Supplementary Information to

Asymmetric Membrane for Digital Detection of Single Bacteria in Milliliters of Complex Water Samples

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Primers

The primers for *Escherichia coli* (*E. coli)* were designed to be specific to a conserved region on the malB gene, and its sequence is as follows[1]:

F3: 5-GCCATCTCCTGATGACGC-3;

B3: 5-ATTTACCGCAGCCAGACG-3;

BIP: 5-CTGGGGCGAGGTCGTGGTATTCCGACAAACACCACGAATT-3;

FIP: 5-CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAATGTTGCT-3;

LF: 5-CTTTGTAACAACCTGTCATCGACA-3;

LB: 5-ATCAATCTCGATATCCATGAAGGTG-3;

The primers for *Salmonella* Typhi were designed to be specific to a conserved region on the STY1607, and its sequence is as follows[2]:

F3: 5- GACTTGCCTTTAAAAGATACCA-3;

B3: 5- AGAGTGCGTTTGAACACTT-3;

BIP: 5-CCTGGGGCCAAATGGCATTATGCACTAAGTAAGGCTGG-3;

FIP: 5-AACTTGCTGCTGAAGAGTTGGACCGAATGACTCGACCATC-3;

LF: 5- TCGGATGGCTTCGTTCCT-3;

LB: 5- CAAGGGTTTCAAGACTAAGTGGTTC-3;

Table S1. Contact angles of LAMP solution on the PC membranes (n = 4).

	Before Bonding	**After Bonding**
PC membrane with 25 µm pore size	40 ± 3°	50 ± 2°
PC membrane with 400 nm pore size	47 ± 3°	54 ± 4°

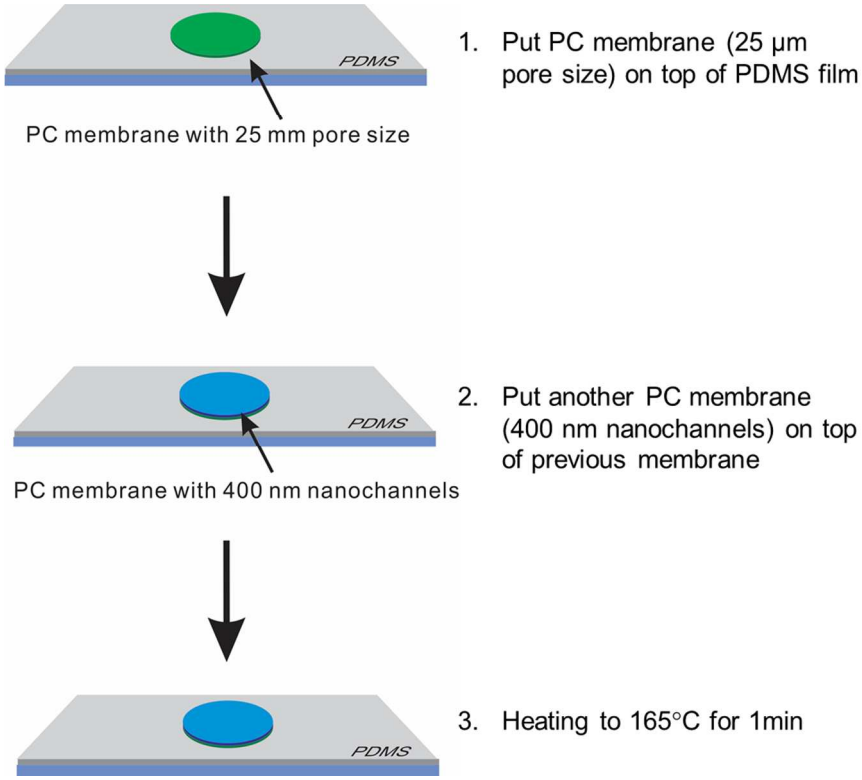

Figure S1. Schematic illustration of the fabrication process for asymmetric membrane.

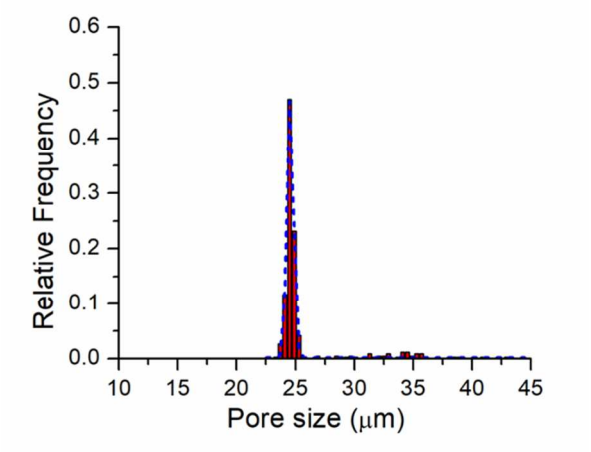

Figure S2. Pore size distribution results of asymmetric membrane at micropore side.

The pore size distribution result illustrated that the micropores on the upper side of asymmetric membrane were uniform.

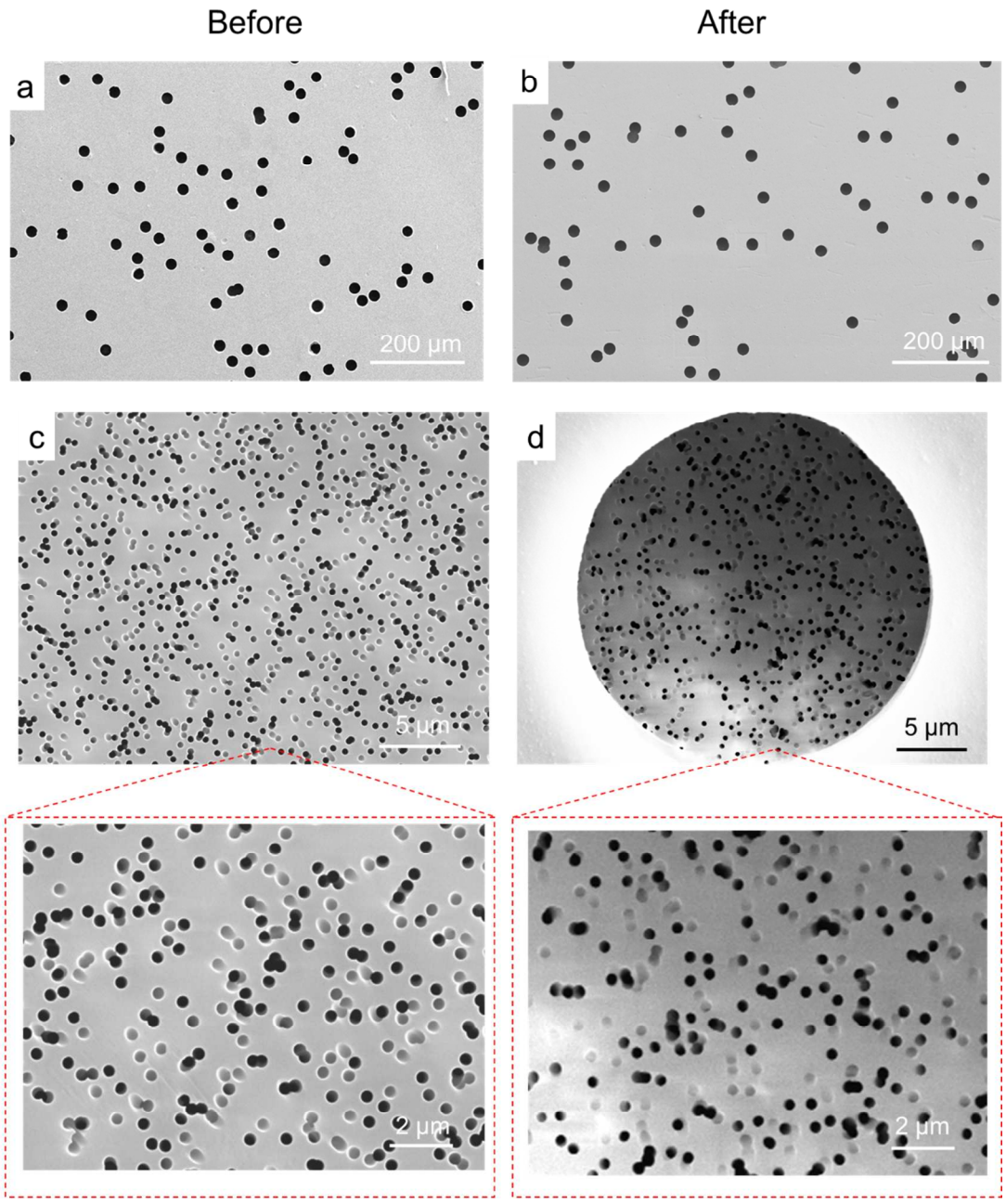

Figure S3. SEM images of membranes before and after thermal bonding. (a) PC membrane with 25 µm pore size before bonding. (b) PC membrane with 25 µm pore size after bonding. (c) PC membrane with 400 nm nanochannels before bonding. Red square shows the enlarged image. (d) PC membrane with 400 nm nanochannels after bonding. Image show one micropore and the underlying nanochannels of asymmetric membrane. Red square shows the enlarged image.

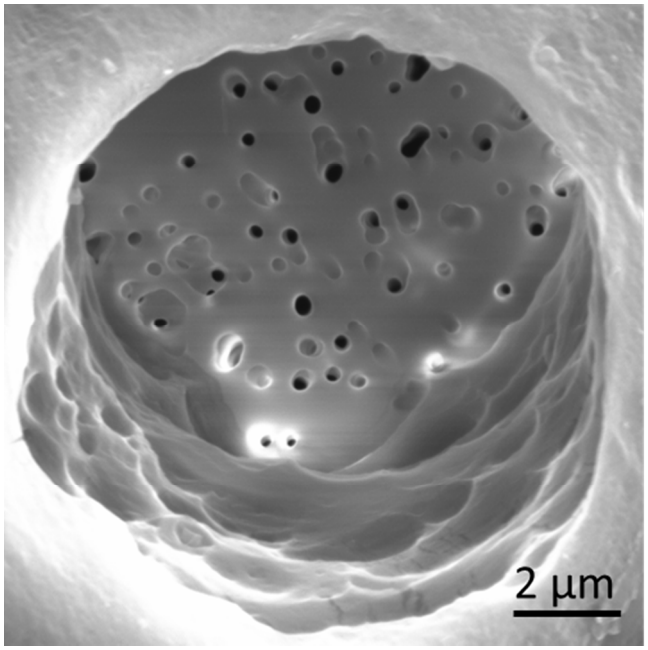

Figure S4. SEM image of asymmetric membrane composed of two materials. The top is a tracked etched polyester membrane with 10 µm pore size, while the bottom is the polycarbonate membrane with 400 nm pore size.

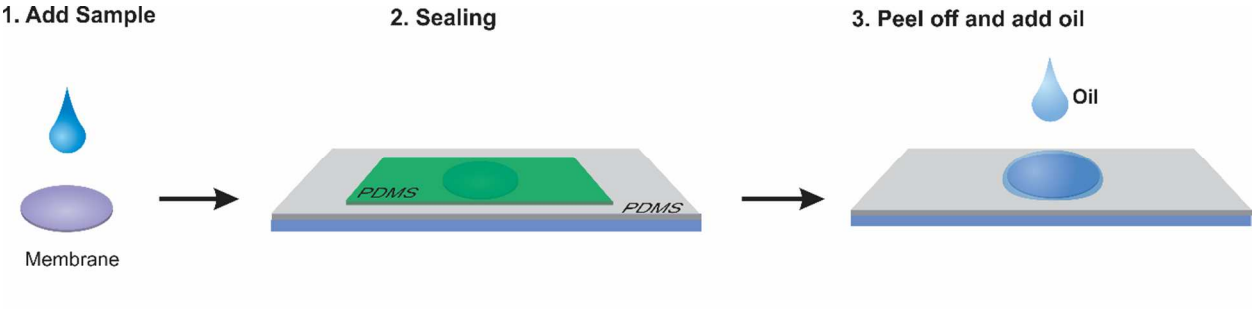

Figure S5. Schematic illustration of reagents loading into each pore of membrane.

Sample was first added on the membrane and waiting for complete wetting. Then, the wetted membrane was sealed between two PDMS films. Subsequently, the top piece of PDMS was peeled off, followed by adding mineral oil to cover the whole membrane for preventing water evaporation.

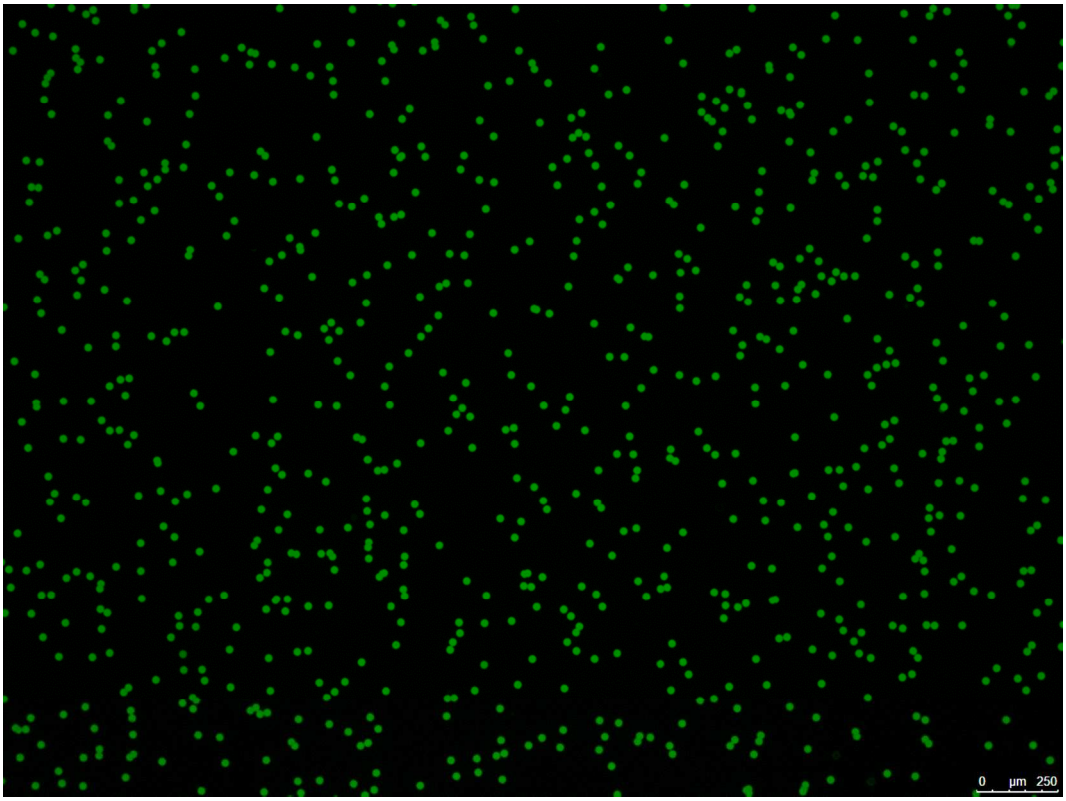

Figure S6. Large-view fluorescence image of membrane after reagents loading.

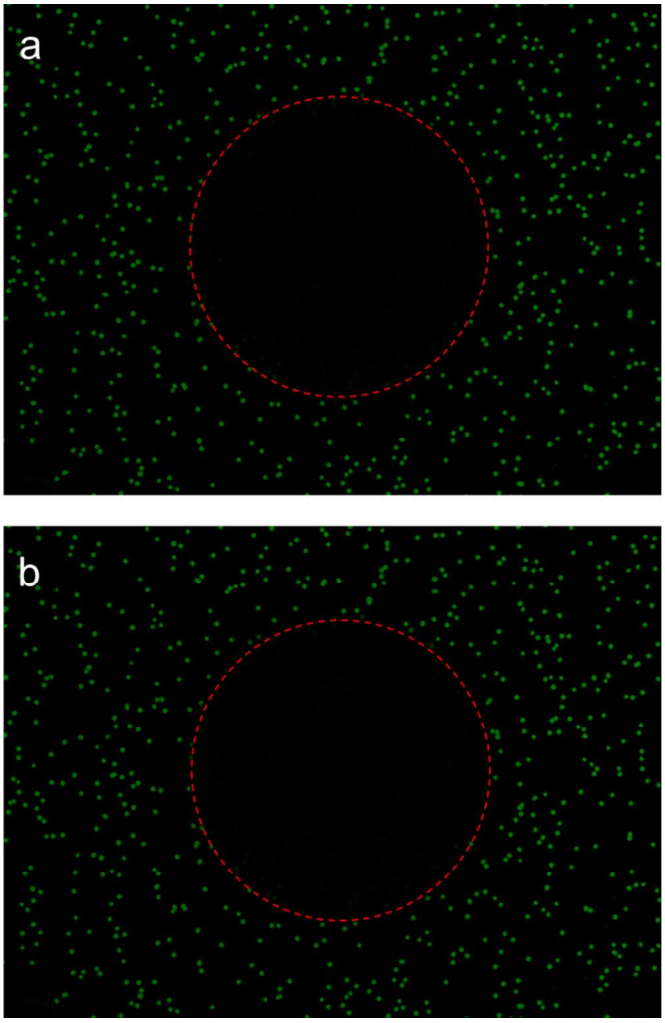

Figure S7. Photobleaching test for cross-contamination. (a) Fluorescence image of membrane after UV exposure. (b) Fluorescence image of membrane after UV exposure and stay in dark for 30 min. The red circles indicate the area for UV exposure.

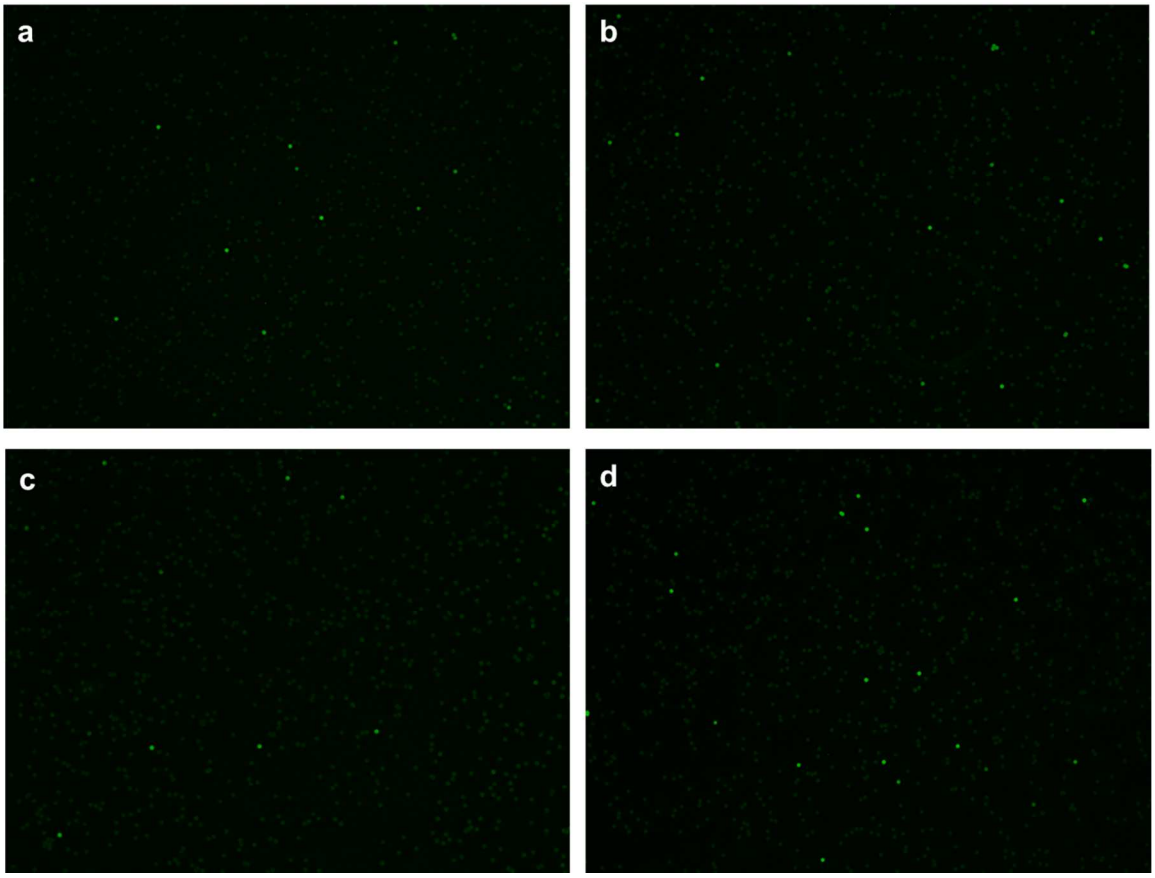

Figure S8. LAMP experiments on asymmetric membranes with low bacteria concentration. The *E. coli* concentration in the sample used here was 100 cells/mL, and the sample volume was 1 mL.

In order to further prove the pore isolation, we also conducted mLAMP experiments on asymmetric membranes at low bacteria concentration (100 cell/mL, 1mL). At such concentrations, ~ 2% of pores are positive pores and are distributed randomly across the whole membrane. If cross-contamination happened, more positive pores would appear at one location on the membrane.[3] After repeating these experiments several times, no clusters of positive pores were found, confirming perfect pore isolation in our system.

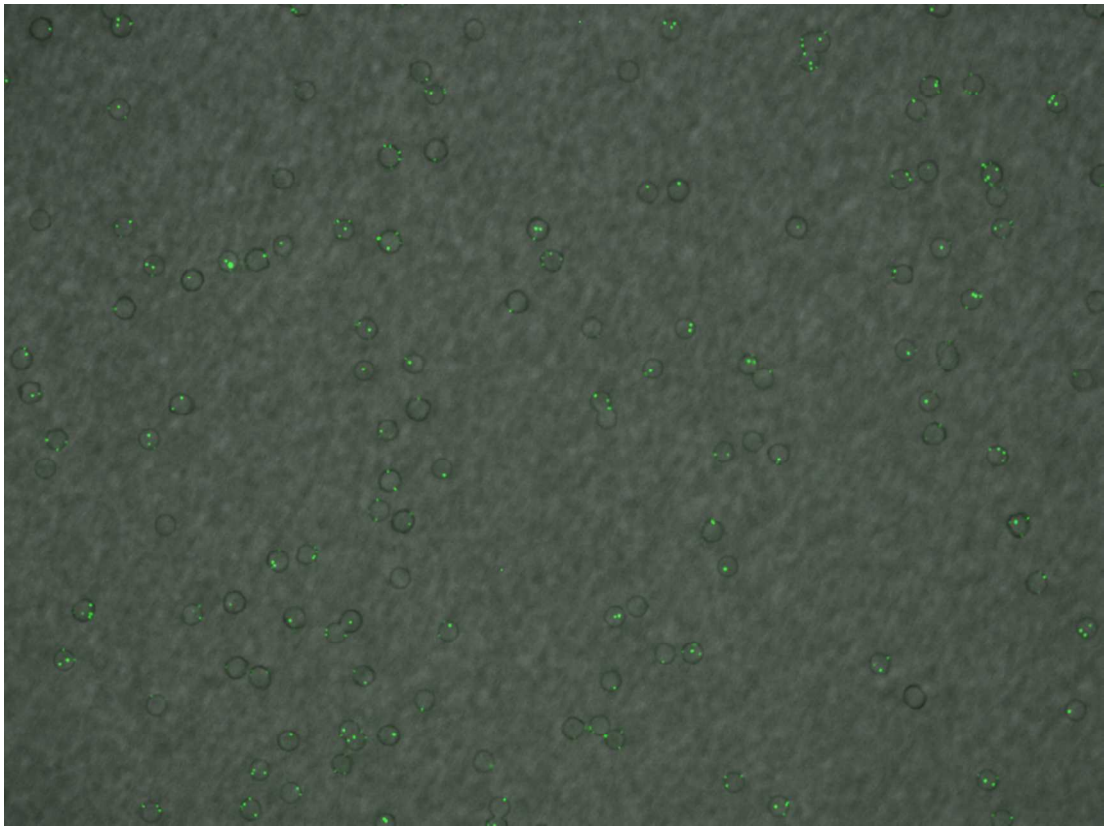

Figure S9. *E. coli* capture image. A high concentration of *E. coli* (20000 cell/mL) were used here.

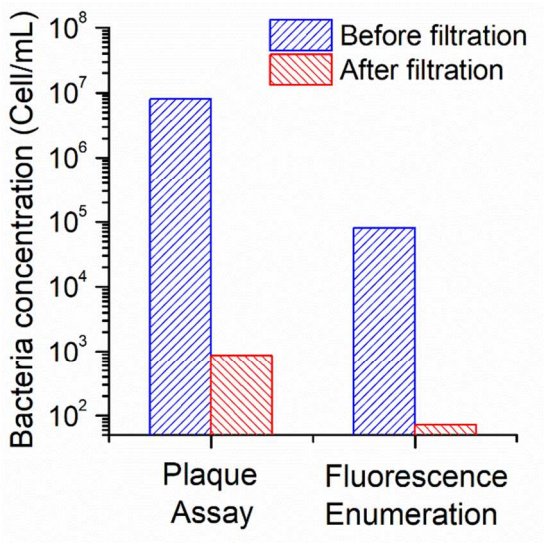

Figure S10. The concentration of *E. coli* measured in the original sample as well as in the filtrate.

The capture efficiency were calculated as,

$$\text{capture efficiency} = 1 - \frac{c_f}{c_0}$$

where c_f is the concentration of *E. coli* in the filtrate, and c_0 is the concentration of *E. coli* in the original sample.

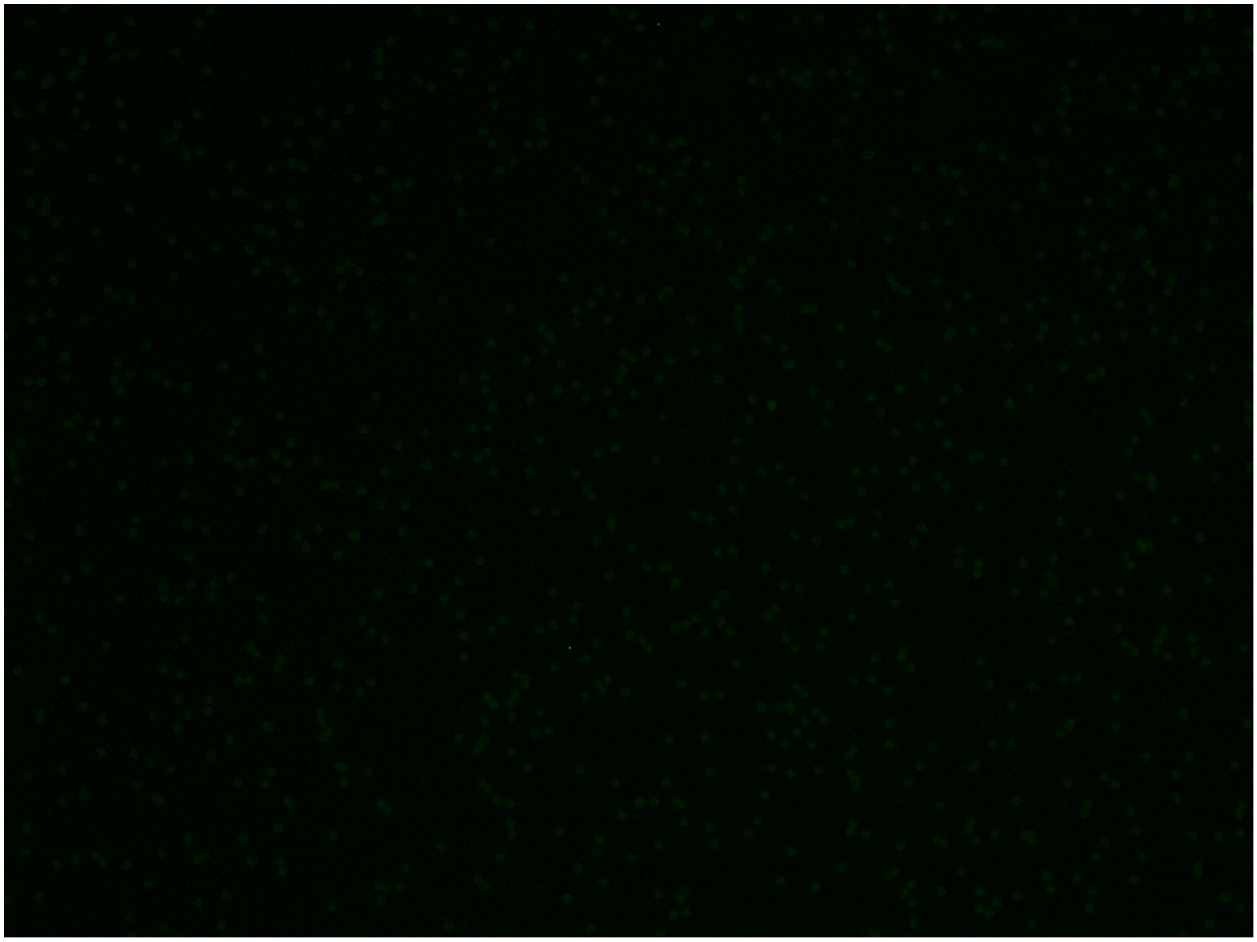

Figure S11. Preliminary result for digital LAMP of *E. coli* on the asymmetric membrane. The *E. coli* sample with concentration of 1000 cell/mL was analyzed. The LAMP reaction contain 1 × isothermal buffer, 6 mM total MgSO4, 1.4 mM dNTP, 640 U/mL *Bst* 2.0 WarmStart polymerase, 1.6 μM FIB and BIP, 0.2 μM F3 and B3, 0.8 μM LF and LB, 1.5 mg/mL BSA, 50 μM calcein, 1 mM $MnCl_2$.

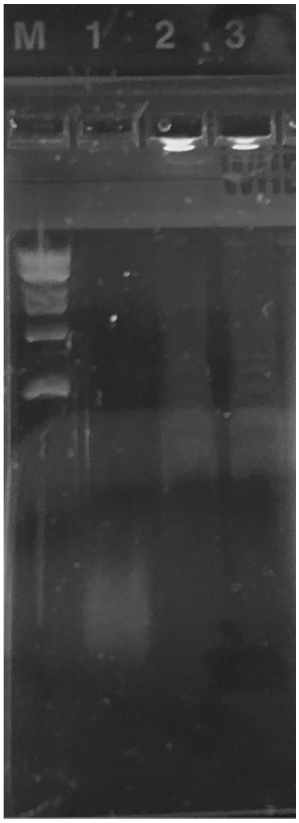

Figure S12. Gel-electrophoresis results of LAMP products. The lane numbers correspond to the following specimens. Lanes: M, 100-bp adder; 1, negative control; 2, positive control; 3, *E. coli*.

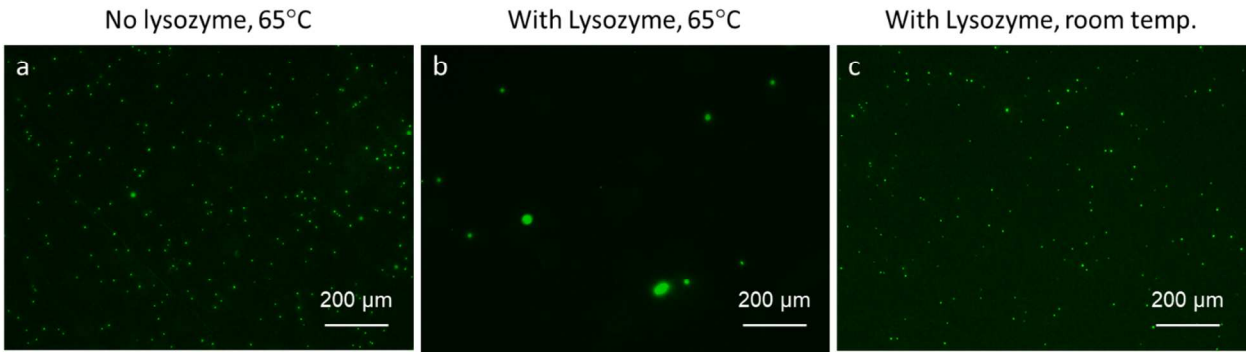

Figure S13. Test of bacterial lysis efficiency by fluorescence enumeration. Bacteria was counted after incubation in a reaction at 65°C for 40 min, (a) without lysozyme, (b) with 0.1 mg/mL lysozyme and (c) With 0.1 mg/mL lysozyme but incubated at room temperature. The reaction contains 1 × isothermal buffer and 1×10^8 cell/mL of *E. coli*.

To test the efficiency of bacteria lysis, we incubate the *E. coli* in the isothermal buffer at 65°C for 40 min with or without lysozyme. After that, the remaining cell in the reaction was stained with SYBR Green, filtrated through a membrane, and enumerated under fluorescence microscope. As shown in **Figure S13**, without including lysozyme into the reaction, most *E. coli* was still intact after 65°C incubation. However, if lysozyme was added, almost all the *E. coli* was lysed after incubation. The remaining large fluorescence point may refer to the aggregate of bacteria debris. Meanwhile, compared to result of **Figure S13c**, the bacteria lysis works better during 65°C incubation. The difference in fluorescence background may result from the variation in adsorption of SYBR Green dye on the PC membrane during filtration.

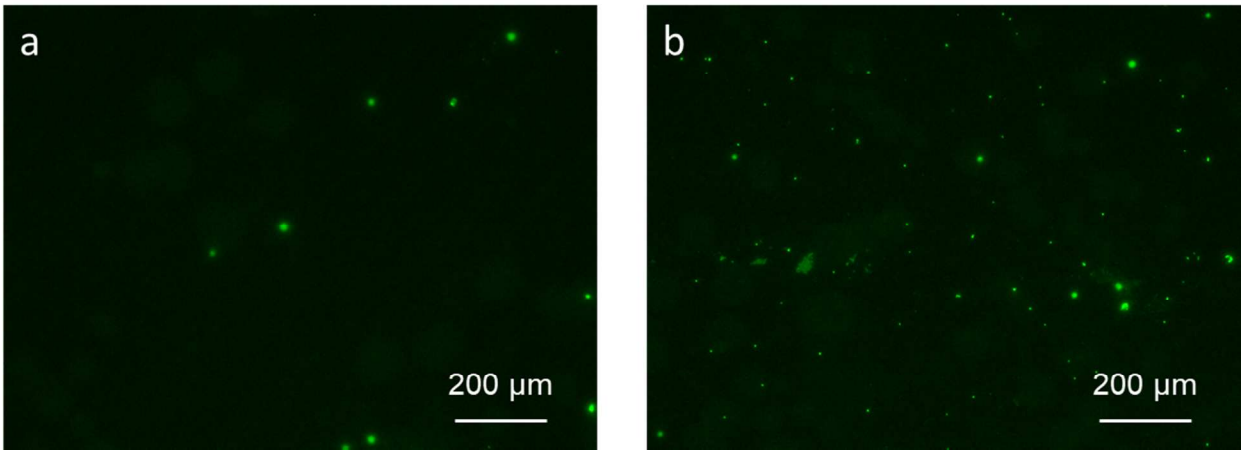

Figure S14. Bacteria lysis efficiency with different concentration of *E. coli*. (a) Reaction contain 1×10^8 cell/mL *E. coli*. (b) Reaction contain 1×10^7 cell/mL *E. coli*.

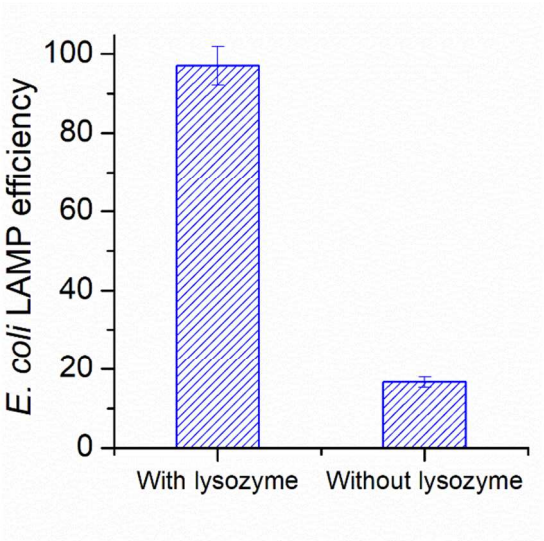

Figure S15. Digital Single *E. coli* LAMP efficiency.

The *E. coli* LAMP efficiency was calculated by measuring the number of stained *E. coli* on the membrane and the number of positive pores on the membrane. Poisson distribution was also introduced for calibration.

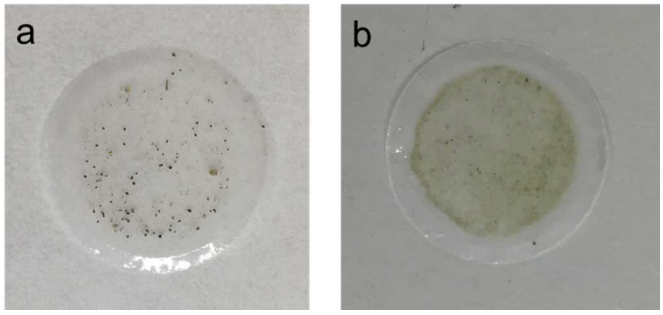

Figure S16. Photo images of pre-filter after environmental sample filtration. (a) Seawater. (b) Pond water.

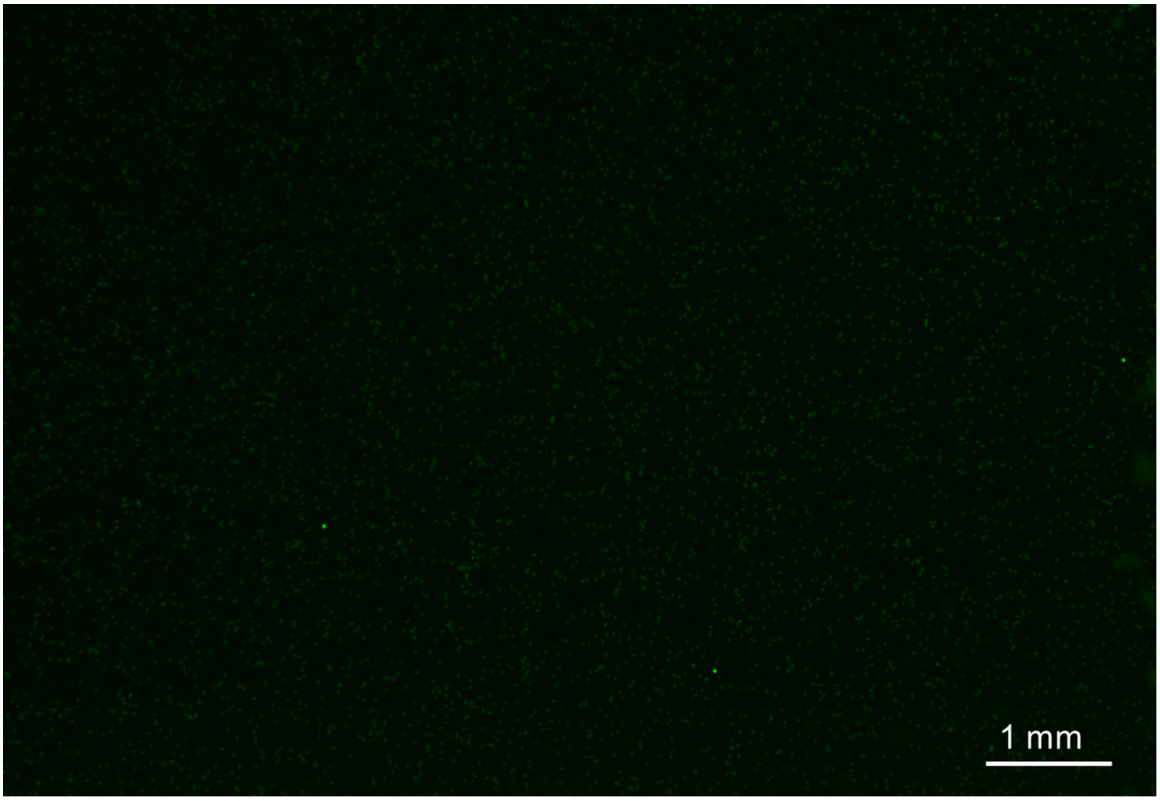

Figure S17. Fluorescence image of membrane after analysis of seawater sample with *E. coli* concentration of 0.3 cell/mL.

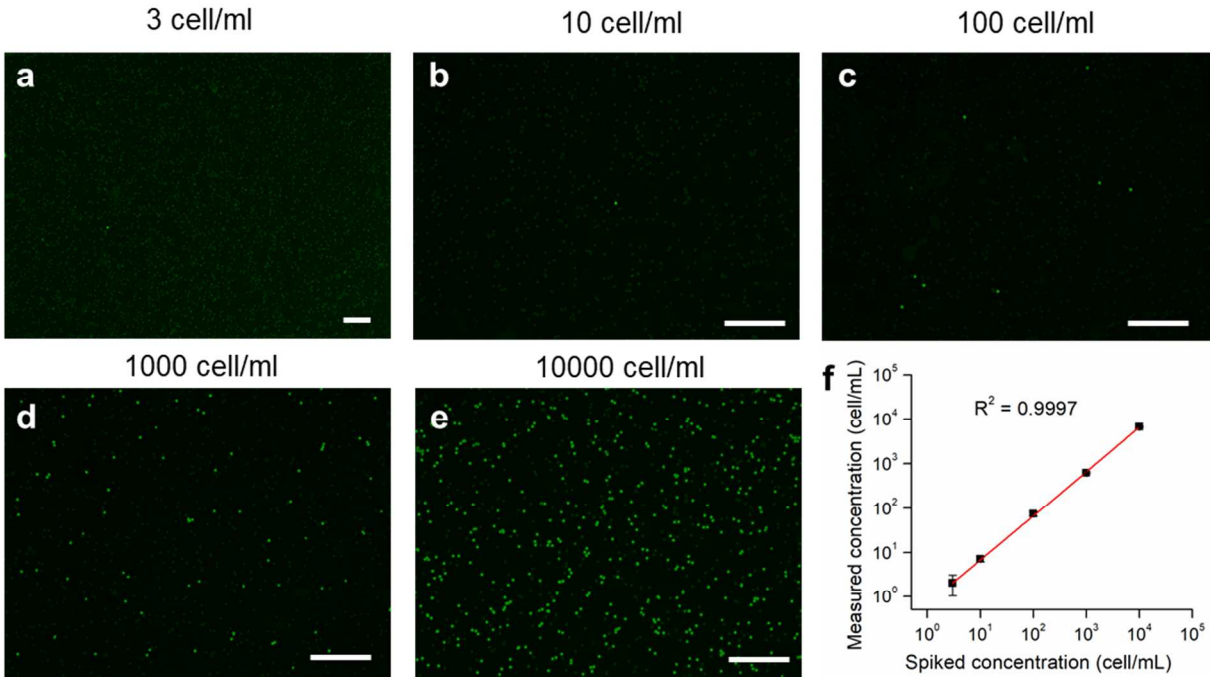

Figure S18. mRT-LAMP performance of *Salmonella* in unprocessed environmental turtle pond water. (a-e) End-point fluorescence image of membrane after mRT-LAMP analysis of pond water with a serial of spiked *Salmonella* concentration. (f) Comparison of measured *Salmonella* concentrations to the spiked concentrations.

Supplementary Video Legends

Supplementary Video S1: COMSOL Stimulated movie showing the trajectories of particles under filtration flow through the asymmetric membrane.

Reference

1. Hill, J.; Beriwal, S.; Chandra, I.; Paul, V. K.; Kapil, A.; Singh, T.; Wadowsky, R. M.; Singh, V.; Goyal, A.; Jahnukainen, T.; Johnson, J. R.; Tarr, P. I.; Vats, A. Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Common Strains of Escherichia Coli. *J. Clin. Microbiol.* **2008**, *46*, 2800-2804.
2. 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.
3. Li, Z.; Liu, Y.; Wei, Q.; Liu, Y.; Liu, W.; Zhang, X.; Yu, Y. Picoliter Well Array Chip-Based Digital Recombinase Polymerase Amplification for Absolute Quantification of Nucleic Acids. *PLoS One* **2016**, *11*, e0153359.