Supplementary information

A self-contained and fully integrated fluidic cassette system for multiplexed nucleic acid detection of bacteriuria

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Figure S1. (A) Schematic depiction of the rotary valve, 1. threaded interface with the Luer syringe; 2. fluid channel; 3. outlet of the fluid channel; 4. venting channel; 5. interface with the rotating connector. (B) Top view of the rotary valve. (C) Side view of the rotary valve. (D) Section view of the cassette along line A, which depicts the Luer syringe and the chamber are connected by the fluid channel inside the rotary valve.

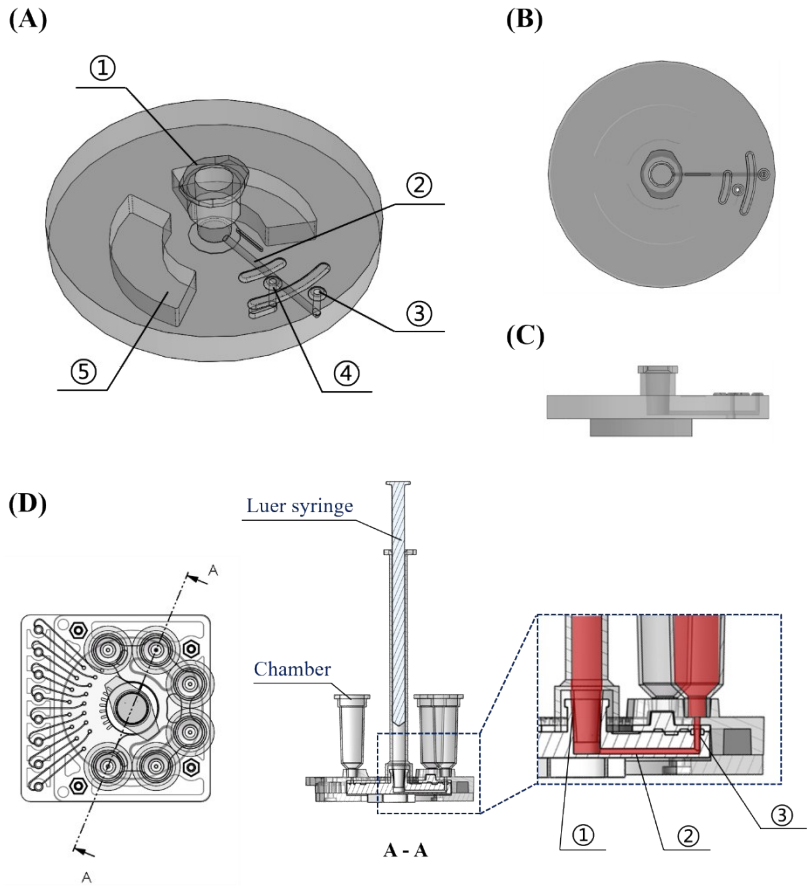


Figure S2. (A) Schematic diagram of magnetic bead movement control system, 1. cassette; 2. focusing magnet; 3. self-locked solenoid; 4. slip ring. (B) A schematic of adjusting the distance between the focusing magnet and the cassette to achieve manipulation of the magnetic beads. (C) Process of magnetic beads enrichment (C1-C4) and resuspending (C5-C8). Yellow dye mixed with magnetic beads turns brown. The magnetic beads are enriched with the magnet close to the bottom of the cassette and the liquid in the left chamber turns yellow, when the mixed solution is slowly passed through the rotary valve. The collected magnetic beads are dispersed with the magnet away from the cassette and the liquid in the left chamber turns brown, by pushing and pulling the syringe rapidly.

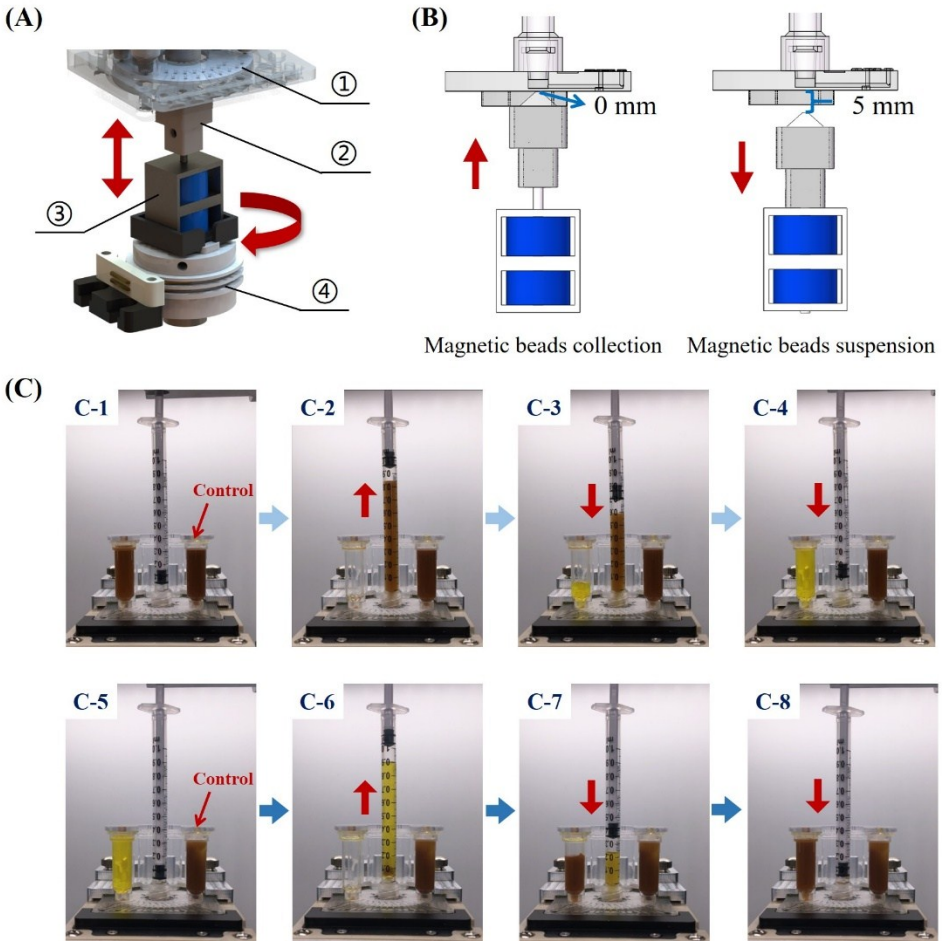


Figure S3. (A) Schematic diagram of the temperature control system in an exploded view, 1. cassette; 2. heating plate; 3. thermocouple; 4. silicone rubber heating film; 5. base. (B) Evaluation performance of the temperature control system, the set LAMP temperature (red line), the temperature of the heating plate (black line), and the temperature of the chamber (blue line) as functions of time. It took about 5 mins for the chambers to raise the temperature from the ambient to the setting of 65℃ and the fluctuation of the temperature is less than 0.2℃.

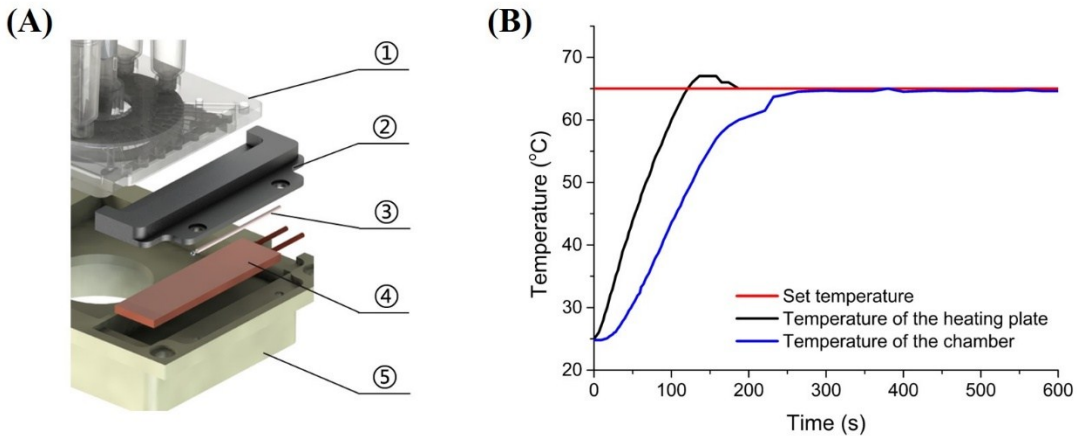
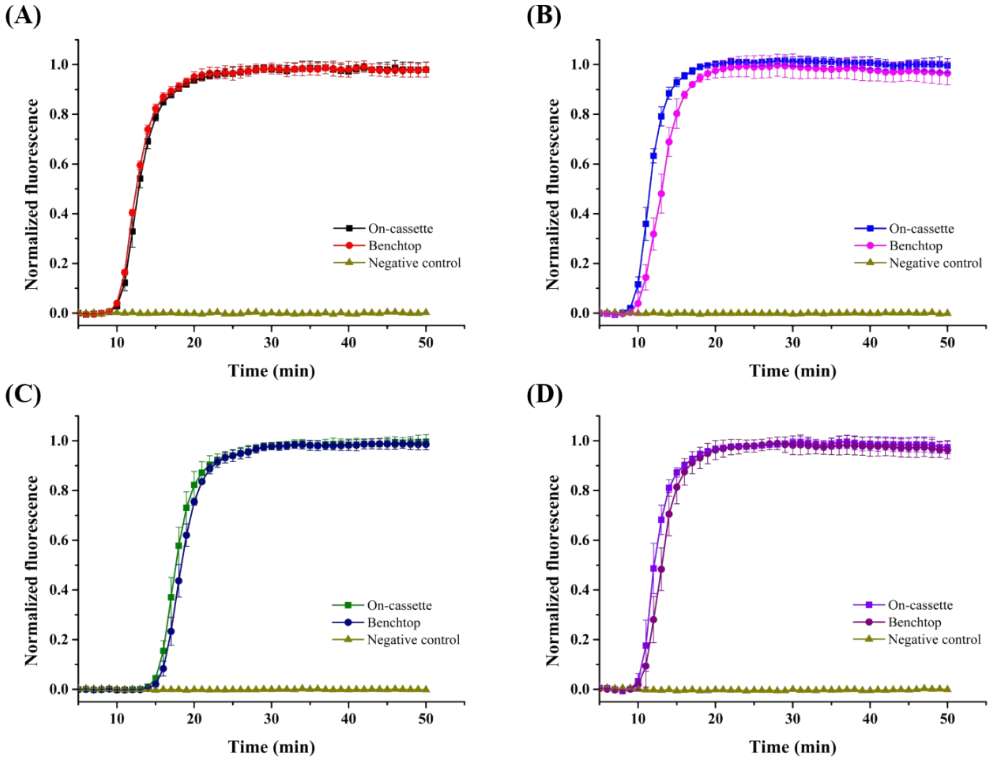


Figure S4. Comparing the efficiency of on-cassette extraction and benchtop extraction using LAMP. (A) *E. coli*, (B) *P. mirabilis*, (C) *S. aureus*, and (D) *S. typhimurium*.



Video S1. Automated processing on the cassette system. The video shows the on-cassette fluid control steps, and the sample and reagents are represented by different coloured dyes.

No.	Platform	Sample volume (μL)	Multiplex	Sensitivity (CFU/mL)	Analytical method	Disposable container	Size and weight of devices	Turnaround time (min)	Price ($)
1	GeneXpert[1-2]	2000	5-plex	10^2	RT-PCR	Cartridge	10 × 29 × 30 cm^3, 4 kg	<120	9.98[a]
2	Filmarray[3]	200	20-plex	10-10^3	Nested PCR	Pouch	17 × 25 × 39 cm^3, 9 kg	60	129[b]
3	Liat[4-5]	200	2-plex	60	RT-PCR	Tube	11 × 19 × 24 cm^3, 3.76 kg	20-90	100[c]
4	ePlex[6]	50	25-plex	10^2 to 10^5	PCR and electrochemical sensor	Cartridge	59 × 48 × 54 cm^3, 49 kg	90	157[d]
5	Liu *et al.*[7]	1000	1-plex	10^3 to 10^6	PCR and electrochemical sensor	Cartridge	_	210	_
6	Chen *et al.*[8]	100	1-plex	10^3 to 10^4	PCR and lateral flow strip	Cassette	_	_	_
7	Czilwik *et al.*[9-10]	200	8-plex	10	Nested PCR	Disk	15 × 18 × 28 cm^3, 2 kg	225	_
8	Our system	50-1000	8-plex	10^3 to 10^4	LAMP	Cassette	21 × 17 × 24 cm^3, 3 kg	100	15[e]

Table S1. Comparison of fully integrated nucleic acid analysis platforms for identification of pathogens.

Note: a. https://web.archive.org/web/20140407082202/http://www.finddiagnostics.org/about/what_we_do/successes/find-negotiated-prices/xpert_mtb_rif.html,
b. https://www.genomeweb.com/pcr/update-biofire-ramping-filmarray-adoption-test-development,

c. https://www.ncbi.nlm.nih.gov/books/NBK401783/,

d. https://www.360dx.com/regulatory-news/fda-clearance-genmarks-eplex-intensifies-competition-highly-plexed-mdx-test-market,

e. Only consider the material cost.

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Table S2. Sequences of the LAMP primers.

Target	Primer	Sequences (5'-3')
E. coli	F3	TGTGGAATTGATCAGCGTT
	B3	TGATTATTGACCCACACTTTG
	FIP	TCTGCATCGGCGAACTGATCGGGTGGGAAAGCGCGTTAC
	BIP	TCTGGTATCAGCGCGAAGTCTAATGAGTGACCGCATCGA
	LF	GCAATTGCCCGGCTTTCTT
	LB	GCAGGCCAGCGTATCGT
P. mirabilis	F3	CCTACGGGGATTGTTTGT
	B3	AGCTTCCCATTGATTTTGAG
	FIP	CAATTTCATCGGGAATATTCCCCTCGATTAAGCCGTTTTCTCGC
	BIP	TCCAATCAGCACCTGCAGTTGCGCATTGTCCATACCCAA
	LF	TTCTGGGCACAGTTTTGGCGG
	LB	GGGGGATCCATCAGCTATATGCTTT
S. aureus	F3	GCAACTGAAACAACAGAAGC
	B3	TTTTGTGTTGGGCGAGC
	FIP	TCACGGATACCTGTACCAGCATCTCTATGGTCCGAGACCGCAATT
	BIP	GGAACATTTGGATATGAAGCGAGACTGCCATCTTGATTTGTCGTTAC
	LF	TTTCACATACTTAGGTGTTTTGT
	LB	CCAAGTGAAACAAATGCATACAAC
S. typhimurium	F3	CGATCTCGATAAAGTCTCTACAG
	B3	TCATTAATCAACAATACGATGCT
	FIP	CGCAAGTTGAGCTTTTTCCAGATACCGTTGATATTACTTGTGCC
	BIP	CAGTTCTTTATTGATTATGGCGTGCGTTATCGTCCAGGCCCTC
	LF	TCTTCACGCCGGCTCTTC
	LB	GCCTGCCGGAAGTATTGTTAC

Table S3. Detection results for clinical samples.

Clinical sample	Test result							
	Cassette system				Culture-based identification			
	Eco	*Pmi*	*Sty*	*Sau*	*Eco*	*Pmi*	*Sty*	*Sau*
1	+	-	-	-	+	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	+	-	-	-	+	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	+	-	-	-	+	-	-	-
8	+	-	-	-	+	-	-	-
9	+	-	-	-	+	-	-	-
10	-	+	-	-	-	+	-	-

Note: "+" represents the positive result, "-" represents the negative result; "*Eco*" represents *E. coli*, "*Pmi*" represents *P. mirabilis*, "*Sty*" represents *S. typhimurium* and "*Sau*" represents *S. aureus*.