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

Electronic Supplementary Information

Paper-embedded thermoplastic microdevice integrating additive-enhanced allele-specific amplification and silver nanoparticle-based colorimetric detection for point-of-care testing

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Table S1 The primer sequences used for loop-mediated isothermal amplification of *Hbb*, *AR*, and *esp* genes. The SNP position in primers is highlighted in bold. The mismatch nucleotide is underlined.

Target gene	Primer name	Primer sequence (5'-3')
Hbb	F3	GCAGCCTAAGGGTGGGAA
	B3	GACACCATGGTGCATCTGA
	FIP	ACTGGGCATGTGGAGACAGAGA-TAGGCAGAGAGAGTCAGTGC
	BIP (HbS)	AGGGCCTCACCACCAACTTC**T**AGAGAAGTCTGCCGTTACTG
	BIP (HbA)	AGGGCCTCACCACCAACTTC**A**AGAGAAGTCTGCCGTTACTG
	LB	TCACCTTGCCCCACAGG
AR (rs6152)	F3	ACATCCTGAGCGAGGCC
	B3	GCCCATGGACACCGACA
	FIP AA	**T**GCCCTCGCTCTCCCGCTGATGCAACTCCTTCAGCAACA
	FIP GG	**C**GCCCTCGCTCTCCCGCTGATGCAACTCCTTCAGCAACA
	BIP	GGCTCCCACTTCCTCCAAGGGGCGTTGTCAGAAATGGTCG
esp	F3	CCAGAACACTTATGGAACAG
	B3	GTTGGGCTTTGTGACCTG
	FIP	CGTGTCTCCGCTCTCTTCTTT-TTATTTGCAAGATATTGATGGTG
	BIP	ATCGGGAAACCTGAATTAGAAGAAG-AACTCGTGGATGAATACTTTC
	LB	TGATGTTGACACAACAGTTAAGGG

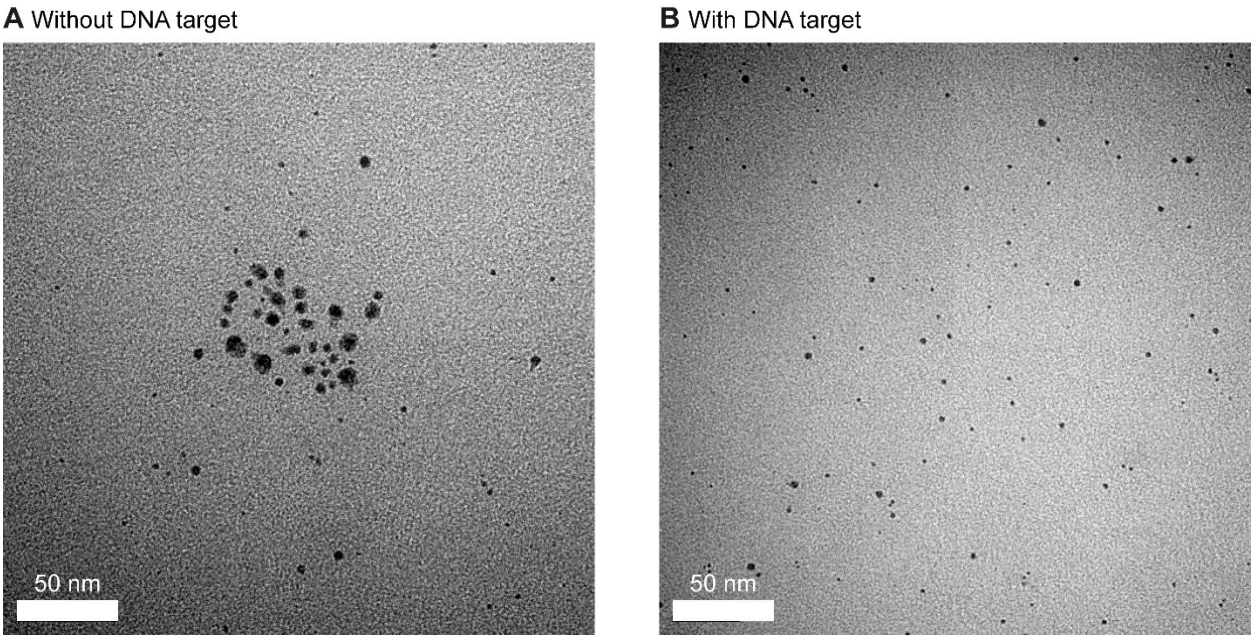

Fig. S1 TEM images of the hydrazine-induced silver nanoparticles (A) in the absence of the target DNA and (B) in the presence of the target DNA.

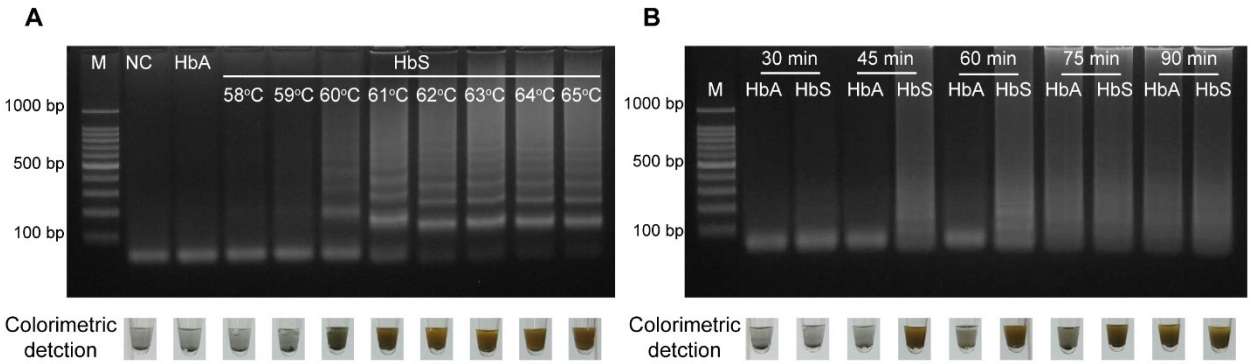

Fig. S2 Effect of different (A) reaction temperatures and (B) reaction times on AS-LAMP reaction for SNP detection. Experiments performed using a HbS primer set and 10^4 copies/μL of DNA template.

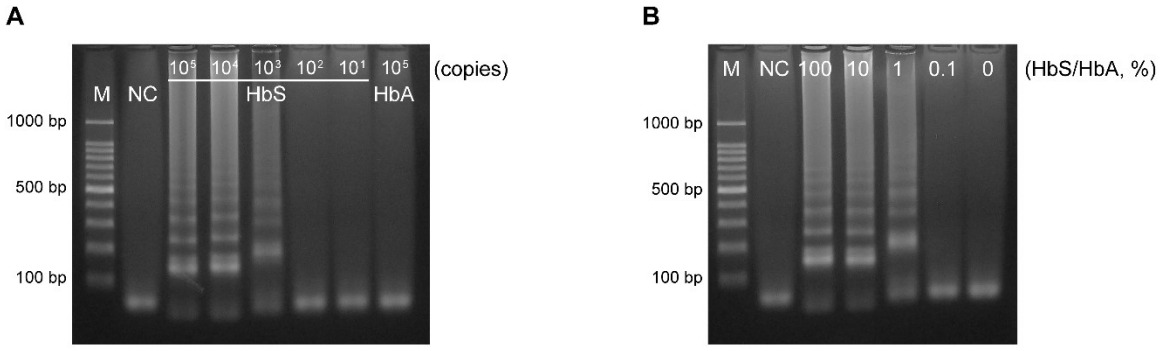

Fig. S3 Agarose gel electrophoresis result showing (A) the sensitivity of the LAMP assay for HbS detection and (B) detection of the HbS target in samples at varying concentrations. M: ladder. NC: negative control.

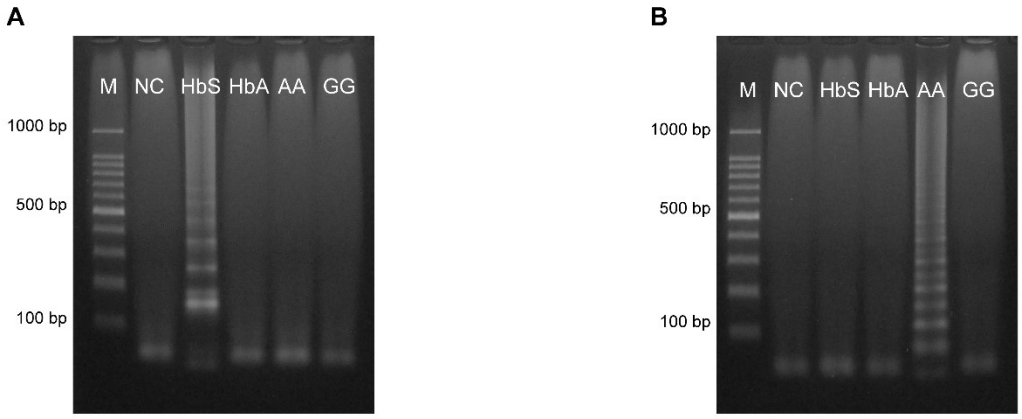

Fig. S4 (A) Agarose gel electrophoresis result showing the selectivity test for HbS detection. Experiments were performed using a HbS primer set and 10^4 copies/μL of DNA template. (B) Agarose gel electrophoresis result showing the selectivity test for rs6152AA detection. Experiments were performed using a rs6152AA primer set and 10^4 copies/μL of DNA template. M: ladder. NC: negative control.

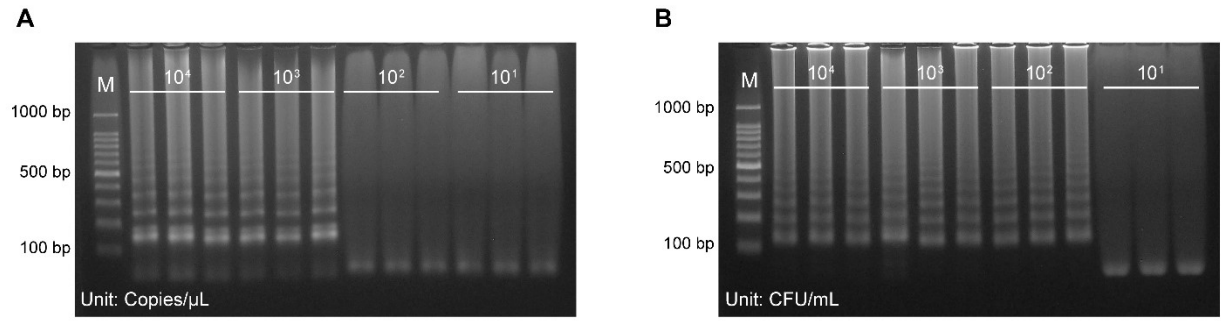

Fig. S5 Agarose gel electrophoresis result showing the sensitivity test of the microdevices for (A) human serum spiked with different concentrations of HbS template performed using a HbS primer set and (B) tap water spiked with different concentrations of *E. faecium* performed using a *esp* primer set. M: ladder. NC: negative control.

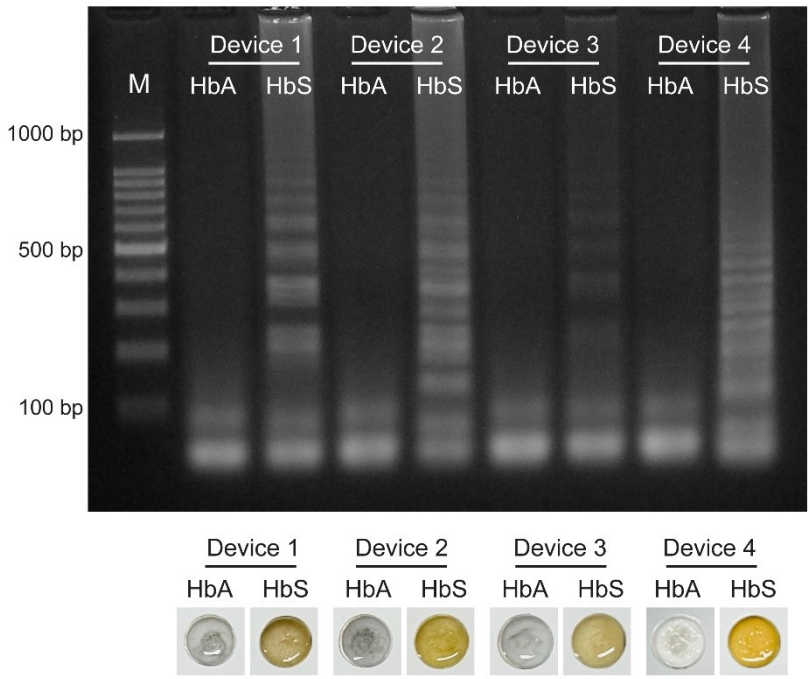

Fig. S6 Results of the reproducibility tests performed using the microdevice for HbS detection in spiked serum. Experiments were performed using a HbS primer set and 10^4 copies/μL of DNA template. The reproducibility was demonstrated by repeating the experiments four times.

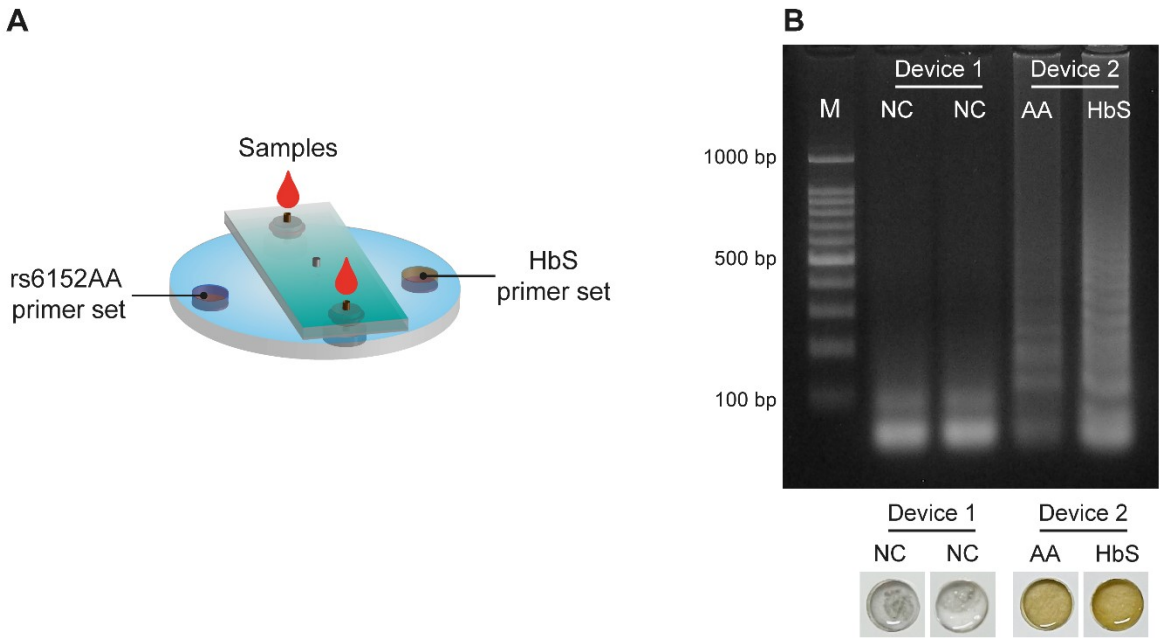

Fig. S7 (A) Experimental design and (B) results of the multiplexing tests performed using the microdevice for rs6152 and HbS detection in spiked serum. Experiments were performed using rs6152AA and HbS primer set and 10^4 copies/μL of DNA template. M: ladder. NC: negative control.

Table S2. Comparison showing several microdevices for point-of-care testing.

Device	Integration	Time	Target	Limit of detection	Sample test	Ref.
Integrated paper-based biosensor	• FTA card-based extraction • LAMP reactions • AuNPs-based detection	80 min	*Escherichia coli* *Streptococcus pneumonia*	10^1-10^3 CFU/mL	Spiked drinking water, milk, blood, and spinach	[1]
Paper-origami device	• Lysis buffer (95°C) • LAMP reactions • Calcein-based fluorescence	60 min	Bovine herpes virus-1 (BoHV-1) *Brucella* *Leptospira*	<1 pg/μL of DNA	Spiked semen	[2]
Origami paper microdevice	• PMA treatment and DNA purification • LAMP reaction • Methylene blue-based detection	120 min	*Escherichia coli* *Salmonella* spp.	10^3 CFU/mL	Not reported	[3]
Pop-up paper-based microdevice	• FTA card-based extraction • LAMP reactions • Colorimetric chemosensor	110 min	*Enterococcus faecium*	10^2 CFU/mL 10^3 CFU/g	Milk and sausage	[4]
Smartphone-assisted genotyping	• On-tube LAMP reaction • 3D-printed cassette-based detection (Cresol red)	90 min	*Salmonella* Pullorum rfbS gene	1500 copies/μL and 3.98 pg/μL	Poultry samples	[5]
Hybridisation chip	• On-tube LAMP reaction • Polycarbonate chips detection (HNB)	70 min	*GRIK4* gene (rs1954787)	10^2 gDNA copies	Buccal swabs	[6]
CD-like microfluidic chip	• LAMP reaction • Colorimetric and fluorescent detection (SYBR Green I and Neutral red)	60 min	CALR mutations	10^1 copies/μL	Parasite lysate	[7]
Lateral flow dipstick	• On-tube LAMP reaction • Lateral flow dipstick	70 min	N51I mutation (*Plasmodium falciparum*)	0.02 ng/reaction	Blood	[8]
Paper-embedded thermoplastic microdevice	• Silica-based preparation • LAMP reaction • AgNPs-based colorimetric detection	70 min	Sickle cell anemia Hair loss- associated SNP (rs6152) *Enterococcus faecium*	10^3 copies/μL 10^2 CFU/mL	Spiked serum and water	This work

***References*:**

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Video S1 The operation process of the paper-embedded thermoplastic microdevice.

Video S2 The time-lapse recording video of time-dependent colorimetric reaction for 15 min.