Supporting Information

for

A Smartphone Based in-Gel Loop Mediated Isothermal Amplification (gLAMP) System Enables Rapid Coliphage MS2 Quantification in Environmental Waters

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Consists of 11 pages, 5 tables and 6 figures

Table S1. MS2 Primer and probe sequences for in-tube real-time LAMP and gLAMP

Primer Name	Genome position	Sequence (5'-3')	Reference
F3	2520-2539	CTTGCGACGATAGACTTATC	1
B3	2776-2759	TAGATGCCTATGGTTCCG	
FIP (F1c + F2)	2658-2638(F1c) + 2586-2607(F2)	ATCGTATCGTCTCGCCATCTA + CCACCAGAGCTATATTCATATC	
BIP (B1c + B2)	2673-2693(B1c) + 2739-2722(B2)	ACAATGGGAAATGGGTTCACA + GGGTCGCTTTGACTATTG	
LF	2636-2619	GATTCCGTAGTGTGAGCG	
LB	2699-2720	GCTAGAGTCCATGATATTCTGG	
5'FAM-FIP	2658-2638(F1c) + 2586-2607(F2)	**FAM**- ATCGTATCGTCTCGCCATCTA + CCACCAGAGCTATATTCATATC	1
qFIP-3'IBFQ		GAGACGATACGAT-**IBFQ**	

For QUASR probe, fluorophore (FAM) was attached to the FIP at 5' end; quencher IBFQ was attached to the quench probe qFIP at the 3' end. IBFQ= Iowa Black® FQ (Integrated DNA Technologies, Coralville, IA) has a broad absorbance spectrum ranging from 420 to 620 nm with peak absorbance at 531 nm. This quencher is ideal for use with fluorescein and other fluorescent dyes that emit in the green to pink spectral range.

Regular LAMP primers were used in post staining gLAMP and in-tube real-time LAMP. The final concentration of F3/B3, FIP/BIP and LF/LB were 0.2 µM, 1.6 µM, and 0.4 µM, respectively. 20x primer mix was prepared to simplify the sample preparation. In QUASR gLAMP, FIP was substituted by 5'FAM-FIP and 2x qFIP-3'IBFQ (3.2 µM) was added to the reaction mix.

LAMP dye (1x final concentration) was used as fluorescent probe in the semi-quantitative in-tube real-time LAMP assay performed with Eppendorf RealPlex2 (Hamburg, Germany). Fluorescence intensity of the reaction mix was monitored every minute for 60 min during 65 ^{o}C incubation. The time-to-detection was defined as the time when the fluorescence of the reaction mix exceeded the initial background fluorescence level due to target amplification. Therefore, shorter time-to-detection correlates to higher original template concentration. For reactions without clear fluorescence increase at the end of reaction, the reaction time (60 min) was used for time-to-detection calculation.

Table S2. MS2 primer and probe sequences for the RT-qPCR assay

MS2 Primer/probe	Genome position	Sequence (5'-3')	Reference
Forward	1630-1650	ATTCCGACTGCGAGCTTATT	2
Reverse	1768-1758	TTCGACATGGGTAATCCTCA	
Probe	1689-1714	FAM-ATTCCCTCAGCAATCGCAGCAAACT-BHQ1	

RT-qPCR reactions were performed using QIAGEN OneStep RT-PCR Kit (Germantown, MD) with Eppendorf RealPlex2 (Hamburg, Germany). Each 25 µL reaction mix included 800 nM forward and reverse primers, 300 nM TaqMan probe, 0.5 mg/mL BSA, 1x RT-PCR buffer, 0.4 mM dNTP, 1 U enzyme mix and 3 µL extracted RNA or water sample. RT-qPCR was performed with an initial reverse transcription step at 50 ^{o}C for 30 min, followed by an initial denaturation at 95 ^{o}C for 10

min, then 40 cycles of 94 °C for 15 seconds and 60 °C for 60 seconds followed by a plate read. A 10x serial dilution of the MS2 stock was prepared in PBS. The samples were directly analyzed by RT-qPCR without RNA extraction. The quantification cycle (Cq) values were plotted in the same figure against plaque assay counts as gLAMP (Figure 4). We also extracted MS2 RNA from the stock MS2 sample using AllPrep PowerViral DNA/RNA Kit (Qiagen, Germantown, MD). Similar 10x diluted RNA samples were prepared in PBS and tested with RT-qPCR. Indeed, the Cq values were almost the same as those of the direct RT-qPCR (data not shown). Therefore, RNA extraction may not be necessary for RT-qPCR for samples with limited inhibition. However, as shown in Figure 5C, direct MS2 RT-qPCR assays were completely inhibited in toilet wastewater. This indicates the value of RNA extraction kit may be more related to inhibitor removal.

Table S3. Water quality parameters of environmental samples

	Lake Water	Pond Water	Toilet Wastewater	Primary Effluent
pH	8.45	7.75	9.16	8.02
EC (mS/cm)	0.794	0.926	14.59	1.377
COD (mg/L)	60	74.7	821.3	1110
DOC (mg/L)	30	101	430	352

Table S4. Primer sequences for *E. coli* and *Salmonella* gLAMP

Target	Primer Name	Genome position	Sequence (5'-3')	Reference
E. coli	F3	3204-3221	GCCATCTCCTGATGACGC	3
	B3	3390-3407	ATTTACCGCAGCCAGACG	
	FIP (F1c + F2)	3284-3305(F1c)+ 3228-3247	CATTTTGCAGCTGTACGCTCGC-AGCCCATCATGAATGTTGCT	
	BIP (B1c + B2)	3316-3335(B1c)+ 3369-3388	CTGGGGCGAGGTCGTGGTAT-TCCGACAAACACCACGAATT	
	LF	3237-3260	CTTTGTAACAACCTGTCATCGACA	
	LB	3344-3368	ATCAATCTCGATATCCATGAAGGTG	
Salmonella	F3	225-246	GGCGATATTGGTGTTTATGGGG	4
	B3	457-483	AACGATAAACTGGACCACGG	
	FIP (F1c + F2)	319-342(F1c)+ 271-292(F2)	GACGACTGGTACTGATCGAT-AGTTTTTCAACGTTTCCTGCGG	
	BIP (B1c + B2)	364-383(B1c)+ 414-434(B2)	CCGGTGAAATTATCGCCAC-ACAAAACCCACCGCCAGG	
	LF	297-319	GACGAAAGAGCGTGGTAATTAAC	
	LB	391-413	GGGCAATTCGTTATTGGCGATAG	

Table S5. Comparison of Microbial Water Quality Monitoring Tools

	Plaque assays	Bio-Rad digital PCR	gLAMP
Cultivation or Genetic	Cultivation	Genetic	Genetic
Capital cost ($)	1,000	80,000	500
Cost per test ($)*	5	10-30	5
Sample to answer time (h)	18-48	3-4	< 0.5
Skill requirements	Medium	High	Low
Tests for specific pathogens	No	Yes	Yes
Point-of-sample analysis	No	No	Yes

* The cost per test only considers consumable costs. The price for plaque assays and digital PCR would be much higher if labor cost was included because of the relatively high technical skill requirements.

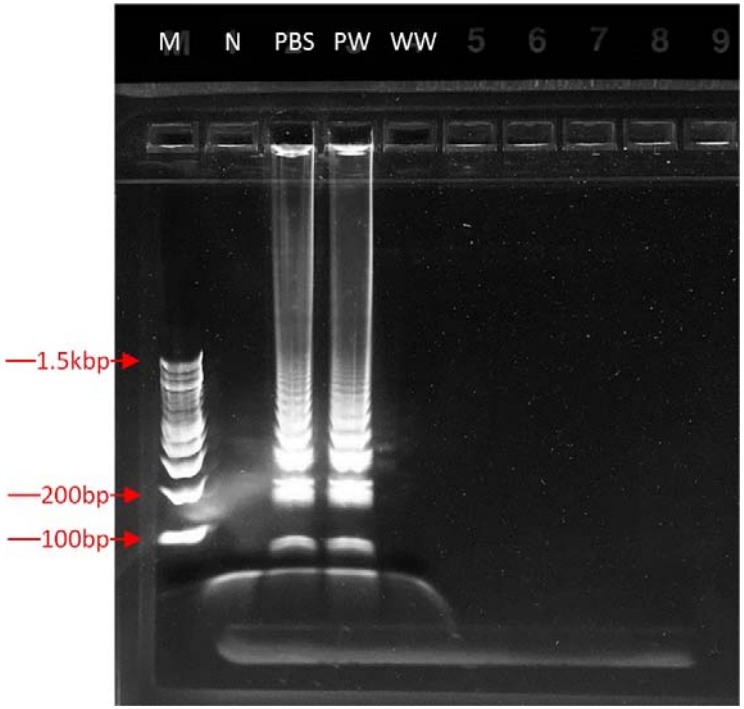

Figure S1. Agarose gel electrophoresis of in-tube real-time LAMP products. M: 100bp DNA ladder; N: no template control; PBS: PBS spiked with MS2; PD: pond water spiked with MS2; WW: toilet wastewater spiked with MS2. Electrophoresed on 2% E-Gel™ EX Agarose Gels with E-Gel® electrophoresis system (Invitrogen, Carlsbad, CA). Image was taken with an iPhone 6s plus.

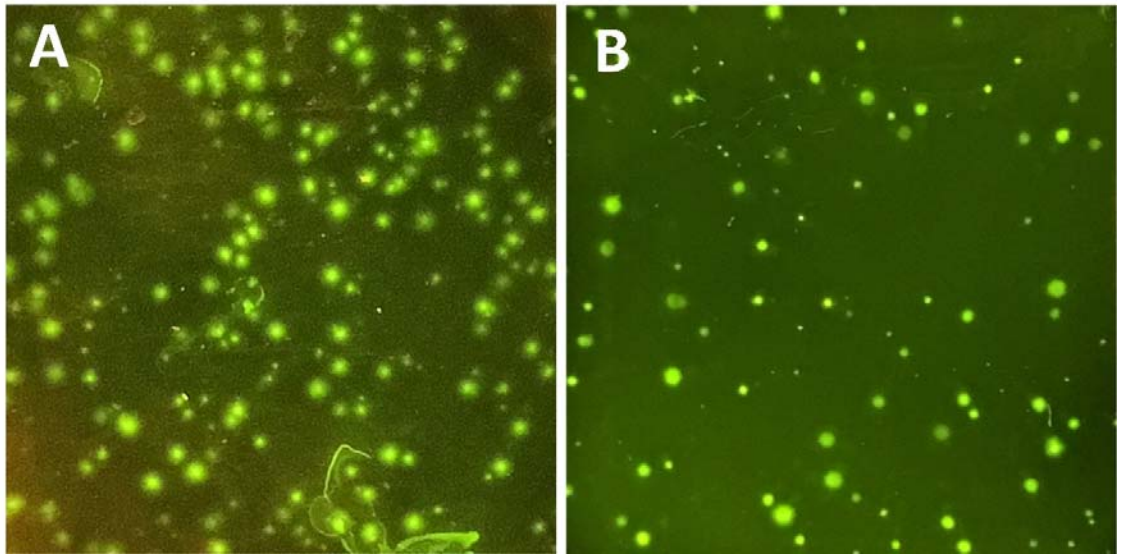

Figure S2. gLAMP detection of *E. coli* (A) and *Salmonella* (B). Extracted *E. coli* and *Salmonella* DNA were used as templates and the reaction time was 20 min. Gels were stained with 0.5x LAMP dye after the reaction. The images were taken by an iPhone 6s Plus.

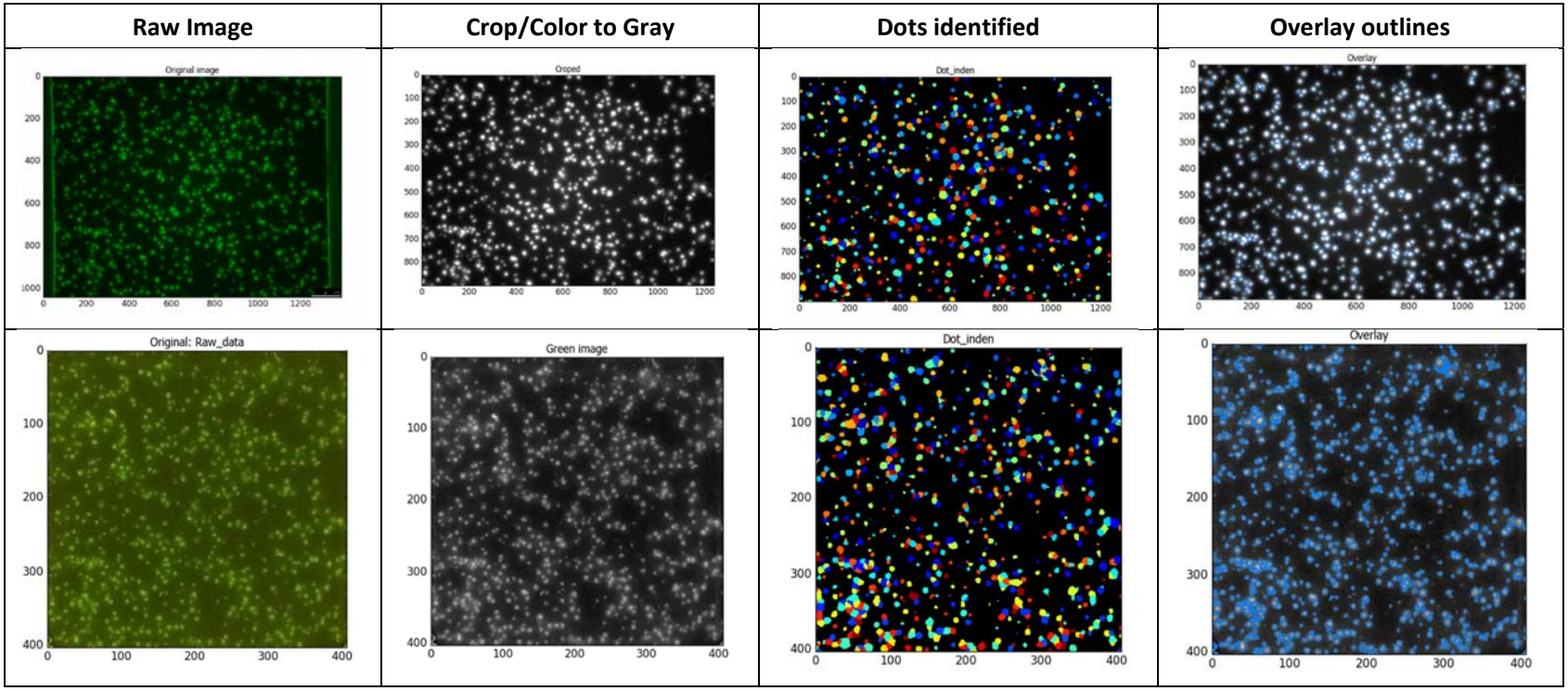

Figure S3. Automatic dot counting with Cellprofiler. Raw images were taken by fluorescent microscope (top) and iPhone 6s Plus (bottom)

Automatic counting of MS2 gLAMP dots was realized by using Cellprofiler. The optimized pipeline included four main steps: 1) Raw image input: add all the raw images to the input folder for batch analysis; 2) Crop/color to gray: cut the image border and split the RBG color channels (in this case only green channel); 3) Dots identification: use command IdentifyPrimaryObjects to identify the amplicon dots. The most important parameter is the Threshold Strategy. We found using pixel intensity combined with object diameter gave the best results; 4) Overlay outlines: generated: create a new image which overlay the segmentation results on the raw data to visually confirm the analysis. Pipelines is available upon request.

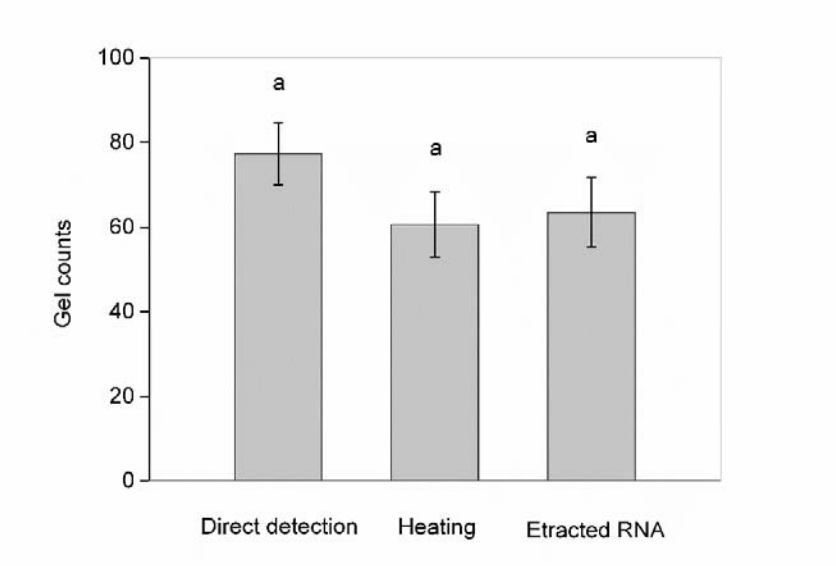

Figure S4. Effect of sample pretreatment procedures on gLAMP counting (Direct detection: no pretreatment; Heating: 95 ^{o}C, 5 min denaturation; and Extracted RNA: virus RNA extraction with AllPrep PowerViral DNA/RNA Kit). MS2 was spiked to PBS buffer solution at the concentration of 2×10^{3}-2×10^{4} PFU/mL (equaling 10-100 PFU/reaction). Error bars represent standard errors of the means. Same letters indicate no significant differences at the $p < 0.05$ level according to one-way ANOVA.

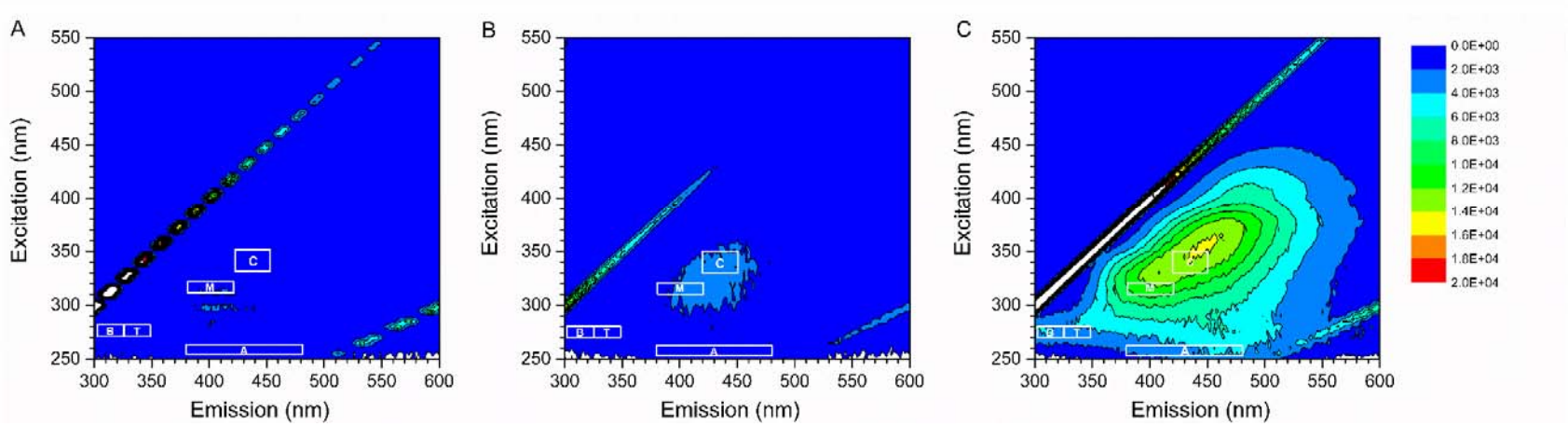

Figure S5. Excitation emission fluorescent spectra (arbitrary fluorescence units) for lake water (A), pond water (B) and toilet wastewater (C). The locations of common fluorescent components are noted.

Fluorescence spectroscopy is employed in this study to investigate the composition of dissolved organic matter in the environmental water samples. The raw water samples were filtered with 0.2 µm syringe filter (GE Whatman, Pittsburgh, PA) to remove large particles. The filtered lake and pond water was analyzed directly, while the toilet wastewater was diluted 4x with Milli-q water to reduce the inner filtering effects due to the high absorption coefficient. Common fluorescent components were measured at the following wavelengths specified in Coble et al.,[5]: (1) humic-acid like A-peak (Ex:250-260 nm; Em 380-480 nm) and C-peak (Ex: 330-350 nm; Em: 420-480 nm); (2) tyrosine-like B-peak (Ex: 270-280 nm; Em 300-320 nm); (3) marine humic like M-peak (Ex: 310-320 nm; Em: 380-420 nm); and (4) typtophan-like T-peak (Ex: 270-280 nm; Em: 320-350 nm). The locations of common fluorescent components are noted.

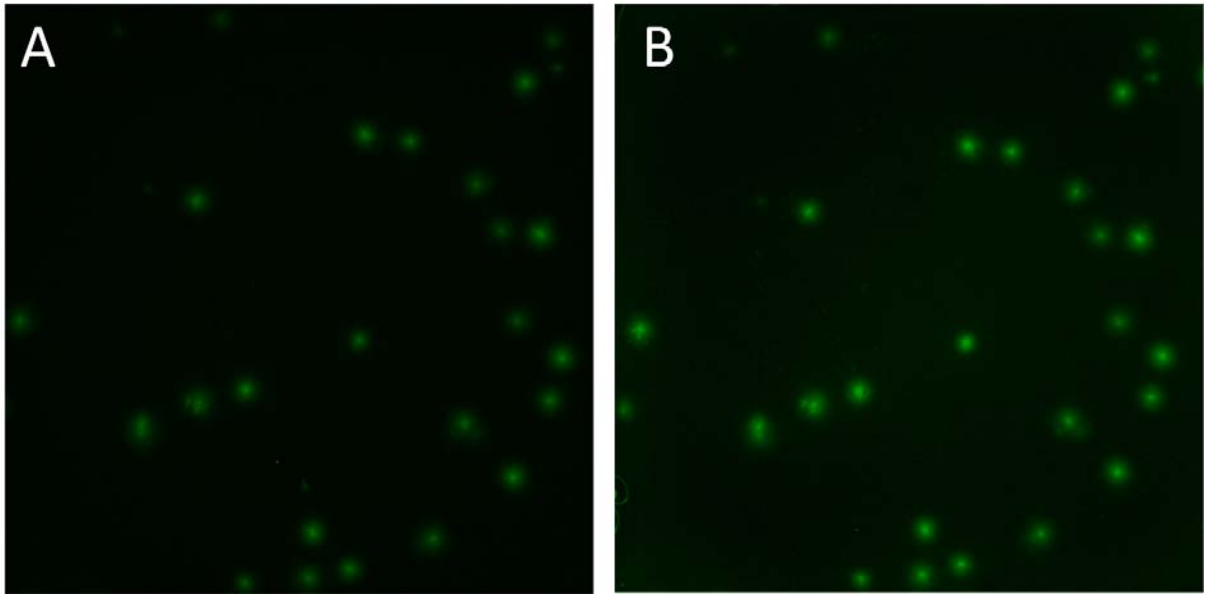

Figure S6. The protection of gel matrix to the amplicon dots. Gel images were taken on (A) Day 1 and (B) 21 days later with fluorescent microscope under the same settings.

The amplicon dots become brighter with time. This is likely because the fluorescent probes were concentrated due to the decrease of water content of the hydrogels through evaporation.

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