTACTGCCC |             |           |
|     | LF     | TCAATTCGAGTTGATGGCTGA |             |           |
|     | LB     | TAACACCAATAGCAGTCCAGATGA |             |           |
| 3   | F3     | TGGCTACTACGATGATGCTCTT | ORF1ab     | Lamb et al. 7 |
|     | B3     | TATGGCCACCACTGCTCCTT |             |           |
|     | FIP    | GCACCGCTACTGAGAATGCTGCTGATTGGAAGAAGAAGAGGAGAG |             |           |
|     | BIP    | TCAACTGGAAGAAGAAGAAGCAAGTATGTGCTCCTACTGCCC |             |           |
|     | LF     | CGCTACTGAGCTGCTCCTAAG |             |           |
|     | LB     | TTGGTAAGAGAAGTATAAAAGGAGC |             |           |
| 4   | F3     | CTGCACCTCATGTTGCTCTT | ORF1a       | Zhang et al. 8 |
|     | B3     | AGGTGTCGCCGCTAATCGA |             |           |
|     | FIP    | AGAGGCAGACACAGGATGGAGCTGCTGATTGGAAGAAGAAGAG |             |           |
|     | BIP    | TCAACTGGAAGAAGAAGAAGCAAGTATGTGCTCCTACTGCCC |             |           |
|     | LF     | CGCTACTGAGCTGCTCCTAAG |             |           |
|     | LB     | TTGGTAAGAGAAGTATAAAAGGAGC |             |           |
| 5   | F3     | TGGCTACCTACCAGAAGAAGCT | N gene      |           |
|     | B3     | TGGAGGACATGATGCTGCTGATTGGAAGAAGAAGAGGAGAG |             |           |
|     | FIP    | AGAGGCAGACACAGGATGGAGCTGCTGATTGGAAGAAGAAGAG |             |           |
|     | BIP    | TCAACTGGAAGAAGAAGAAGCAAGTATGTGCTCCTACTGCCC |             |           |
|     | LF     | CGCTACTGAGCTGCTCCTAAG |             |           |
|     | LB     | TTGGTAAGAGAAGTATAAAAGGAGC |             |           |
| 6   | F3     | TGGCTACCTACCAGAAGAAGCT | N gene      |           |
|     | B3     | TGGAGGACATGATGCTGCTGATTGGAAGAAGAAGAGGAGAG |             |           |
|     | FIP    | AGAGGCAGACACAGGATGGAGCTGCTGATTGGAAGAAGAAGAG |             |           |
|     | BIP    | TCAACTGGAAGAAGAAGAAGCAAGTATGTGCTCCTACTGCCC |             |           |
|     | LF     | CGCTACTGAGCTGCTCCTAAG |             |           |
|     | LB     | TTGGTAAGAGAAGTATAAAAGGAGC |             |           |
| 7   | F3     | ACAGGAGGGACCTACAGAAGAGCT | N gene      |           |
|     | B3     | TGCAAGCAATGTTGGACAGGAT |             |           |
|     | FIP    | AGAGGCAGACACAGGATGGAGCTGCTGATTGGAAGAAGAAGAG |             |           |
|     | BIP    | TCAACTGGAAGAAGAAGAAGCAAGTATGTGCTCCTACTGCCC |             |           |
|     | LF     | CGCTACTGAGCTGCTCCTAAG |             |           |
|     | LB     | TTGGTAAGAGAAGTATAAAAGGAGC |             |           |
| 8   | F3     | ACAGGAGGGACCTACAGAAGAGCT | N gene      |           |
|     | B3     | TGCAAGCAATGTTGGACAGGAT |             |           |
|     | FIP    | AGAGGCAGACACAGGATGGAGCTGCTGATTGGAAGAAGAAGAG |             |           |
|     | BIP    | TCAACTGGAAGAAGAAGAAGCAAGTATGTGCTCCTACTGCCC |             |           |
|     | LF     | CGCTACTGAGCTGCTCCTAAG |             |           |
|     | LB     | TTGGTAAGAGAAGTATAAAAGGAGC |             |           |
Table S1 (continued)

| No. | Primer | Sequence (5’ to 3’) | Target gene | Reference         |
|-----|--------|---------------------|-------------|-------------------|
|     | F3     | TGCTTCAGTCAGCTGATG  | ORF1ab      | El-Tholoth et al.⁹ |
| 9   | B3     | TTAATTTGTCATCTTCTGCTTT |             |                   |
|     | FIP    | TCACTAAGTGCTGCAATGCCTCTT |             |                   |
|     | BIP    | TCTAGACAGGTTTGTGAACATCTATCTT | ORF1ab      | El-Tholoth et al.⁹ |
|     | LF     | CTGCATTTACAGCGCA      |             |                   |
|     | LB     | GTAGCTGGTTCTGTTCTCC   |             |                   |
|     | F3     | GTTCCTGATCAGTCGTCG    | ORF1ab      | El-Tholoth et al.⁹ |
| 10  | B3     | GTTGGGCTTTGTGTGTGTG   |             |                   |
|     | FIP    | GCCAGCCATTCTAGCAGAGCAGCTTTTGAGAAAATTCAACTCC | N gene | Ganguli et al.⁵ |
|     | BIP    | GATGCTGCTTTGCTTACAGACATTTTTGCTCTCA      | N gene | Ganguli et al.⁵ |
|     | LB     | GCTGCTGACAGATTGAAACCTG |             |                   |
|     | F3     | GTCACTGCTGAAATAAGCATAT |             |                   |
| 11  | B3     | GAGTCACACGTCATCATG    |             |                   |
|     | FIP    | TAAGGCCTGAGTTTCTACGCACTTACGTAACAAACATTCCA | N gene | Ganguli et al.⁵ |
|     | BIP    | CAAGACAGAGAAAACAGCAAATCTGATTTGTTGGAATTTGTTGAG | ORF1ab      | El-Tholoth et al.⁹ |
|     | LB     | GTGACTCTCTCTCGCTGAGATT |             |                   |

Table S1. Sequences and the corresponding target genes for 11 published RT-LAMP primer sets targeting SARS-CoV-2.⁵-⁹
Table S2. Assay recipes and temperature protocols summarized for (a) primer screening, (b) in-assay viral lysis using primer set 3, (c) in-assay viral lysis using primer set 11, and (d) quenching probe selection and optimization.

| Test | Assay recipe | Varying component | Temperature protocol |
|------|--------------|-------------------|---------------------|
| a    | Each 20-μL reaction contained 2 μL of template RNA for samples or nuclease-free water for no-template controls (NTCs), 10 μL of 2× WarmStart LAMP Master Mix, 0.4 μL of 50× LAMP Fluorescent Dye, 2 μL of 10× primer mix, and 5.6 μL of nuclease-free water. | Primer mix identity | 40 cycles of 65 °C for 1 min followed by 10 min of 80 °C heat inactivation in qPCR platform. |
| b    | Each 20-μL reaction contained 10 μL of 2× WarmStart LAMP Master Mix, 0.4 μL of 50× LAMP Fluorescent Dye, 2 μL of 10× primer mix, 1 μL of 20 U/μL SUPERase•In™ RNase Inhibitor (Invitrogen, Carlsbad, CA), 2 μL of sample or water, and complementary amount of nuclease-free water. | Concentration of Triton™ X-100 (0.3%, 0.5%, and 0.7%) or Triton™ X-405R (0.5%). | 60 cycles of 63 °C for 1 min followed by 10 min of 80 °C heat inactivation in qPCR platform. |
| c    | Each 20-μL reaction contained 10 μL of 2× WarmStart LAMP Master Mix, 0.4 μL of 50× LAMP Fluorescent Dye, 2 μL of 10× primer mix, 2 μL of sample or water, and complementary amount of nuclease-free water. | Concentration of Triton™ X-100 or Triton™ X-405R. | 60 cycles of 65 °C for 1 min followed by 10 min of 80 °C heat inactivation in qPCR platform. |
| d    | Each 20-μL reaction contained 10 μL of 2× WarmStart LAMP Master Mix, 2 μL of 10× primer mix, 1.2 μL of 5M Betaine (PCR reagent, Sigma Aldrich, St. Louis, MO), 2 μL of sample or water, and complementary amount of nuclease-free water. | Quenching probe identity and concentration (1.6 μM, 2.4 μM, and 3.2 μM) | 65 °C for 40 min, heat inactivation at 80 °C for 10 min, and cooling at 20 °C for 2 min in qPCR platform. |
Table S3

| Primer set # | RNA copies/reaction | NTC |
|--------------|---------------------|-----|
|              | 186 | 93  | 18.6 | 9.3 |     |
| 1            | 3/3 | 3/3 | 2/3  | 2/3 | 1/3 |
| 2            | 3/3 | 0/3 | 0/3  | 0/3 | 0/3 |
| 3            | 3/3 | 3/3 | 0/3  | 0/3 | 0/3 |
| 4            | 2/3 | 1/3 | 0/3  | 0/3 | 0/3 |
| 5            | 1/3 | 0/3 | 0/3  | 0/3 | 0/3 |
| 6            | 3/3 | 3/3 | 2/3  | 2/3 | 0/3 |
| 7            | 2/3 | 2/3 | 1/3  | 0/3 | 0/3 |
| 8            | 3/3 | 0/3 | 2/3  | 1/3 | 0/3 |
| 9            | 3/3 | 0/3 | 0/3  | 0/3 | 0/3 |
| 10           | 3/3 | 3/3 | 2/3  | 1/3 | 0/3 |
| 11           | 3/3 | 3/3 | 1/3  | 1/3 | 0/3 |

Table S3. Primer set screening based on sensitivity of detection. The 11 sets of published RT-LAMP primer sets were tested using extracted RNA templates spiked at 9.3 to 186 copies per 20µL of reaction. Triplicate samples were tested. Each data represents the number of positive amplifications observed out of the triplicates tested. For target-spiked samples, green indicates true positives observed for 3 out of 3 samples, and yellow indicates false negatives occurred with 1-3 samples failed to amplify. For NTCs, blue indicates all triplicated were true negatives, and red indicates false positive was observed.

Table S4

| Triton | Concentration | Copies/reaction | NTC |
|--------|---------------|-----------------|-----|
|        |               | 186 | 93  | 18.6 | 9.3 |     |
| X-100  | 0.3%          | 3/3 | 3/3 | 3/3  | 2/3 | 3/3 |
|        | 0.5%          | 3/3 | 3/3 | 3/3  | 2/3 | 3/3 |
|        | 0.7%          | 3/3 | 3/3 | 3/3  | 1/3 | 3/3 |
| X-405R | 0.3%          | 1/3 | 1/3 | 1/3  | 1/3 | 2/3 |
|        | 0.5%          | 0/3 | 2/3 | 1/3  | 0/3 | 2/3 |
|        | 0.7%          | 0/3 | 0/3 | 1/3  | 1/3 | 2/3 |
| Extracted RNA | 3/3 | 3/3 | 1/3  | 1/3 | 3/3 |
| SARS-CoV-2 particles | 0/3 | 0/3 | 1/3  | 0/3 | 0/3 |

All triplicates had time-to-threshold earlier than all NTCs

Had sample with time-to-threshold later than at least one NTC

True negatives for all triplicates

With false positives
Table S4. RT-LAMP results for assays containing 0.3%, 0.5%, and 0.7% Triton X-100 or X-405R, using primer set 11. Triton-added samples were spiked with Zeptometrix SARS-CoV-2 particles. No-Triton controls were included for samples spiked with the same concentration of SARS-CoV-2 particles and template RNA extracted using commercial kit. NTCs were included for each recipe tested. The fluorescence emitted by a DNA-intercalating LAMP dye was monitored for 60 cycles of 1 min RT-LAMP reaction. For target-spiked samples, green indicates that the time-to-threshold was earlier than all the NTCs of the corresponding recipe for 3 out of 3 samples, and yellow indicates that the time-to-threshold was later than at least one NTC of the corresponding recipe for 1-3 out of 3 samples. For NTCs, blue indicates all triplicated were true negatives, and red indicates false positive was observed.
Table S5

| NA | 1 | 2 | 3 | 4 |
|----|---|---|---|---|
| 0  | 1.4 | 1.5 | 1.6 | 1.7 |
| 0  | 2.0 | 2.1 | 2.2 | 2.3 |
| 0  | 3.0 | 3.1 | 3.2 | 3.3 |
| 0  | 4.0 | 4.1 | 4.2 | 4.3 |
| 0  | 5.0 | 5.1 | 5.2 | 5.3 |
| 0  | 6.0 | 6.1 | 6.2 | 6.3 |
| 0  | 7.0 | 7.1 | 7.2 | 7.3 |
| 0  | 8.0 | 8.1 | 8.2 | 8.3 |
| 0  | 9.0 | 9.1 | 9.2 | 9.3 |
| 0  | 10.0 | 10.1 | 10.2 | 10.3 |

Note: This table contains data entries that are not clearly visible due to the quality of the image.
**Table S5.** Theoretical screening of QUASR probe design for primer set 11. For 5’-FAM-labeled FIP and BIP, quenching probes of 7 to 17 nt. The $T_m$ and $\Delta G^{25\text{o}}$ values of probe-primer hybrid, primer-product hybrid, as well as interference of the quenching probe with other primers were calculated using DINAMelt webserver. The bolded quenching probe names (qFIP12nt, qFIP17nt, qBIP10nt, and qBIP15nt) were the ones ordered for experimental tests. The sequences of which are also listed in **Section 2.2.**

**Table S6**

| QUASR quencher | Conc.  | Positive sample fluorescence intensity | NTC fluorescence intensity | S/N ratio |
|----------------|-------|--------------------------------------|---------------------------|----------|
|                |       | 1     | 2     | 3     | Mean | 1     | 2     | 3     | Mean |       |
| qFIP12nt       | 1.6µM | 250   | 255   | 249   | 251.3 | 203   | 199   | 200   | 200.7 | 1.25  |
|                | 2.4µM | 255   | 254   | 243   | 250.7 | 172   | 171   | 161   | 168.0 | 1.49  |
|                | 3.2µM | 252   | 255   | 255   | 254.0 | 139   | 136   | 123   | 132.7 | 1.91  |
| qFIP17nt       | 1.6µM | 231   | 221   | 229   | 227.0 | 142   | 132   | 141   | 136.3 | 1.64  |
|                | 2.4µM | 197   | 206   | 198   | 200.3 | 82    | 80    | 78    | 80.0  | 2.50  |
|                | 3.2µM | 190   | 192   | 197   | 193.0 | 73    | 75    | 78    | 75.3  | 2.56  |
| qBIP10nt       | 1.6µM | 255   | 255   | 254   | 254.7 | 193   | 191   | 196   | 193.3 | 1.32  |
|                | 2.4µM | 254   | 252   | 255   | 253.7 | 147   | 144   | 148   | 146.3 | 1.73  |
|                | 3.2µM | 255   | 255   | 254   | 254.7 | 142   | 144   | 136   | 140.7 | 1.81  |
| qBIP15nt       | 1.6µM | 255   | 255   | 252   | 254.0 | 130   | 129   | 131   | 130.0 | 1.95  |
|                | 2.4µM | 255   | 253   | 254   | 254.0 | 101   | 103   | 100   | 101.3 | 2.51  |
|                | 3.2µM | 252   | 255   | 246   | 251.0 | 96    | 95    | 98    | 96.3  | 2.61  |

**Table S6.** The fluorescence intensity of regular RT-LAMP reactions using qFIP12nt, qFIP17nt, qBIP10nt, and qBIP15nt quenching probes. The fluorescence intensity in the green channels was measured in MATLAB using the interactive impixelinfo function. For each tube, 3 points were randomly selected for measurements. S/N was calculated based on the ratio of fluorescence intensity of target-spiked samples and NTCs.
Table S7. Detection sensitivity of RT-LAMP reaction using the 4 QUASR quenchers at the concentration optimized for positive-negative sample fluorescence contrast. Each data represents the number of positive amplifications observed out of the triplicates tested. Green indicates true positives observed for 3 out of 3 samples, and yellow indicates false negatives occurred with 1-3 samples failed to amplify.

Table S8. Primer sequences for *E. coli* and *S. Typhi*. 

| Target | Primer name | Sequence (5’-3’) | Reference |
|--------|-------------|-----------------|-----------|
| *E. coli* | F3 | GCCATCTCCCTGATGACGC | Hill et al., 2008 |
| | B3 | ATTTACGCAGCCAGGCA | |
| | FIP | CATTCTTCAGCTCGTCGCGAGCCCATTGAATGTTGCT | |
| | BIP | CTCGGGCGAGGTCTGTTGATTTCCGACAAACACCACTACGAATT | |
| | LF | CTGTTGTAACATCGTCATCGACA | |
| | LB | GTTCTCGATATCCATGAAGGTG | |
| *S. Typhi* | F3 | GACGTGCTTTAAAAAGATACCA | Fan et al., 2015 |
| | B3 | AGAGTGCTGTAGTTGGACCCAGTGAAGTGTTGCT | |
| | FIP | AAACAGTGCTGGGTTAGGGTCATCGCGCGATCCATG | |
| | BIP | CCTAGGCTGTGGGAAAGTTGGACCCAGTGAAGTGTTGCT | |
| | LF | TCGATGCTGCTTCTTCTT | |
| | LB | GAAGGTTCAAGACTAAGTGTCGTC |
Supplementary Figures

Figure S1

Figure S1. The location of surface water sampling marked in ArcGIS. The samples were collected from a suburban river in Godawari Botanical Garden, Godawari Khola, Nepal (27°35'50"N 85°22'59"E).
**Figure S2.** Pretreatment process including dislodging process and 3-tier filtration (schematic illustration and actual photo) for environmental water and wastewater samples.
Figure S3

Figure S3. Portable mgLAMP prototype design with major components labeled.

Figure S4

Figure S4. Real-time fluorescence monitoring during RT-LAMP amplification using the 4 primer sets with lowest preliminary LOD. For each primer set, amplification curves are shown for triplicate samples with target RNA spiked at 186 copies per reaction.
**Figure S5.** RT-LAMP reactions using (a) molecular beacons and (b) QUASR probes of primer set 3 taken by a smartphone under the E-gel imager. The molecular beacon sequences for MB-LF1 is 5’FAM-TGAGCCTCATATTGAGTTGATGGCTCA-3’IBFQ and for MB-LF2 is 5’FAM-CGACTCTCATATTGAGTTGATGGCTCAAGTCG-3’IBFQ. The QUASR quenching probe sequence for qBIP12nt is 5’-TCTTCAGGTTGA-3’IBFQ.

**Figure S6.** Smartphone images taken for mgLAMP samples corresponding to microscope images in Figure 3a-d. Effect of varying gel concentration tested with (a) 2.5%, (b) 5%, (c) 7.5%, and (d) 10% gel.
Figure S7

(a) 30 min  (b) 35 min  (c) 40 min  (d) 40 min-NTC

Figure S7. Effect of mgLAMP incubation time. Example fluorescence images of mgLAMP with 5% gel after an RT-LAMP incubation time of (a) 30 min, (b) 35 min, and (c) 40 minutes with SARS-CoV-2 particles spiked at $1.92 \times 10^4$ copies/mL, and (d) after 40-minute incubation of NTC. Scale bar, 2mm.

Figure S8

Comparison of mgLAMP measured ATCC SARS-CoV-2 concentration and RT-qPCR count number (Ct) to the spiked concentration in Milli-Q water. For mgLAMP estimations, the blue dots represent results based on 1 mL spiked Milli-Q water, the navy dot represents 10 mL, and the purple dot represents 100 mL. The black dots are RT-qPCR. The dashed lines represent linear fitting for mgLAMP and RT-qPCR with equations shown.
Figure S9

Figure S9. DLS analysis results of (a-c) raw influent, (d) primary effluent and (e) surface water after the dislodging process as shown in Figure S2. The raw influent sample was tested in triplicates due to visible large particles that easily settled.

Figure S10

Figure S10. Performance of mgLAMP for E. coli and S. Typhi cells spiked Milli-Q water. (a, e) No template control. (b-d, f-h) mgLAMP amplicon dots for varying concentrations of E. coli and S. Typhi cells spiked Milli-Q water samples. All images were taken by the google pixel 3 (Google, Mountain View, CA) under the E-gel Safe imager. (i) Comparisons of measured E. coli and S. Typhi concentrations to the spiked concentrations in Milli-Q water.
References

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