Supplementary Information to

Digital Loop-Mediated Isothermal Amplification on a Commercial Membrane

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Table S1. Primers and probe sequences for mLAMP or mRT-LAMP

	Primer Name	**Sequence (5'-3')**	**Reference**
Escherichia coli	F3	GCCATCTCCTGATGACGC	1
	B3	ATTTACCGCAGCCAGACG	
	LF	CTTTGTAACAACCTGTCATCGACA	
	LB	ATCAATCTCGATATCCATGAAGGTG	
	FIP	CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAATGTTGCT	
	BIP	CTGGGGCGAGGTCGTGGTATTCCGACAAACACCACGAATT	
Enterococcus faecalis	F3	GCCGGAAATCGATGAAGA	2
	B3	TCCAGCAACGTTGATTGT	
	LF	AAATGCTGCGCCAGCTCG	
	LB	TCCAATGTGGAACTTAAACGTACC	
	FIP	CACTTTTTGTTGTTGGTTTTCGCTTTATTATCTGCTTGGGGTGC	
	BIP	ATCTGCAGACAAAGTAGTAATTGCTCCAAGCTTTTAAGCGTGTC	
Salmonella Typhi	F3	GACTTGCCTTTAAAAGATACCA	3
	B3	AGAGTGCGTTTGAACACTT	
	LF	TCGGATGGCTTCGTTCCT	
	LB	CAAGGGTTTCAAGACTAAGTGGTTC	
	FIP	AACTTGCTGCTGAAGAGTTGGACCGAATGACTCGACCATC	
	BIP	CCTGGGGCCAAATGGCATTATGCACTAAGTAAGGCTGG	
MS2	F3	CTTGCGACGATAGACTTATC	4
	B3	TAGATGCCTATGGTTCCG	
	LF	GATTCCGTAGTGTGAGCG	
	LB	GCTAGAGTCCATGATATTCTGG	
	5'FAM-FIP	**FAM**- ATCGTATCGTCTCGCCATCTA + CCACCAGAGCTATATTCATATC	
	qFIP-3'IBFQ	GAGACGATACGAT-**IBFQ**	
	BIP	ACAATGGGAAATGGGTTCACA + GGGTCGCTTTGACTATTG	

Table S2. Primers and probe for qPCR.

	Sequence (5'–3')
Forward primer	5'CGGTGAATACGTTCYCGG3' where Y is either C or T
Reverse primer	5'GGWTACCTTGTTACGACTT3', where W is either A or T
TaqMan probe	FAM-5'CTTGTACACACCGCCCGTC3'

The PCR thermocycling involves 3 minutes of initialization at 95 °C, and 42 cycles of denaturation at 95 °C for 15 seconds, followed by annealing/extension at 55 °C for 30 seconds. The primers and probe are targeting the universal 16s rRNA gene. The sequences are listed above.

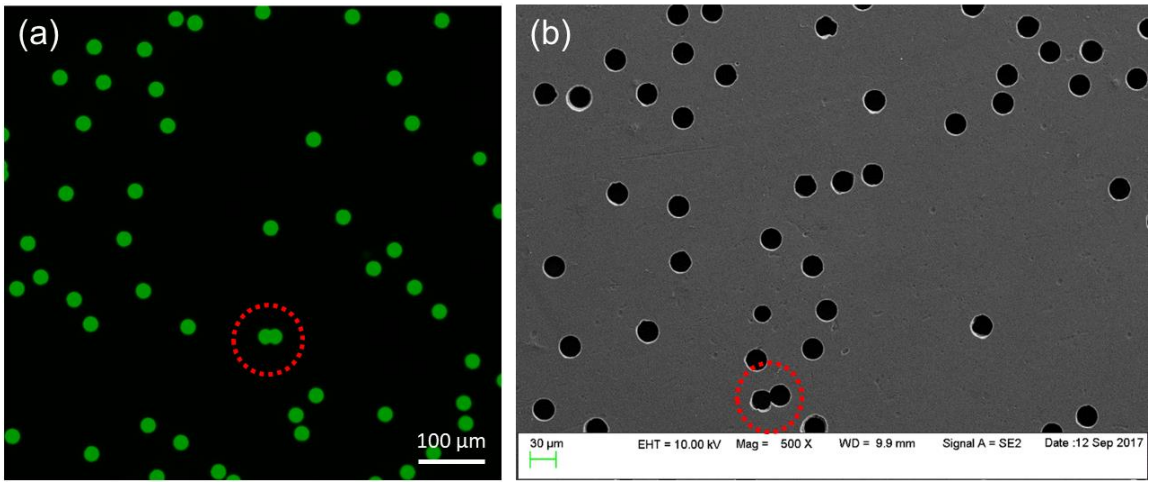

Figure S1. (a) Fluorescence image of the membrane when pores were filled with fluorescent solution. The red circle indicates overlapped micropores. (b) Top-view SEM image of a membrane. The red circle denotes the overlapped micropores

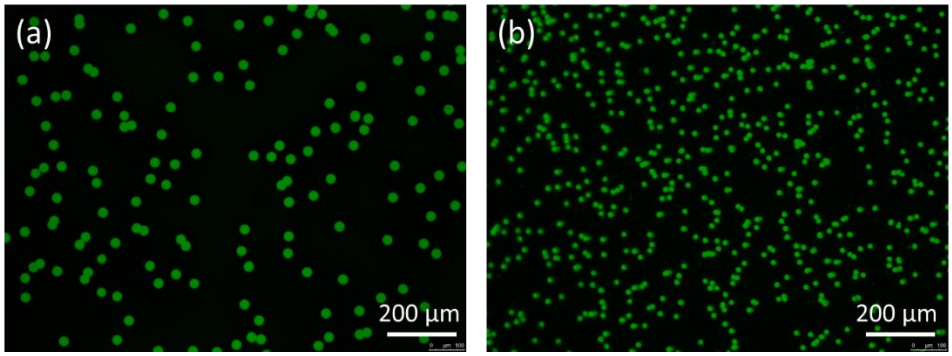

Figure S2. Droplet formation on PCTE membranes with different nominal pore sizes. (a) 30 µm. (b) 14 µm.

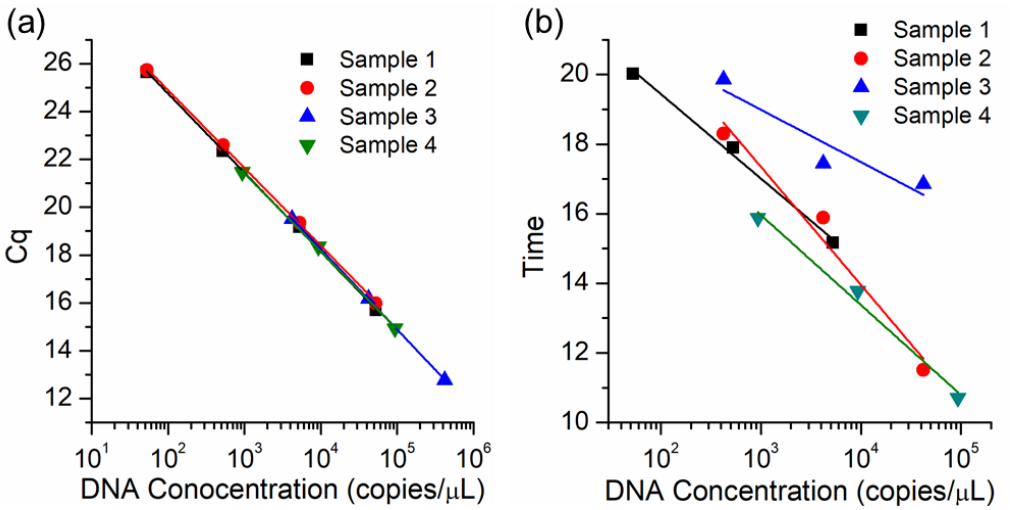

Figure S3. Calibration curves obtained from a series of dilutions of different *E. coli* DNA samples. (a) qPCR. (b) qLAMP.

As can be seen in **Figure S3**, the qPCR results based on Cq show identical calibration curves for different *E. coli* DNA samples. However, the qLAMP results based on Tt show disordered calibration curves for different samples. Even multiple samples with the same DNA concentration show quite different Tt values.

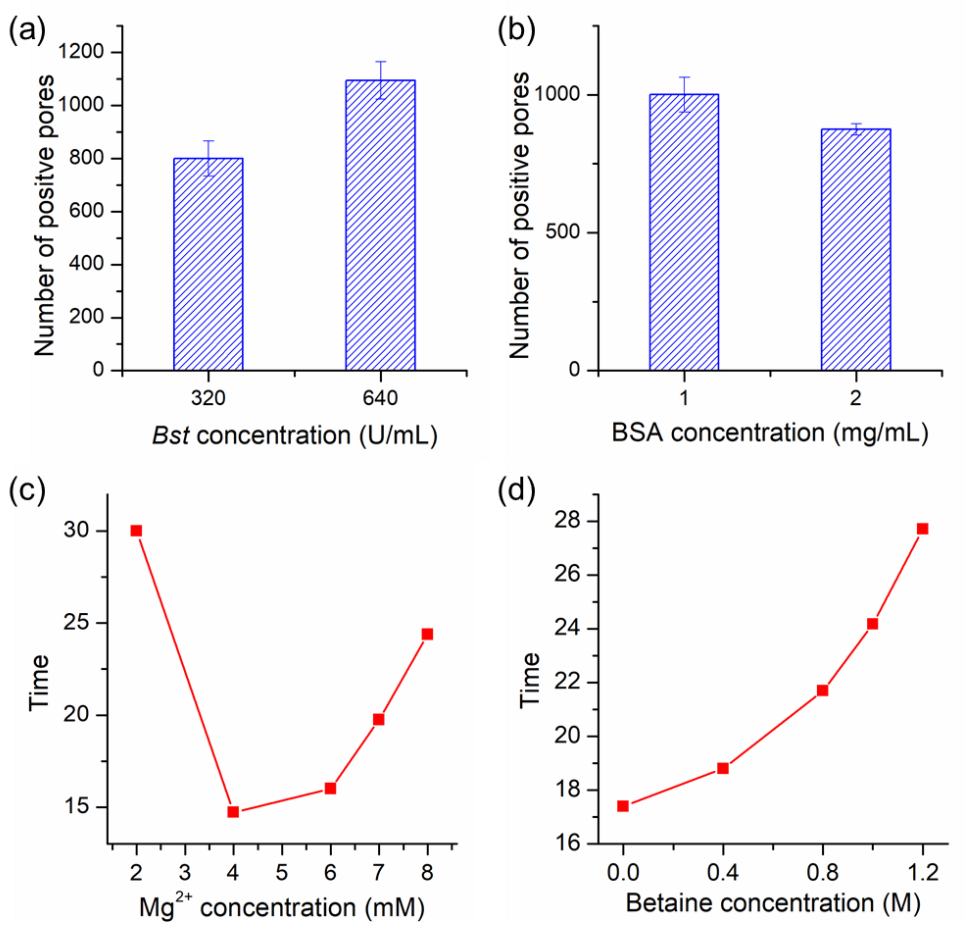

Figure S4. Optimization of LAMP reaction. (a,b) Effects of *Bst* (a) and BSA (b) concentrations on the number of positive pores obtained on the membrane. (c,d) Effects of Mg^{2+} (c) and betaine concentration (d) on Tt value for LAMP performed in the tube. A smaller Tt value means less time is needed for nucleic acid amplification.

According to the optimization results (**Figure S4**), the final 25 µL LAMP reactions contained 1 × isothermal buffer, 6 mM total $MgSO_4$, 1.4 mM dNTP, 640 U/mL *Bst* 2.0 WarmStart polymerase, primer mix (1.6 uM FIB and BIP, 0.2 uM F3 and B3, 0.8 uM LF and LB), 1 mg/mL BSA, 50 µM calcein, 1 mM $MnCl_2$ and 2.5 µL of template.

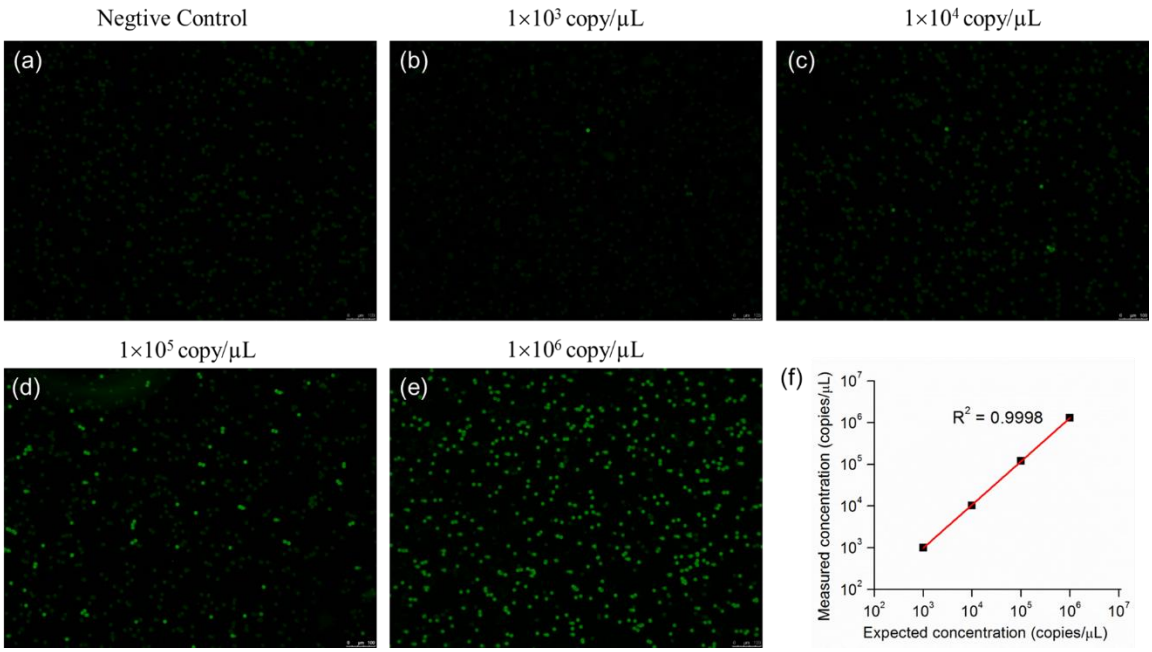

Figure S5. (a-e) End-point fluorescence images of membranes (14 μm pore size) after mdLAMP with a series concentration of target *E. coli* DNA. (f) Comparison of measured *E. coli* DNA concentrations to the expected concentrations.

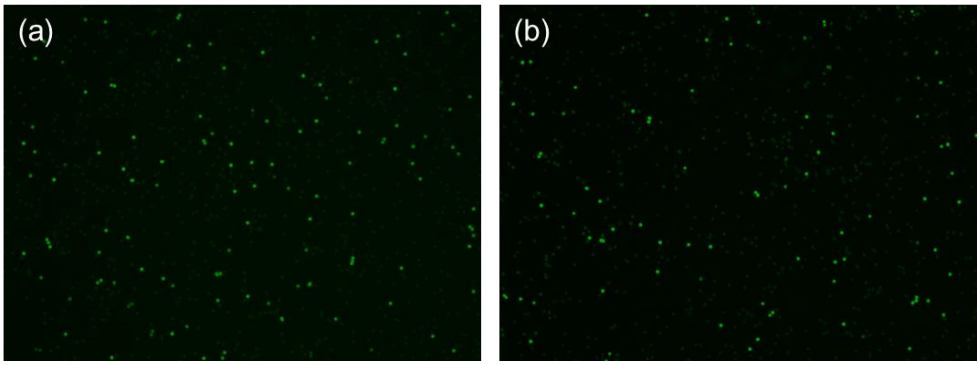

Figure S6. mdLAMP analysis of DNA samples extracted from *Enterococcus faecalis* (a) and *Salmonella* Typhi (b).

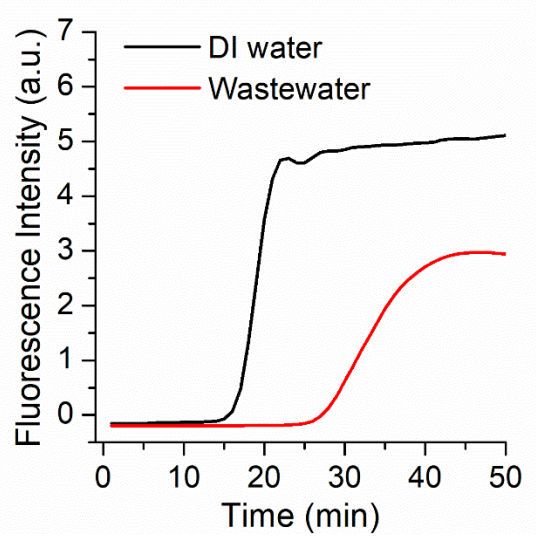

Figure S7. Real-time RT-LAMP results of MS2 quantification in DI water and wastewater.

As shown in **Figure S7**, the qRT-LAMP in the tube was strongly inhibited, resulting in an increased Tt value.

Supplementary Movie Legends

Supplementary Movie S1: Time lapse movie showing the removal of fluorescent solution on the membrane surface when peeling off the PDMS films. In order to be seen clearly by naked eye, an over-exposure time was used to increase the fluorescence intensity. Therefore, the pores looks larger than original.

1. Hill, J.; Beriwal, S.; Chandra, I.; Paul, V. K.; Kapil, A.; Singh, T.; Wadowsky, R. M.; Singh, V.; Goyal, A.; Jahnukainen, T., et al. Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Common Strains of Escherichia Coli. *J. Clin. Microbiol.* **2008**, *46*, 2800-2804.
2. Kato, H.; Yoshida, A.; Ansai, T.; Watari, H.; Notomi, T.; Takehara, T. Loop-Mediated Isothermal Amplification Method for the Rapid Detection of Enterococcus Faecalis in Infected Root Canals. *Oral Microbiol. Immunol.* **2007**, *22*, 131-135.
3. Fan, F.; Yan, M.; Du, P.; Chen, C.; Kan, B. Rapid and Sensitive Salmonella Typhi Detection in Blood and Fecal Samples Using Reverse Transcription Loop-Mediated Isothermal Amplification. *Foodborne Pathog. Dis.* **2015**, *12*, 778-786.
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