Supplementary Information

Supplementary figures

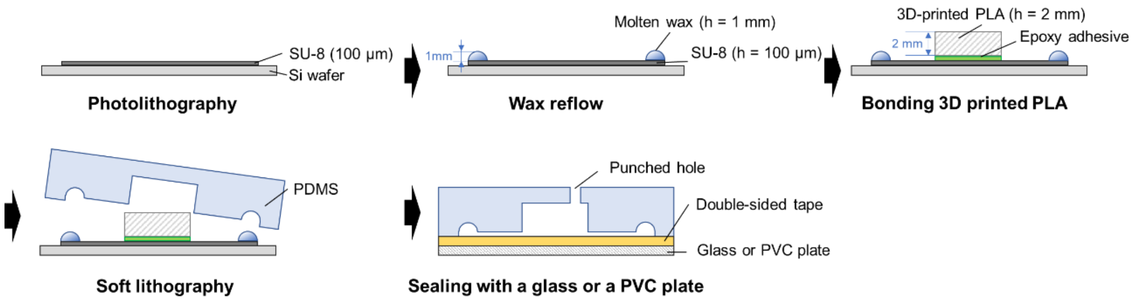

Fig. S1 Fabrication process for the PDMS-based centrifugal microfluidic device through a modified soft-lithography process combined with a wax reflow process and an adhesive bonding process of 3D printed PLA parts to create deep semi-elliptical microchambers and fan-shaped inlet/waste reservoirs, respectively.

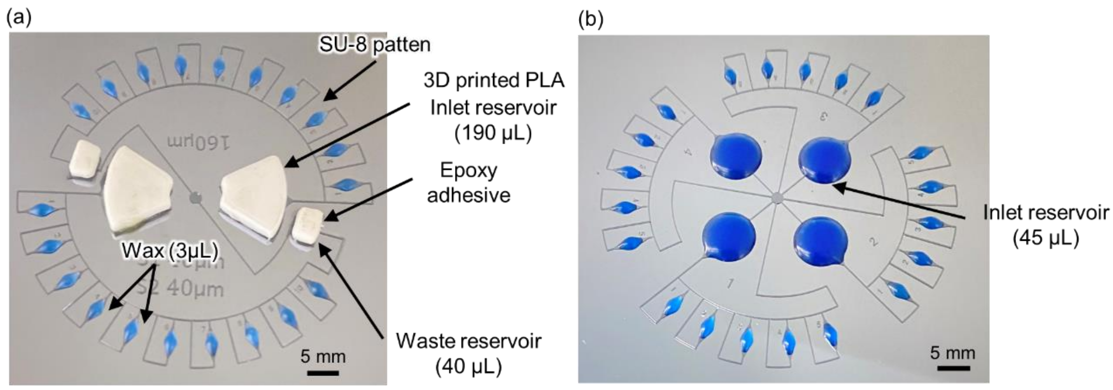

Fig. S2 Photographs of the master mold fabricated on a Si wafer. (a) SU-8 master mold pattern, in which reversed wax molds for two sets of 10 semi-elliptical microchambers were formed through the reflow process, and subsequently, 3D printed PLA parts for two sets of fan-shaped inlet/waste reservoirs were manually bonded with an epoxy adhesive. (b) SU-8 master mold pattern, in which reversed wax molds were formed to create four sets of five semi-elliptical microchambers and four spherical inlet reservoirs.

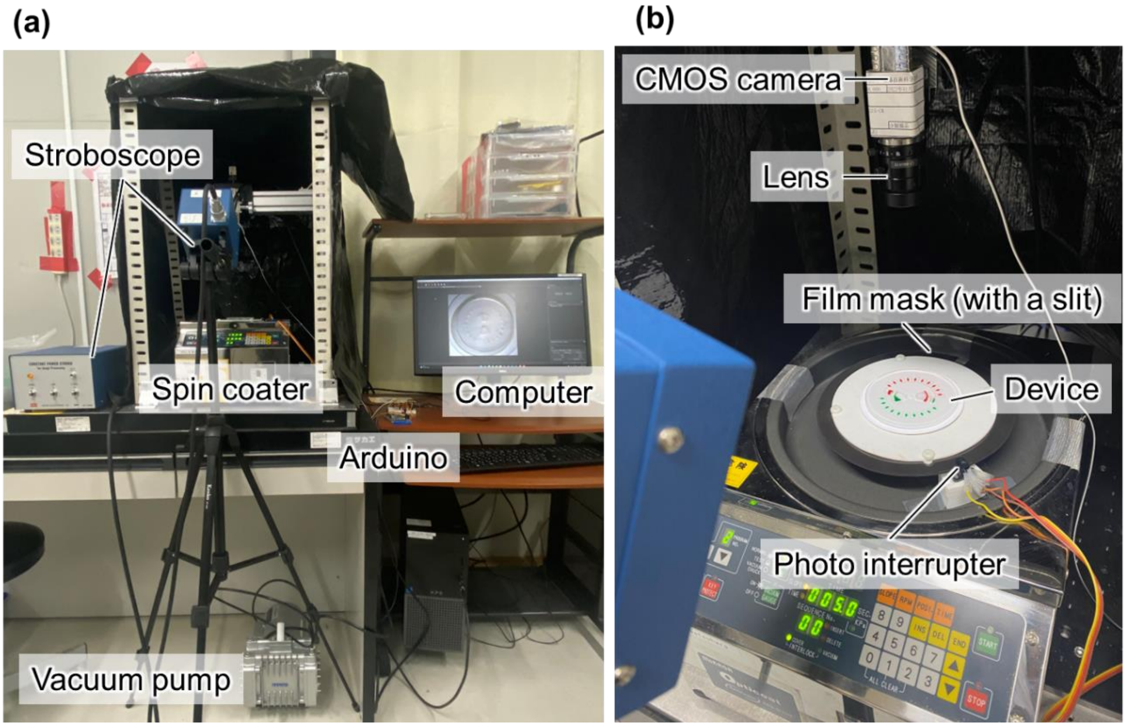

Fig. S3 Experimental setup for centrifugal microfluidic devices. (a) General view of the setup, consisting of a spin coater, stroboscope, microcomputer, and desktop computer. (b) Close-up view of the device placed on spin coater.

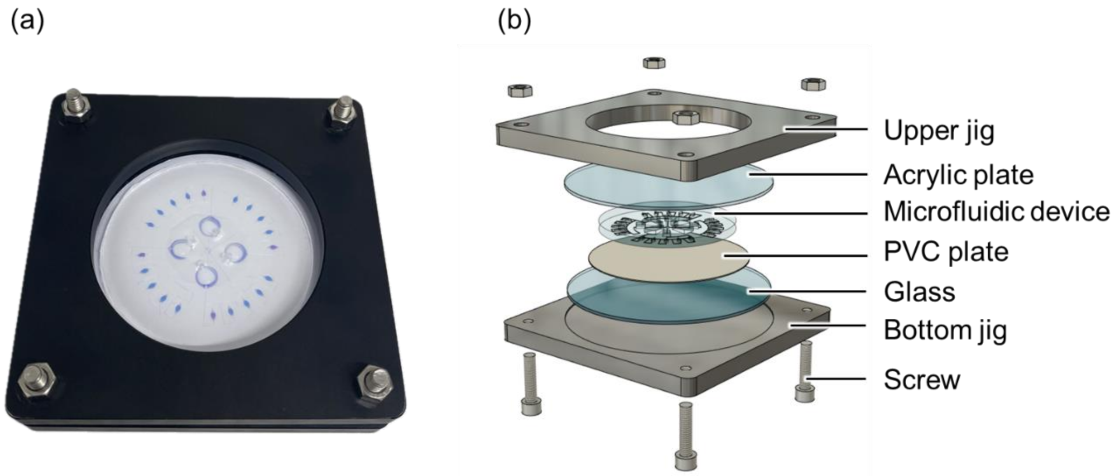

Fig. S4 Schematic of the microfluidic device mechanically clamped with a jig for colorimetric LAMP assays in a hot water bath. (a) Photograph of the device clamped with a jig. (b) Configuration of centrifugal microfluidic device and jig.

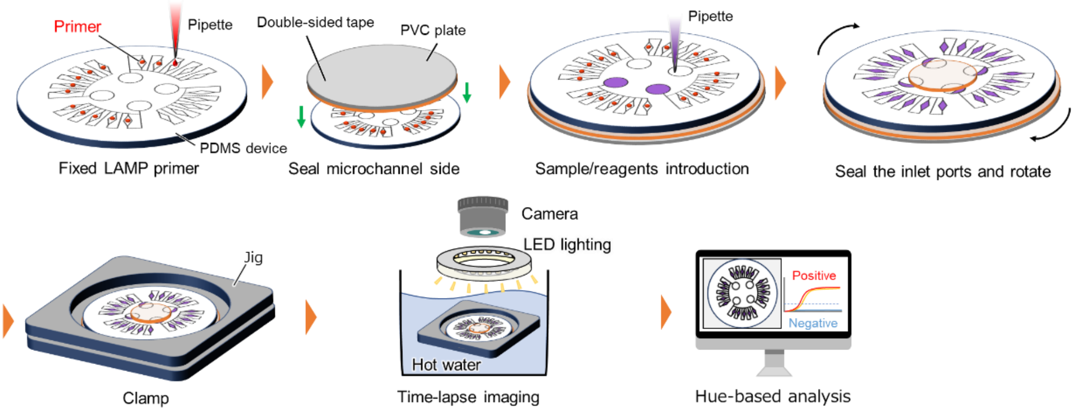

Fig. S5 Operating procedure for multiplexed colorimetric LAMP detection of target pathogens in the centrifugal microfluidic device.

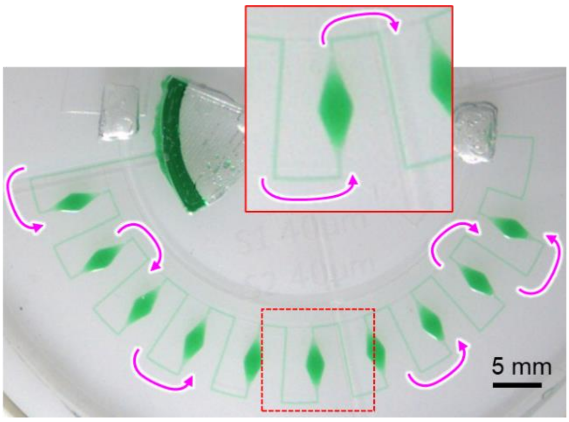

Fig. S6 Experimental result showing the influence of burst pressure P_2 of permanent stop valve S_2 on the performance of sequential liquid dispensing at a rotational speed of 1500 min^{-1}. The liquid flowed over all the valve S_2 (P_2 = 2.50 kPa for g_2 = 42.1 μm) because it was smaller than that of the design constraint (P_2 > 3.07 kPa).

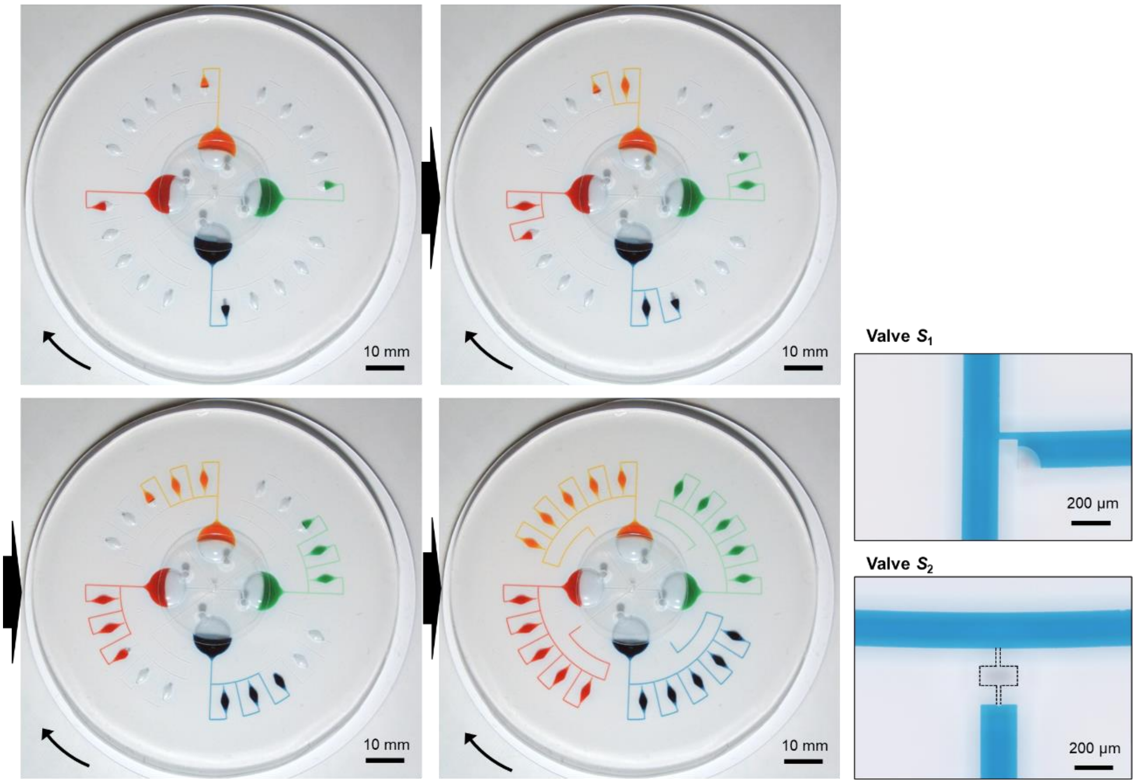

Fig. S7 Experimental results showing the sequential dispensing of four colored waters at a rotational speed of 1500 min^{-1} and close-up views of temporary stop valve S_1 and permanent stop valve S_2 after dispensing colored water.

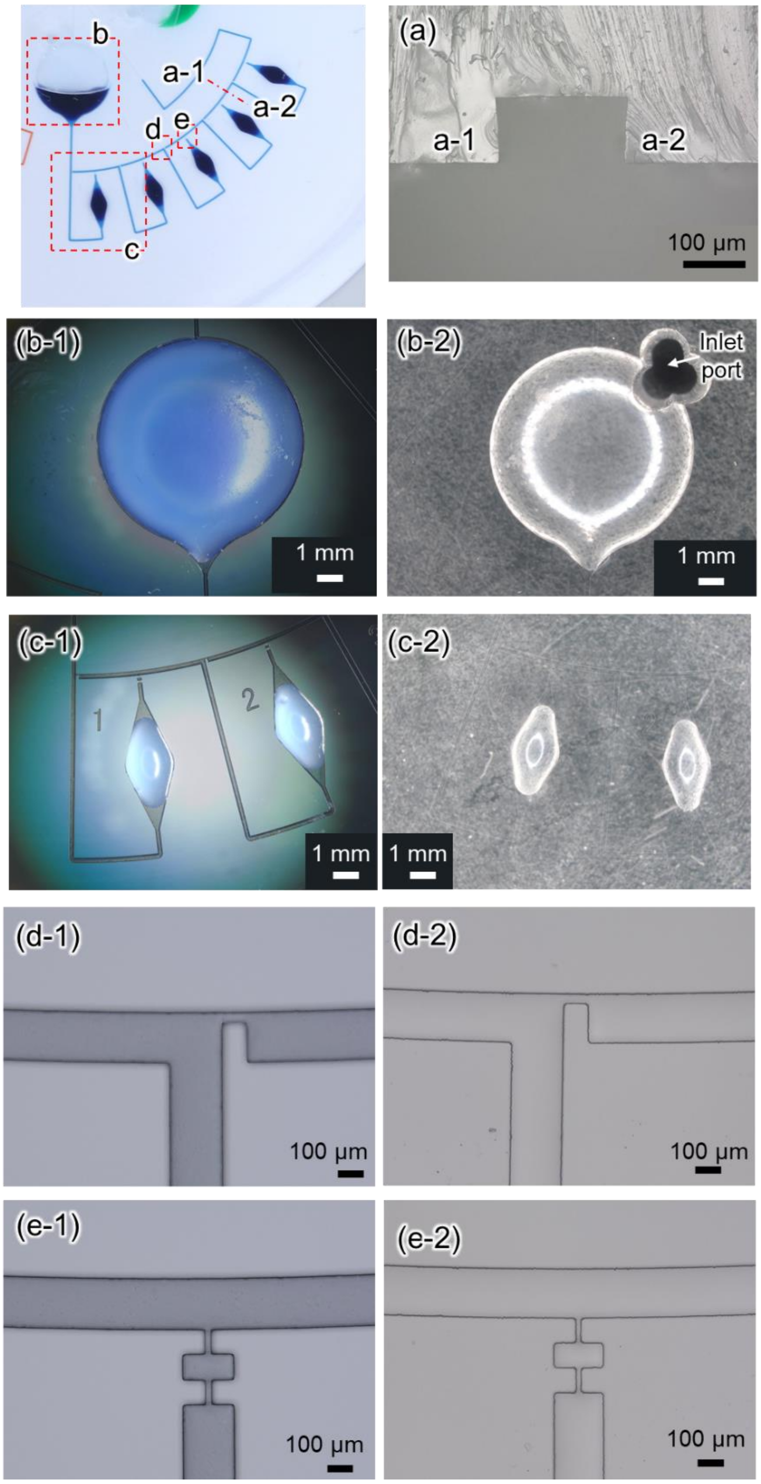

Fig. S8 Optical microscopy images of the fabricated master mold and a replicated PDMS device. (a) Cross-section of a PDMS microchannel. Inlet reservoir shapes of (b-1) the master mold and (b-2) the PDMS device. Microchamber shapes of (c-1) the master mold and (c-2) the PDMS device. Temporary stop valve S_1 structure of (d-1) the master mold and (d-2) the PDMS device. Permanent stop valve S_2 structure of (e-1) the master mold and (e-2) the PDMS device.

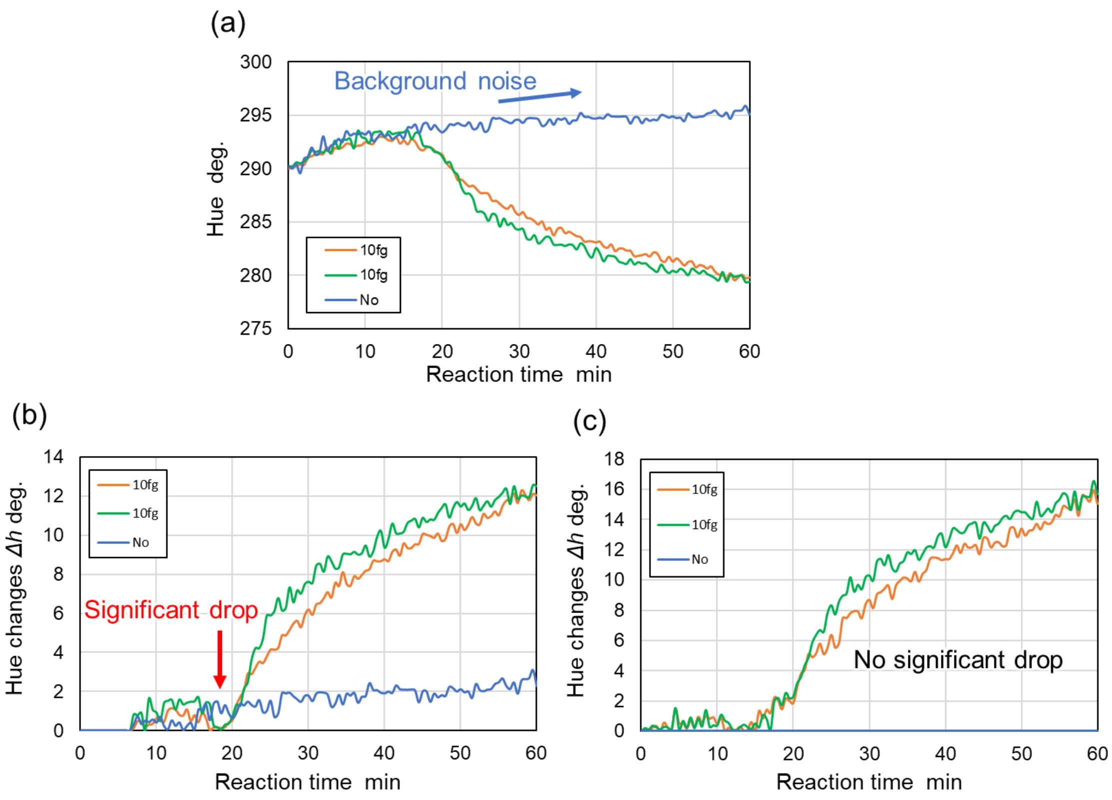

Fig. S9 Comparison between the previous and modified methods for baseline correction to obtain DNA amplification curves. (a) Color change in hue angle (*h*) as a function of LAMP reaction time. (b) The DNA amplification curve obtained using the previous baseline correction, in which a significant drop was observed immediately before starting DNA amplification. (c) DNA amplification curve obtained using modified baseline correction, where no significant drop was observed.

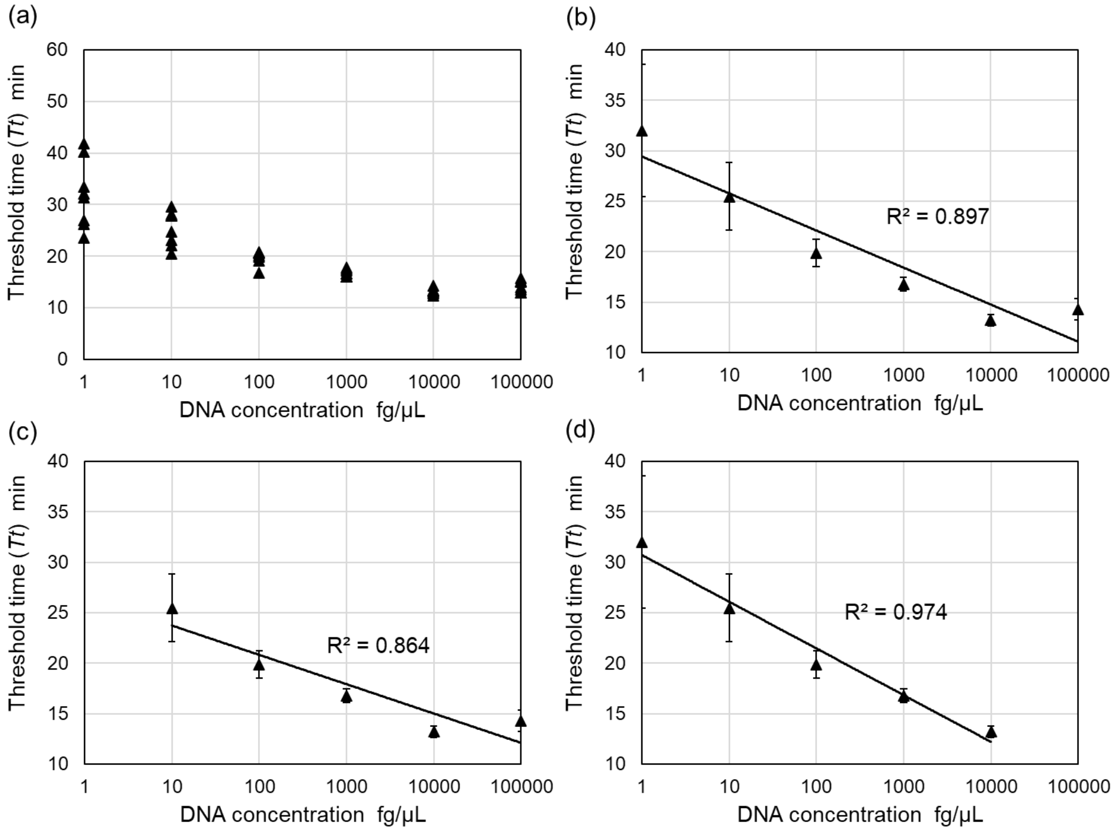

Fig. S10 Experimental results showing the quantitative analysis of *Salmonella* DNA template in the centrifugal microfluidic device (3 μL reaction volume). (a) *Tt* values were plotted against DNA concentrations ranging from 1 fg/μL to 100 pg/μL, with eight experiment replicates for each DNA concentration. The evaluation of the correlation coefficients of the standard curves with DNA concentrations ranging from (b) 1 fg/μL to 100 pg/μL, (c) 10 fg/μL to 100 pg/μL, and (d) 1 fg/μL to 10 pg/μL.

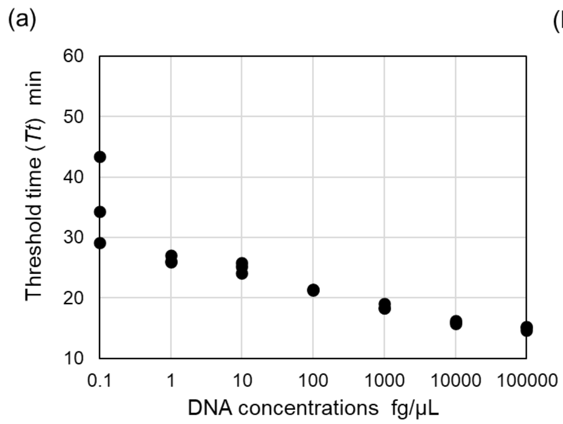

Fig. S11 Experimental results showing the quantitative analysis of *Salmonella* DNA template using real-time turbidity measurements in PCR tubes (25 μL reaction volume). (a) *Tt* values were plotted against DNA concentrations ranging from 0.1 fg/μL to 100 pg/μL, with three experiment replicates for each DNA concentration. (b) The evaluation of the correlation coefficient of the standard curve with DNA concentrations ranging from 1 fg/μL to 10 pg/μL.

Supplementary tables

Table S1 LAMP primer and template sequences for the detection of *Salmonella* spp.[41]

Primer name	Primer sequence
F3	GAACGTGTCGCGGAAGTC
B3	CCACCGAAATACCGCCAAT
FIP	GCGCGGCATCCGCATCAATAATGGTATGCCCGGTAAACAG
BIP	AGGGAAAGCCAGCTTTACGGTTTAATGATGCCGGCAATAGCG
LF	CCTTCAAATCGGCATCAATACTCAT
LB	CCTTTGACGGTGCGATGAAGTTTAT
DNA Template	GTTATTGGCGATAGCCTGGCGGTGGGTTTTGTTGTCTTCTCTATTGTCA CCGTGGTCCAGTTTATCGTTATTACCAAAGGTTCAGAACGTGTCGCGG AAGTCGCGGCCCGATTTTCTCTGGATGGTATGCCCGGTAAACAGATG AGTATTGATGCCGATTTGAAGGCCGGTATTATTGATGCGGATGCCGCG CGCGAACGGCGAAGCGTACTGGAAAGGGAAAGCCAGCTTTACGGTT CCTTTGACGGTGCGATGAAGTTTATCAAAGGTGACGCTATTGCCGGC ATCATTATTATCTTTGTGAACTTTATTGGCGGTATTTCGGTGGGGATGA CTCGCCATGGTATGGATTTGTCCTCCGCCCTGTCTACTTATACCATGCT GACCATTGCGTCTGTCGA

Table S2 LAMP primer and template sequences for the detection of *Vibrio parahaemolyticus*.[42]

Primer name	Primer sequence
F3	TATCGACCTAGACATACACG
B3	TTCAATCGCTTTAATGAACATG
FIP	CAGTGACAATCTTGGCTTACGA-CGTGAAAACATCGCAAACA
BIP	GTTCGAGTGGAGCGCATCAA-TGGTTAGTGCGGTTGGTA
LF	CTCGATCATCGCATTGGTG
LB	TTCGAGTGGAGCGCATCAAC
DNA Template	CATGGAAATCGCGTTAGAAGTATTTGCACGCCGTGGTATTGGCCGTGG TGGTCACGCAGATATTGCTGAAATTGCGCAAGTGTCTGTTGCAACCG TTTTTAACTACTTCCCAACTCGCGAAGATTTGGTTGATGAAGTTCTCA ACCATGTTGTCCGTCAGTTCTCGAATTTCCTTTCGGATAATATCGACCT AGACATACACGCTCGTGAAAACATCGCAAACATCACCAATGCGATGA TCGAGCTCGTAAGCCAAGATTGTCACTGGCTGAAAGTTTGGTTCGAG TGGAGCGCATCAACTCGTGATGAAGTTTGGCCTCTATTTGTGTCTACC AACCGCACTAACCAATTATTGGTTCAAAACATGTTCATTAAAGCGATT GAACGCGGTGAAGTGTGTGACCAACACGATTCAGAACACTTGGCAA ACCTATTCCACGGTATTTGTTACTCGCTGTTCGTACAAGCAAACCGCT TCAAAGGTGAAGCCGAGTTGAAAGA

Table S3 Components and volume used for colorimetric LAMP assays in the microfluidic device

Component	Concentration	Volume (μL)
2× Reaction mixture	–	18.75
Enzyme mix	–	1.5
DNA template	10 fg/μL to 1 ng/μL for *Salmonella* spp. 1 ng/μL for *V. parahaemolyticus* 1/10 diluted of *Campylobacter* spp. kit 1/10 diluted of norovirus GII kit	3.75
Primers for *Salmonella* spp.[a] and *V. parahaemolyticus* [a]	FIP 9.6 pmol/μL BIP 9.6 pmol/μL F3 1.2 pmol/μL B3 1.2 pmol/μL LF 4.8 pmol/μL LB 4.8 pmol/μL	(0.5) [b]
Primers for *Campylobacter* spp. [a] and norovirus GII [a]	N/A [c]	(0.3) [b]
Hydroxynaphthol blue	4.2 mM	1.34
Distilled water	–	12.16
Total		37.5 [b]

[a] Primer sets were pre-spotted and dried in each reaction chamber at 80 ℃ for 3 min.

[b] Total volume does not include the volume of the primer.

[c] The concentrations of each primer are unknown owing to them being commercial products.

Table S4 Components and volumes used for conventional off-chip LAMP assays for detection of *Salmonella* spp. performed in a 0.2-mL PCR tube using a real-time turbidimeter

Component	Concentration	Volume (μL)
2× Reaction mixture	–	12.5
Enzyme mix	–	1.0
DNA template	10^3–10^8 pg/μL for *Salmonella*	2.5
Primers for *Salmonella* spp.	FIP 30.8 pmol/μL BIP 30.8 pmol/μL F3 3.85 pmol/μL B3 3.85 pmol/μL LF 15.38 pmol/μL LB 15.38 pmol/μL	1.3
Distilled water	–	5.0
Total		25.0