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

Electronic Supplementary Information (ESI)

A rapid and eco-friendly isothermal amplification microdevice for multiplex detection of foodborne pathogens

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Table S1. The primer sequences used for loop-mediated isothermal amplification of four microorganisms

Target gene	Primer name	Primer sequence (5'-3')
*inv*A gene (*Salmonella*)	F3	CCA ACA ATC CAT CAG CAA G
	B3	AGC ATA TGT TTT GTT TCC TGA A
	FIP	AAC ACA TAG CCA AGC TCC CGC AGT CAG TAT TTC TGG GTA AC
	BIP	GAA CGC GCT TGA TGA GCT TTC GAA ATA TTC ATT GAC GTT GC
	LF	GAG TTT CTC CCC CTC TTC ATG C
	LB	ACC ACT GTC TGG CGG TGA
nuc gene (*S. aureus*)	F3	AAC AGT ATA TAG TGC AAC TTC AA
	B3	CTT TGT CAA ACT CGA CTT CAA
	FIP	ATG TCA TTG GTT GAC CTT TGT ACA TAA ATT ACA TAA AGA ACC TGC GA
	BIP	GTT GAT ACA CCT GAA ACA AAG CAT CAT TTT TTT CGT AAA TGC ACT TGC
	LF	CCG TAT CAC CAT CAA TCG CTT T
	LB	AGG TGT AGA GAA ATA TGG TCC TGA
*eae*A gene (*E.coli* O157:H7)	F3	TCT GAT GCC AAT GAC GAA T
	B3	GGT AGC ATC ATC GAG AGG
	FIP	CAT TCA AAA TCT CAA GAG CGA AGT TGA TTT ATG GTG TCT GTG GGA
	BIP	CAA ACT TAT GGT TTG CAG AGA ATG GAA ACG TAA CGC TAA CAA CAG
	LF	AGA GTT ATC TGT CGG GAA TT
	LB	TGG CCC ATA TCT GTG CTA
Large subunit ribosomal RNA gene (C. *polykrikoides*)	F3	TCC TGA CAC ACG TCT ACG AC
	B3	TCA CCA TCT TTC GGG TCC TA
	FIP	AGC CCA AGC ACT CGC ACA TAT GGT TCT TTC CGA CCC GTC TTG
	BIP	CAT GCG CGC AAC GAA AGT GAA CGT CTC GCA ATT GAT CAG T
	LF	AGA CTC CTT GGT CCG TGT TT
	LB	GCT GAG ATC TTT GCA CCA GCA

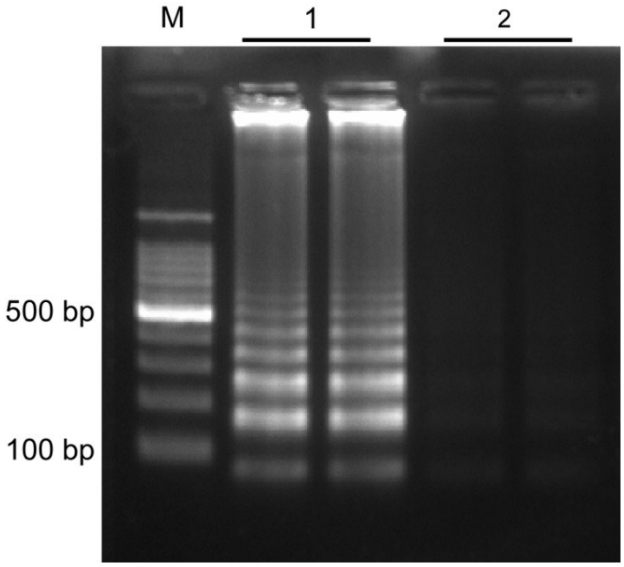

Fig. S1 Evaluation of the reaction temperatures for LAMP. Lane M shows 100 bp DNA ladder. Lane 1 shows reaction performed at 65 ^{o}C. Lane 2 shows reaction performed at 60 ^{o}C.

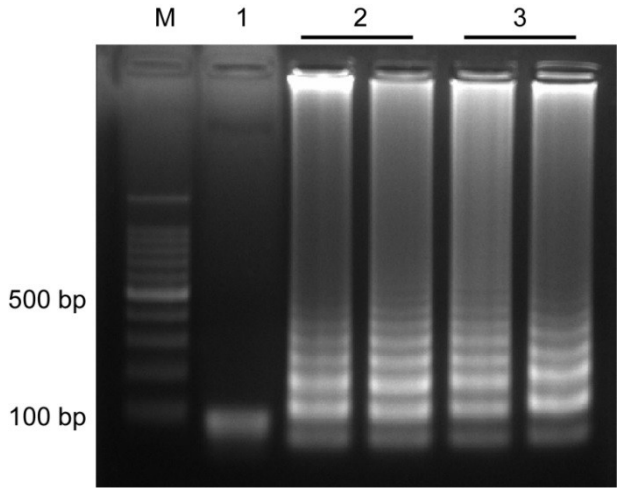

Fig. S2 Evaluation of the reaction time when the reaction temperature was 65 ^{o}C. Lane M shows 100 bp DNA ladder. Lane 1 shows negative control result. Lane 2 shows the results when reactions were performed for 60 min. Lane 3 shows the results when reactions were performed for 30 min.

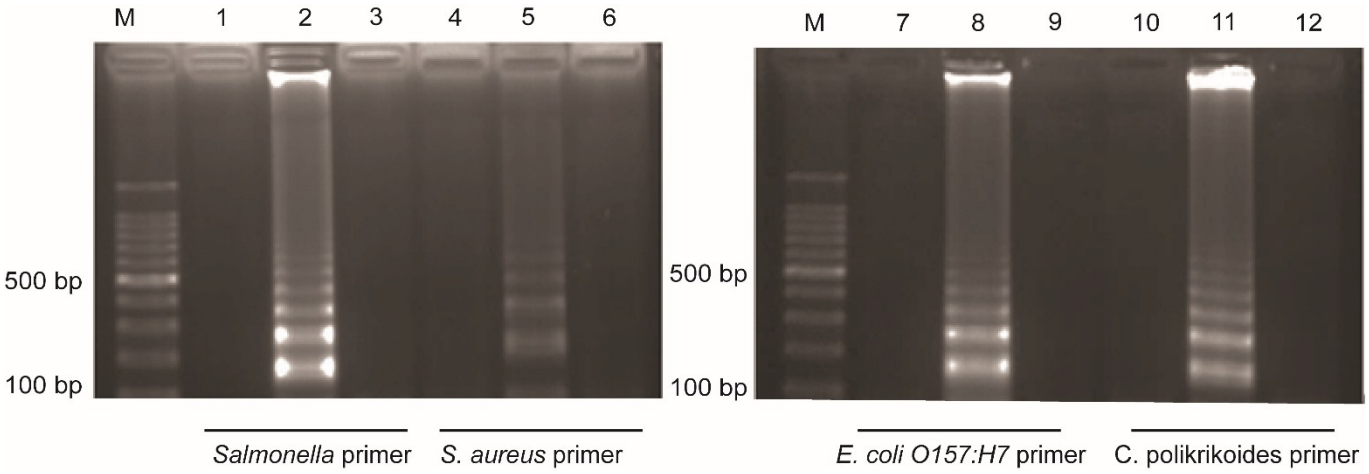

Fig. S3 Results demonstrating specificity of LAMP primers. Lane M shows 100 bp DNA ladder. Lane 1 shows negative control result when *Salmonella* primer was used. Lane 2 shows the result when *Salmonella* template and its primers were used. Lane 3 shows the result when LAMP reaction was carried out using *Salmonella* spp. primer and mixed templates of *S. aureus*, *E.coli* O157:H7, and *C. polykrikoides*. Lane 4 shows negative control result when *S. aureus* primer was used. Lane 5 shows the result when *S. aureus* template and its primers were used. Lane 6 shows the result when LAMP reaction was carried out using *S. aureus* primer and mixed templates of *Salmonella*, *E.coli* O157:H7, and *C. polykrikoides*. Lane 7 shows negative control result when *E.coli* O157:H7 primer was used. Lane 8 shows the result when *E.coli* O157:H7 template and its primers were used. Lane 9 shows the result when LAMP reaction was carried out using *E.coli* O157:H7 primer and mixed templates of *Salmonella*, *S. aureus*, and *C. polykrikoides*. Lane 10 shows negative control result when *C. polykrikoides* primer was used. Lane 11 shows the result when *C. polykrikoides* template and its primers were used. Lane 12 shows the result when LAMP reaction was carried out using *C. polykrikoides* primer and mixed templates of *Salmonella*, *S. aureus*, and *E. coli* O157:H7.

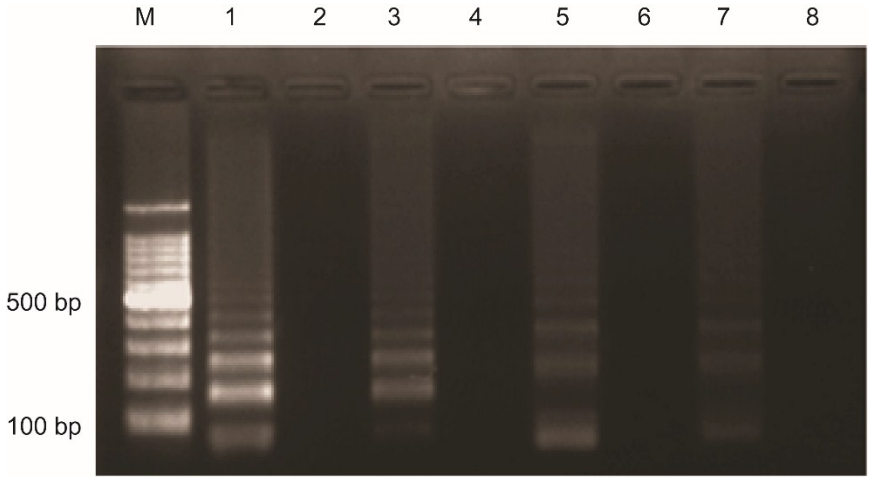

Fig. S4 Gel electrophoresis results of on-chip LAMP for four target genes. Lane M shows 100 bp DNA ladder. Lane 1 shows the result from the positive chamber for *Salmonella* spp.. Lane 2 shows the result from the negative chamber for *Salmonella*.spp.. Lane 3 shows the result from the positive chamber for *E.coli* O157:H7. Lane 4 shows the result from the negative chamber for *E.coli* O157:H7. Lane 5 shows the result from the positive chamber for *C. Polykrikoides.* Lane 6 shows the result from the negative chamber for *C. polykrikoides*. Lane 7 shows the result from the positive chamber for *S. aureus*. Lane 8 shows the result from the negative chamber for *S. aureus*.

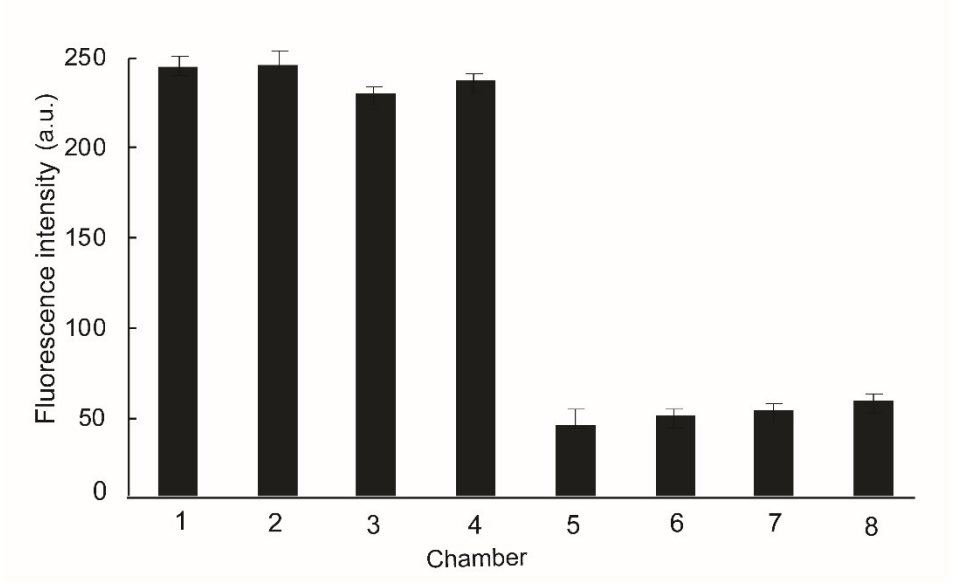

Fig. S5 The intensity of the fluorescence signals for four target genes after the on-chip LAMP. “1” shows the result of positive chamber for *Salmonella* spp.. *“*2” shows the result of positive chamber for *E.coli* O157:H7. “3” shows the result of positive chamber for *C. polykrikoides*. “4” shows the result of positive chamber for *S. aureus*. “5” shows the result of negative chamber for *Salmonella* spp.. “6” shows the result of negative chamber for *E.coli* O157:H7. “8” shows the result of negative chamber for *C. Polykrikoides*. “8” shows the result of negative chamber for *S. aureus*.

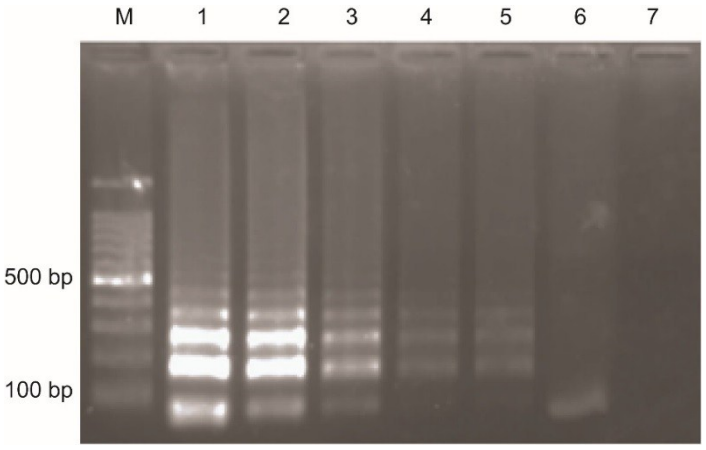

Fig. S6 Gel electrophoresis results of LAMP showing sensitivity results when performing serial 10-fold dilutions of the initial genomic DNA of *E.coli* O157:H7 from 1335 ng μL^{-1} to 0.0133 ng μL^{-1}. Lane M shows 100 bp DNA ladder. Lanes 1–6 show the results of positive reactions containing DNA with initial genomic concentrations of 1335, 133.5, 13.35, 1.335, 0.1335, and 0.0133 ng μL^{-1}. Lane 7 shows the result of negative control.

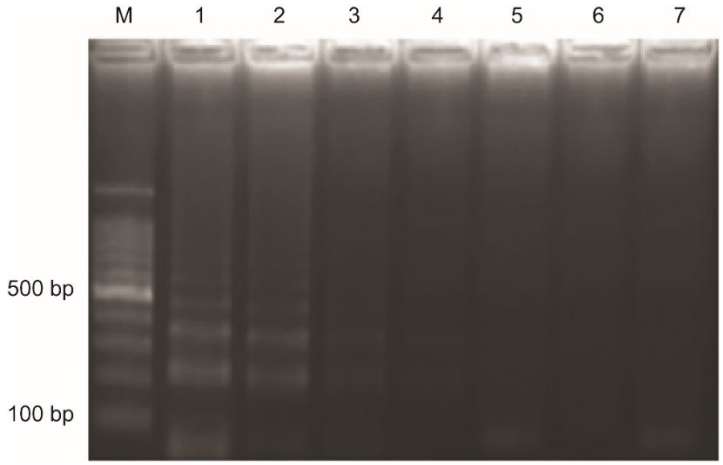

Fig. S7 Gel electrophoresis results of LAMP showing sensitivity results when performing serial 10-fold dilutions of the initial genomic DNA of *S.aureus* from 120 ng μL^{-1} to 0.0012 ng μL^{-1}. Lane M shows 100 bp DNA ladder. Lanes 1–5 show the results of positive reactions containing DNA with initial genomic concentrations of 120, 12, 1.2, 0.12, and 0.012 ng μL^{-1}. Lanes 6 and 7 show the results of negative controls.

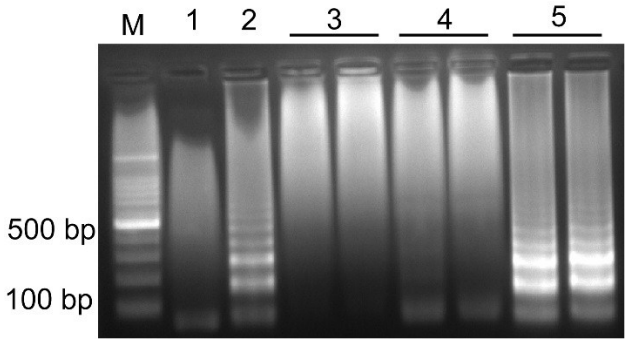

Fig. S8 Gel electrophoresis results of LAMP obtained when *Salmonella* spp. was extracted from real milk sample using polydopamine-coated paper. Lane M shows 100 bp DNA ladder. Lane 1 shows negative control result. Lane 2 shows DNA extracted using a commercialized kit. Lane 3 show the results when milk solution was treated only with polydopamine-coated paper. Lane 4 show the results when the milk solution was treated only with heat. Lane 5 show the results when milk solution was treated with heat followed by polydopamine-coated paper.

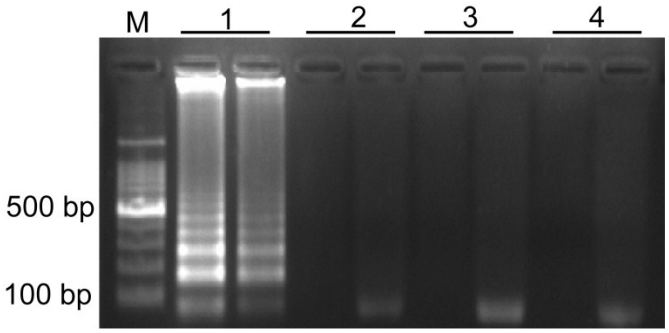

Fig. S9 Gel electrophoresis results of LAMP reaction realized using the microdevice when the concentration of *Salmonella* spp. was 7 x 10^4 CFU mL^{-1}. Lane M shows 100 bp DNA ladder. Lane 1 show the results of chambers with *Salmonella* spp. primer set. Lane 2 show the results of chambers with *E. coli* O157:H7 primer set. Lane 3 show the results of chambers with *C. polykrikoides* primer set. Lane 4 show the results of chambers with *S. aureus* primer set.

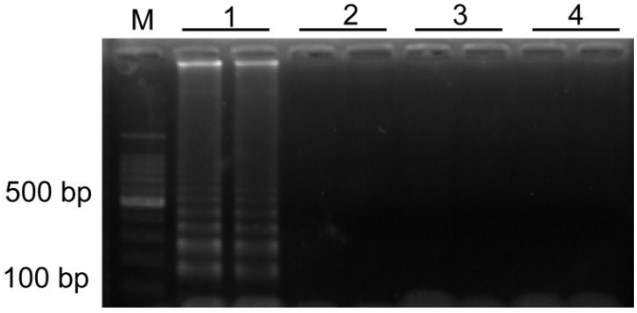

Fig. S10 Gel electrophoresis results of LAMP reaction realized using the microdevice when the concentration of *Salmonella* spp. was 1.7 x 10^3 CFU mL^{-1}. Lane M shows 100 bp DNA ladder. Lane 1 show the results of chambers with *Salmonella* spp. primer set. Lane 2 show the results of chambers with *E. coli* O157:H7 primer set. Lane 3 show the results of chambers with *C. polykrikoides* primer set. Lane 4 show the results of chambers with *S. aureus* primer set.

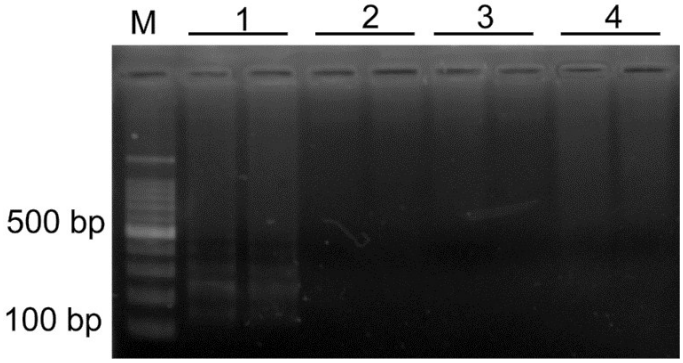

Fig. S11 Gel electrophoresis results of LAMP reaction realized using the microdevice when the concentration of *Salmonella* spp. was 1.7 x 10^2 CFU mL^{-1}. Lane M shows 100 bp DNA ladder. Lane 1 show the results of chambers with *Salmonella* spp. primer set. Lane 2 show the results of chambers with *E. coli* O157:H7 primer set. Lane 3 show the results of chambers with *C. polykrikoides* primer set. Lane 4 show the results of chambers with *S. aureus* primer set.

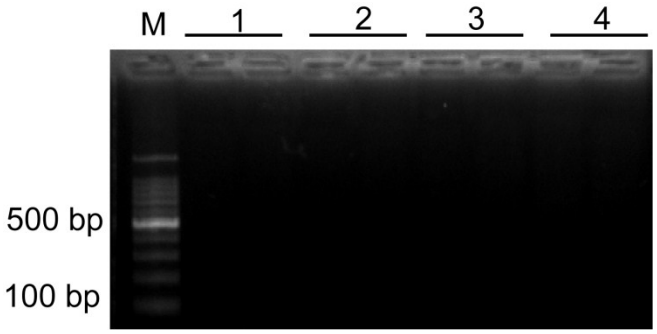

Fig. S12 Gel electrophoresis results of LAMP reaction realized using the microdevice when the concentration of *Salmonella* spp. was 1.7 x 10^1 CFU mL^{-1}. Lane M shows 100 bp DNA ladder. Lane 1 show the results of chambers with *Salmonella* spp. primer set. Lane 2 show the results of chambers with *E. coli* O157:H7 primer set. Lane 3 show the results of chambers with *C. polykrikoides* primer set. Lane 4 show the results of chambers with *S. aureus* primer set.

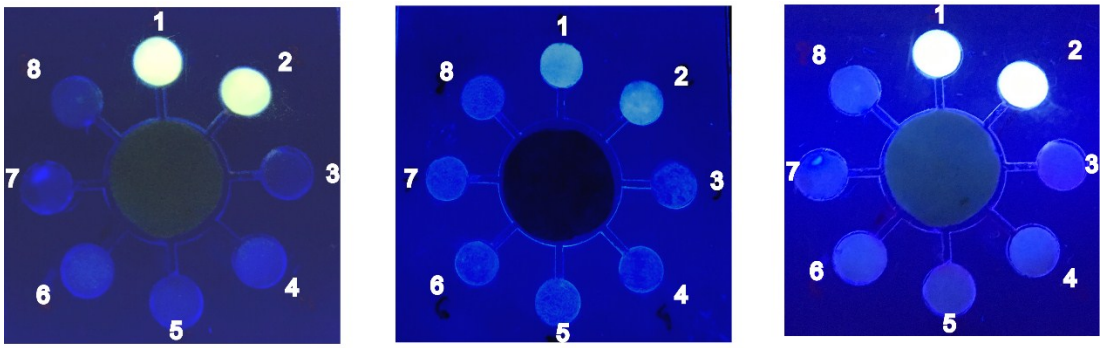

Fig. S13 Fluorescence images taken directly from the microdevice for detecting *Salmonella* spp. at the concentration of 1.7 x 10^4 CFU mL^{-1}. Each analysis was repeated three times. Chambers 1 and 2 contained *Salmonella* spp. primer set. Chambers 3 and 4 contained *E. coli* O157:H7 primer set. Chambers 5 and 6 contained *C. polykrikoides* primer set. Chambers 7 and 8 contained *S. aureus* primer set.