Electronic Supplementary Material (ESI) for Lab on a Chip.

Sensitive and rapid detection of pathogenic bacteria from urine samples using multiplex recombinase polymerase amplification

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Supplementary materials

Figure S1. Pre-treatment of spiked urine samples. (A) The structure of the filter-based pipette for enrichment of bacteria. (B) First, the three-way switch was placed on a position to connect the tip and the white syringe. When we pressed and then released the white syringe, a volume of 5 mL urine was steadily sucked into the syringe through a 0.22 μm filter by the bounce of the spring in white syringe, and the bacteria were thus captured and enriched on the membrane. (C) After that, we rotated the three-way switch for 90° to connect the tip with the blue syringe. (D) The bacterial suspension was injected into the chip with the pure water stored in the blue syringe.

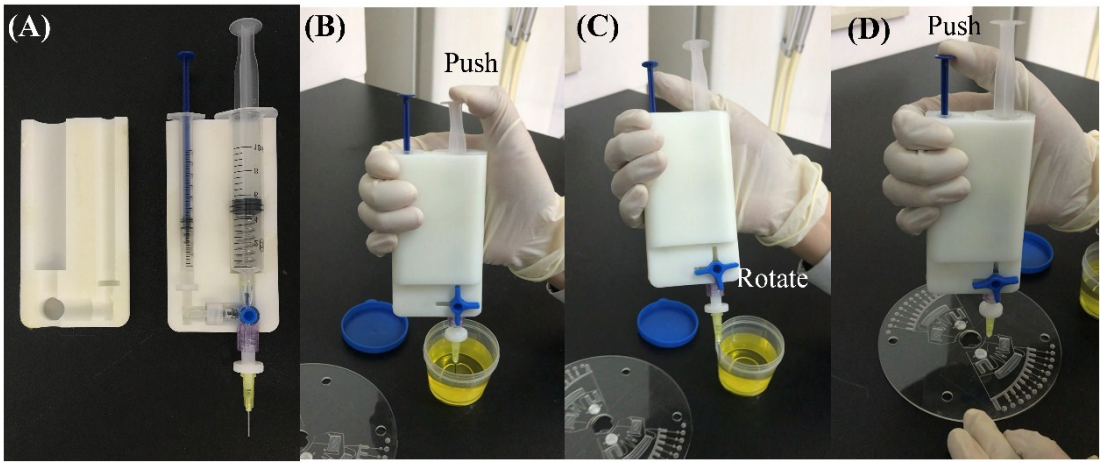

Figure S2. Off-chip RPA detection of five target bacteria (*E. coli*, *S. aureus*, *S. typhimurium*, *P. mirabilis*, and *P. aeruginosa*) in the urine samples. (RFU: relative fluorescence unit; NC: negative control).

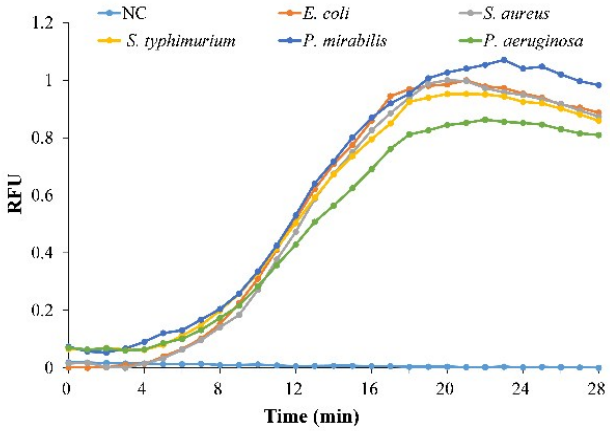

Figure S3. Specificity tests of RPA primers in the tube. (A) Specificity test for detecting *E. coli*. (B) Specificity test for detecting *S. aureus*. (C) Specificity test for detecting *S. typhimurium*. (D) Specificity test for detecting *P. mirabilis*. (E) Specificity test for detecting *P. aeruginosa*.

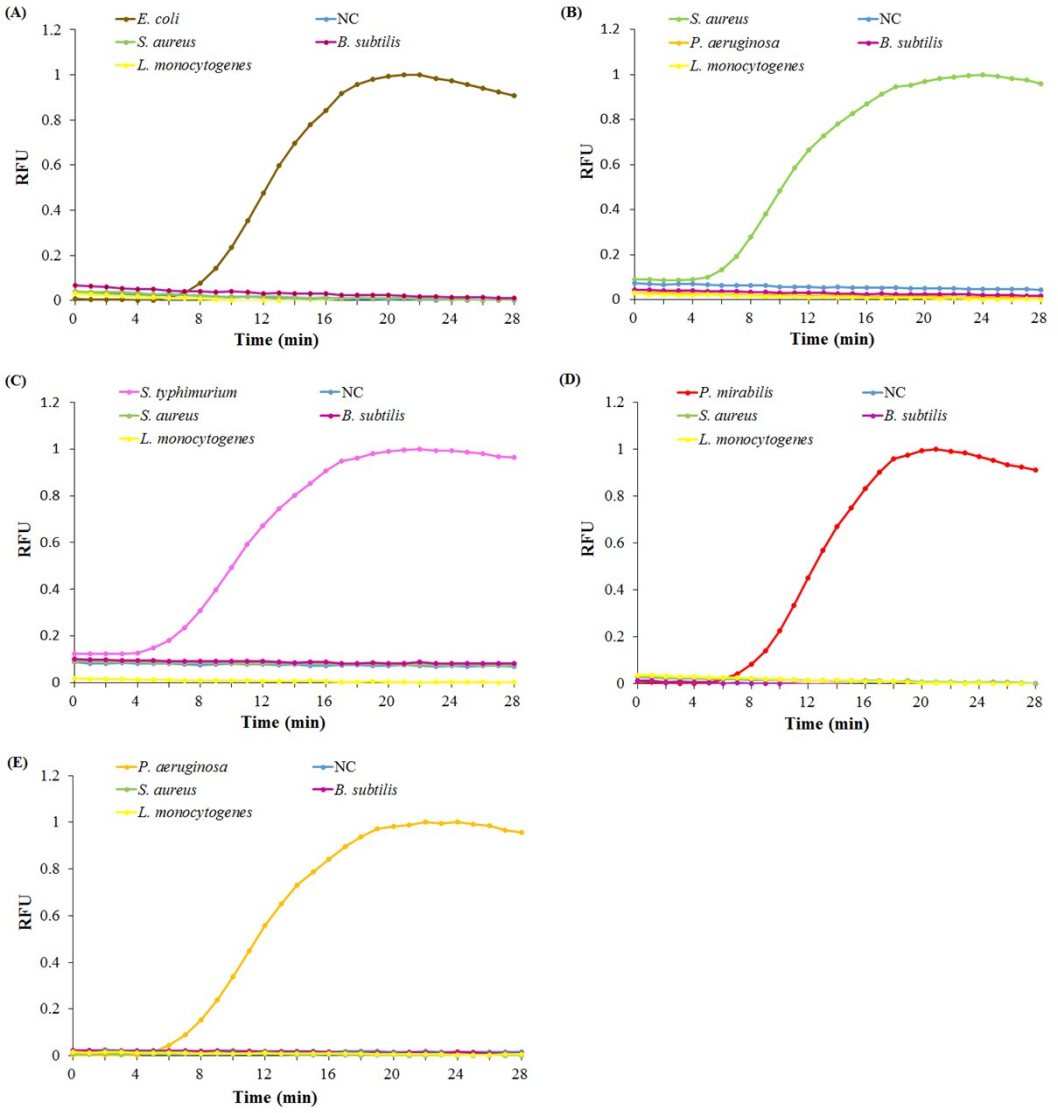

Figure S4. Optimization of bead-beating lysis. (A) Optimization of the rotate speed of magnets for Gram-positive bacteria (S. aureus); (B) Optimization of the lysis time for Gram-positive bacteria (S. aureus).

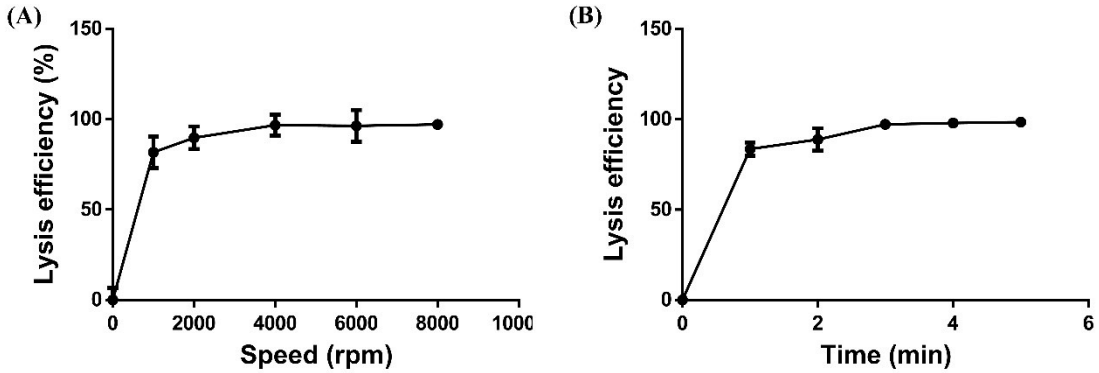

Figure 5S. Specificity of on-chip real-time RPA. 10^3 CFU/mL of *E. coli* and *B. subtilis* were spiked in the urine.

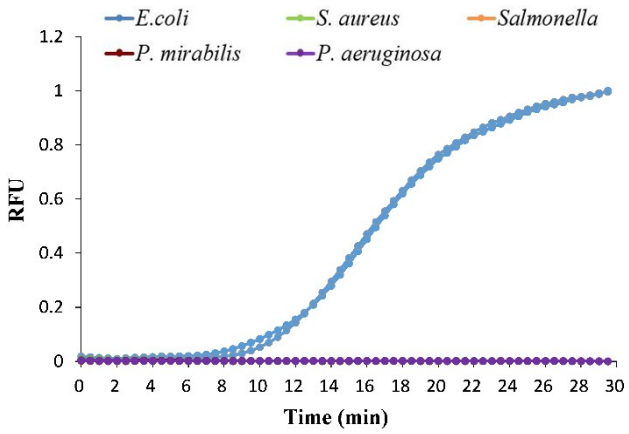

Table S1. Sequences of RPA primers for the various pathogens.

Pathogen	Target gene	Nucleotide sequence 5' to 3'
E. coli	endA	Primer F: GTACGTTTTATTGCGGATGTAAAATTAACTG Primer R: ATAGACCGGATCTTTAGCGCAGTTTTTAC Probe: GGGCAAAAAAGGCGTTGTTGATCTGCAA-T(FAM)C-THF-T(BHQ-1)-GCGGCTATCAGGTGCGC-GGATGAAATTAACGAAG-C6 Spacer
S. aureus	MecA	Primer F: CGATATCGAGGCCCGTGGATTTAGTCGTGA Primer R: GTGACTTCGACACCTTTTTCAAAGGCATGTAC Probe: CGCAAACGTGGCGAAGAATTCTTTTGGTCAA-T(FAM)-THF-A-T(BHQ-1)-GGATGAAATTAACGAAG-C6 Spacer
S. typhimurium	STM4599	Primer F: GTCGAAATCTAGCTAATCTACTGGTTCTTCC Primer R: CTGTGCTGTATTAAGATCATGAAACGCAGTAT Probe: CTGGTGTTTCAGCCAAAACATCAGCAGGCGA-T(FAM)-C-THF-A-T(BHQ-1)-GGCGGCATACAGGC-C6 Spacer
P. mirabilis	ureR	Primer F: GTATATGGTGCAAAAGGTGAGATTTGTATTA Primer R: TTGTAATTCAGTTTCAGACAGTACTAAGGTAT Probe: GATTATTCCTAAATATAGTCAAGTTTCTTG-T(FAM)-THF-A-T(BHQ-1)-GTGACAAATTTTTTC-C6 Spacer
P. aeruginosa	ETA	Primer F: GTGCTGCACTACTCCATGGTCCTGGAGGG Primer R: GTTCGTGGATGAACACCTTGATGTTCGAGG Probe: GAAGGTGGCGTCGAGCCGAACAAGCCGG-T(FAM)-G-THF-GC-T(BHQ-1)-ACAGCTACACGC-C6 Spacer

Table S2. Pre-storage of RPA master mix in real-time RPA chambers.

Reagents	Volume
10 μM species-specific forward primer	0.42 μL
10 μM species-specific reverse primer	0.42 μL
10 μM species-specific probe	0.12 μL
280 mM magnesium acetate	0.50 μL
50 mg/mL BSA	0.10 μL

Table S3. Spinning program of the complete assay.

No.	Speed (rpm)	Time (s)	Operation
A	0	180	Cell lysis
B	100	10	Siphon
C	3000	10	Transfer the bacterial lysate into the quantitative chamber
	50	10	Siphon
D	2000	30	Transfer the lysate and RPA master mix into the mixing chamber
	500 ⟷ 4000	60	Mixing of bacterial lysate and RPA master mix
	50	10	Siphon
E	1000	30	Pre-distribution of the mixture
F	4000	10	Dispensing of the sample into ten reaction chambers