Supporting Information

CUT-LAMP: Contamination-Free Loop-Mediated Isothermal Amplification Based on the CRISPR/Cas9 Cleavage

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Experimental Section

Cas9 Expression and Purification. The pET28a/Cas9-Cys was a gift from Hyongbum Kim (Addgene plasmid #53261; http://n2t.net/addgene:53261; RRID: Addgene_53261). Plasmid pET28a/Cas9-Cys was transformed into *Escherichia coli* Rosetta 2 (DE3). *E. coli* was shocked (250 rpm/min) for 4 h in TB-kanamycin (30 μg/mL) medium at 37 °C. Once the OD_{600} reached 0.6–0.8, IPTG (1 mM) was supplied to induce Cas9 expression at 18 °C overnight. Bacterial cultures were then harvested and resuspend in lysis buffer (20 mM Tris-HCl, pH 7.5, 1 mM NaCl, 10% glycerin), followed by supersonic treatment on ice for 6 mins. Then, lysate was centrifuged at 4 °C and supernatants were collected. The protein was purified and eluted through Ni-NTA affinity chromatography. The purity of Cas9 was explained by 6% SDS-PAGE gel scan analysis.

Synthesis of sgRNAs. DNA template that contains sgRNA-encoding sequence and T7 promoter sequence was prepared by a fill-in PCR reaction. PCR reaction system was consisted of PrimeSTAR DNA Polymerase mixture, a forward customized primer containing the T7 promoter and the targeting sequence, and a reverse universal primer encoding the sgRNA scaffold. The two-step PCR was performed with the following cycling conditions: 33 cycles of 98 °C for 10 s and 68 °C for 10 s. PCR product was verified by gel electrophoresis and purified using SanPrep Column PCR Product Purification Kit. This purified product was transcribed (T7 polymerase, NTPs and RNAase inhibitor) at 37 °C for 4 h in transcription buffer (40 mM Tris-HCl, 6 mM $MgCl_2$, 1 mM DTT, 2 mM spermidine) to create sgRNA. After transcription, sgRNAs were purified by RNA Purification Kit and quantified by the NanoDrop 2000.

Synthesis of crRNAs. DNA template which contains crRNA-encoding sequence and T7 promoter sequence was synthesized by annealing two complementary oligonucleotides (10 μM). DNA template was heated at 95 °C for 5 min and then cooled down to 25 °C with 2 °C/min in 1× hybridization buffer (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM $MgCl_2$).

Annealed product was purified and served as template to produce a crRNA with T7 polymerase, NTPs and RNAase inhibitor at 37 °C for 4 h in transcription buffer (40 mM Tris-HCl, 6 mM $MgCl_2$, 1 mM DTT, 2 mM spermidine). Transcribed RNAs were purified by RNA purification kit and subsequently stored at –80 °C. The concentration of the obtained crRNA was measured and quantified by Nanodrop 2000.

Bacterial Culture and DNA Extraction. *Salmonella enterica* and *Methicillin-resistant staphylococcus aureus* were kept in our Laboratory collection. Cultures were grown in Luria Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract and 5 g/L NaCl) at 37 °C for approximately 12–18 h. Genomic DNA was extracted by Bacteria Genomic DNA Extraction Kit, and quantitatively determined by 1% agarose gel electrophoresis. Concentration of genomic DNAs was measured by NanoDrop 2000, and genomic DNAs was then stored at –20 °C. The genomic DNAs from *N. meningitidis* were obtained from Hubei provincial centre for Disease Control and Prevention. The Genomic DNAs of *Zika* was obtained from Sun Yat-Sen University.

In vitro CRISPR/Cas9 Cleavage and Characterization. Cas9 and sgRNA were pre-incubated in cleavage buffer (20 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA) for 10 min at 25 °C to formed a binary complex. 1 μL of target DNA was then added to Cas9/sgRNA mixture in 10 μL system. The mixture was incubated at 25 °C or 37 °C for 30 min. The assay was stopped by heating at 65 °C for 10 min. CRISPR/Cas9 cleavage products were evaluated with fragment analysis by 10% PAGE at 140 V for 1 h followed by silver staining.

Supporting Figures

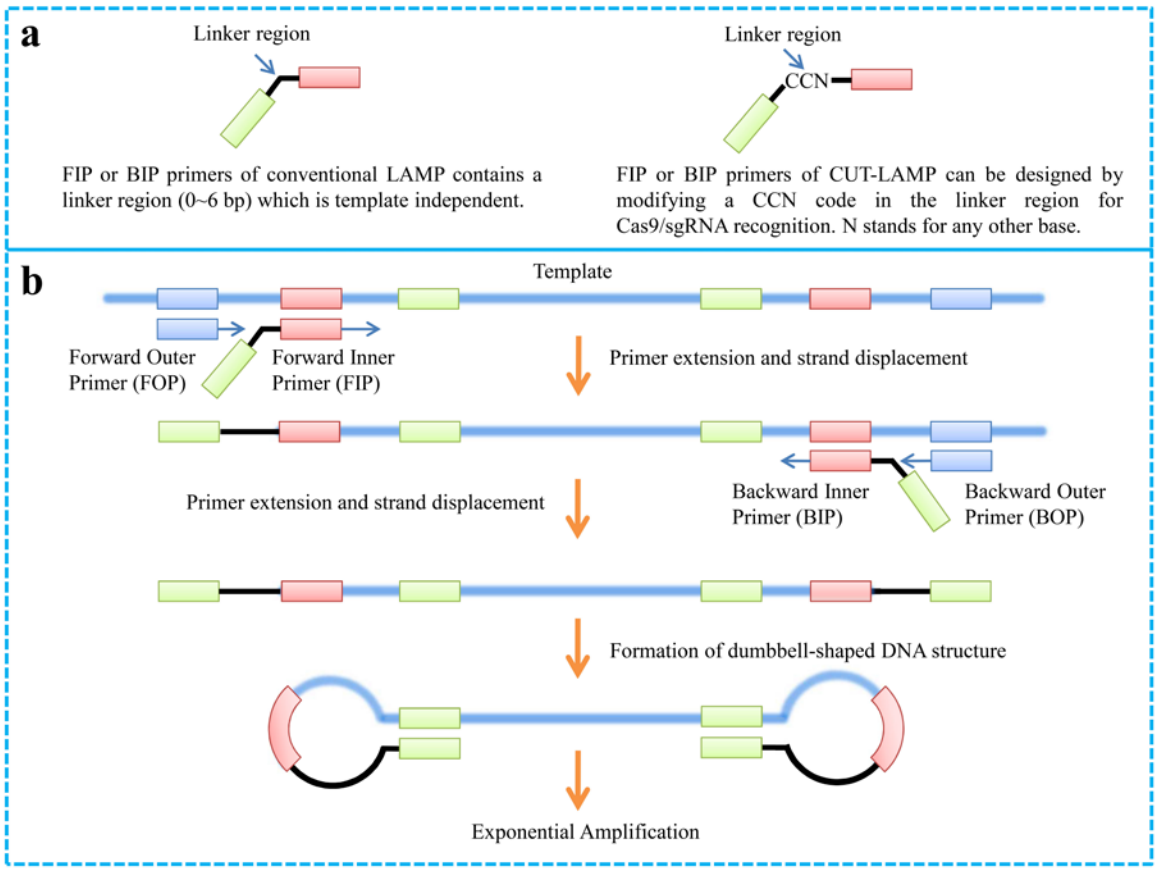

Figure S1. (a) Structure comparison of FIP or BIP of the CON-LAMP and the CUT-LAMP reaction. (b) Process of generating dumbbell-shaped DNA structure in the CUT-LAMP reaction. The dumbbell-shaped DNA structure is used as a template for exponential amplification. Since the dumbbell-shaped DNA structure includes a CCN code, so the LAMP products will contain a PAM site-NGG code near the sgRNA target region.

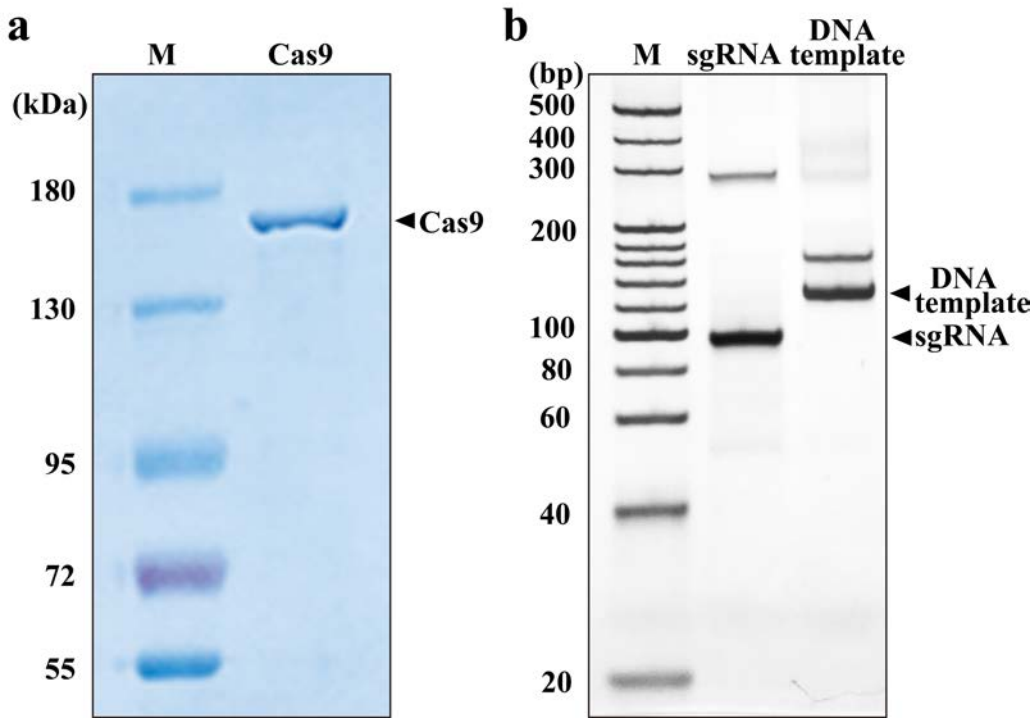

Figure S2. 6% SDS-PAGE electrophoresis characterization of the home-made Cas9 protein and 10% native PAGE electrophoresis characterization of sgRNA. Two bands are appeared in sgRNA electrophoresis. They may represent the native single-stranded RNA and a secondary structure formed by sgRNA.

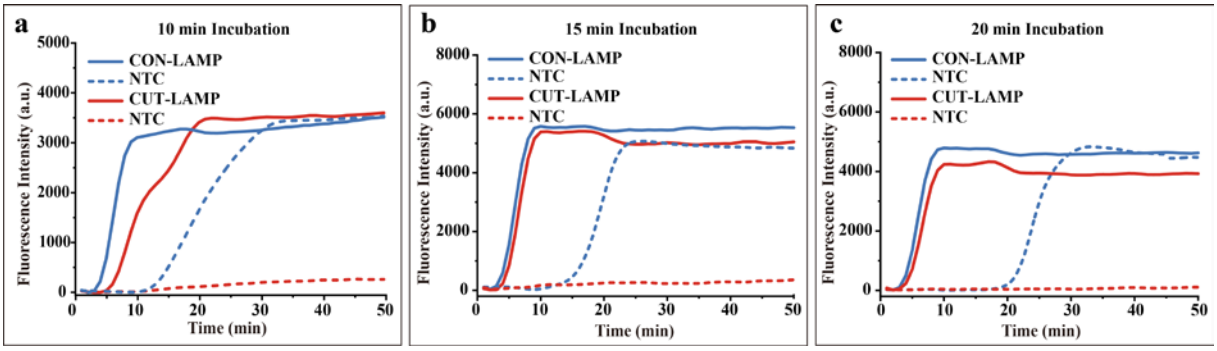

Figure S3. Test of cleavage ability of the CUT-LAMP reaction with various pre-incubation time. Compared to the CON-LAMP, the CUT-LAMP contains a pre-incubation step before amplification. In the presence of 10 pg contaminants, different pre-incubation time of 5 min (Figure 4c), (a) 10 min, (b) 15 min, and (c) 20 min were evaluated, and the results indicate that the false-positive amplification caused by contaminants disappeared with 5 min pre-incubation. NTC stands for no template control.

Figure S4. Sequences of the conventional FIPs or BIPs and PAM site-modified FIPs or BIPs, and the corresponding sgRNA sequences for targeting the sequence adjacent to the PAM site

to detect five kinds of *N. meningitidis* serotypes by using the CUT-LAMP methods. The PAM sites are highlighted in yellow and the red arrows indicate the cutting position of Cas9/sgRNA.

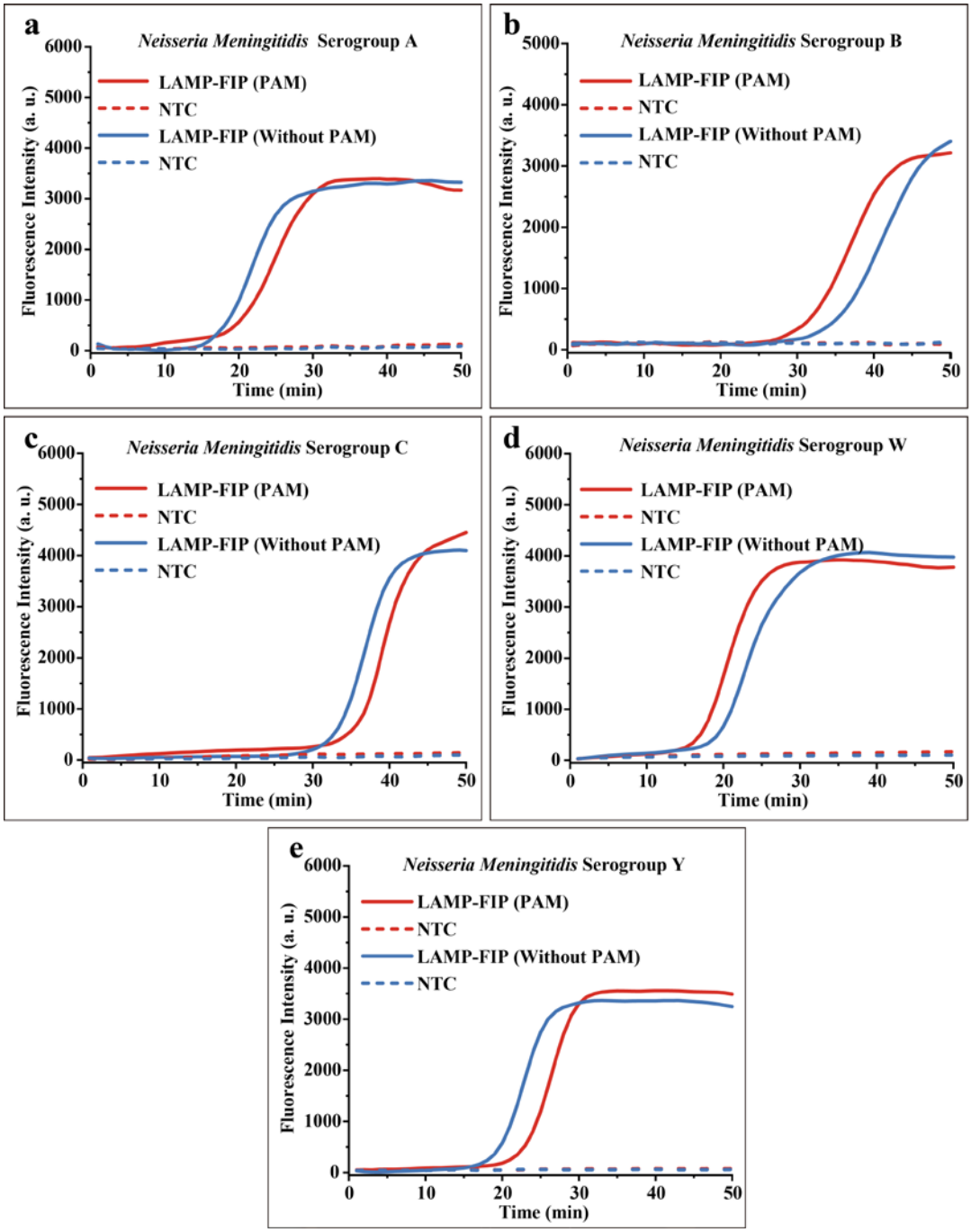

Figure S5. Test of the CON-LAMP amplification efficiency and specificity by using conventional FIPs or BIPs without PAM site and modified FIPs or BIPs with PAM site. (a) The LAMP amplification of *sacB* gene of *N. meningitidis* serogroup A by using conventional primer and modified primer. (b) The LAMP amplification of *siaD* gene of *N. meningitidis* serogroup B by using conventional primer and modified primer. (c) The LAMP amplification of *siaD* gene of *N. meningitidis* serogroup C by using conventional primer and modified primer. (d) The LAMP amplification of *synG* gene of *N. meningitidis* serogroup W by using

conventional primer and modified primer. (e). The LAMP reaction of *synF* gene of *N. meningitidis* serogroup Y by using conventional primer and modified primer. NTC stands for no template control.

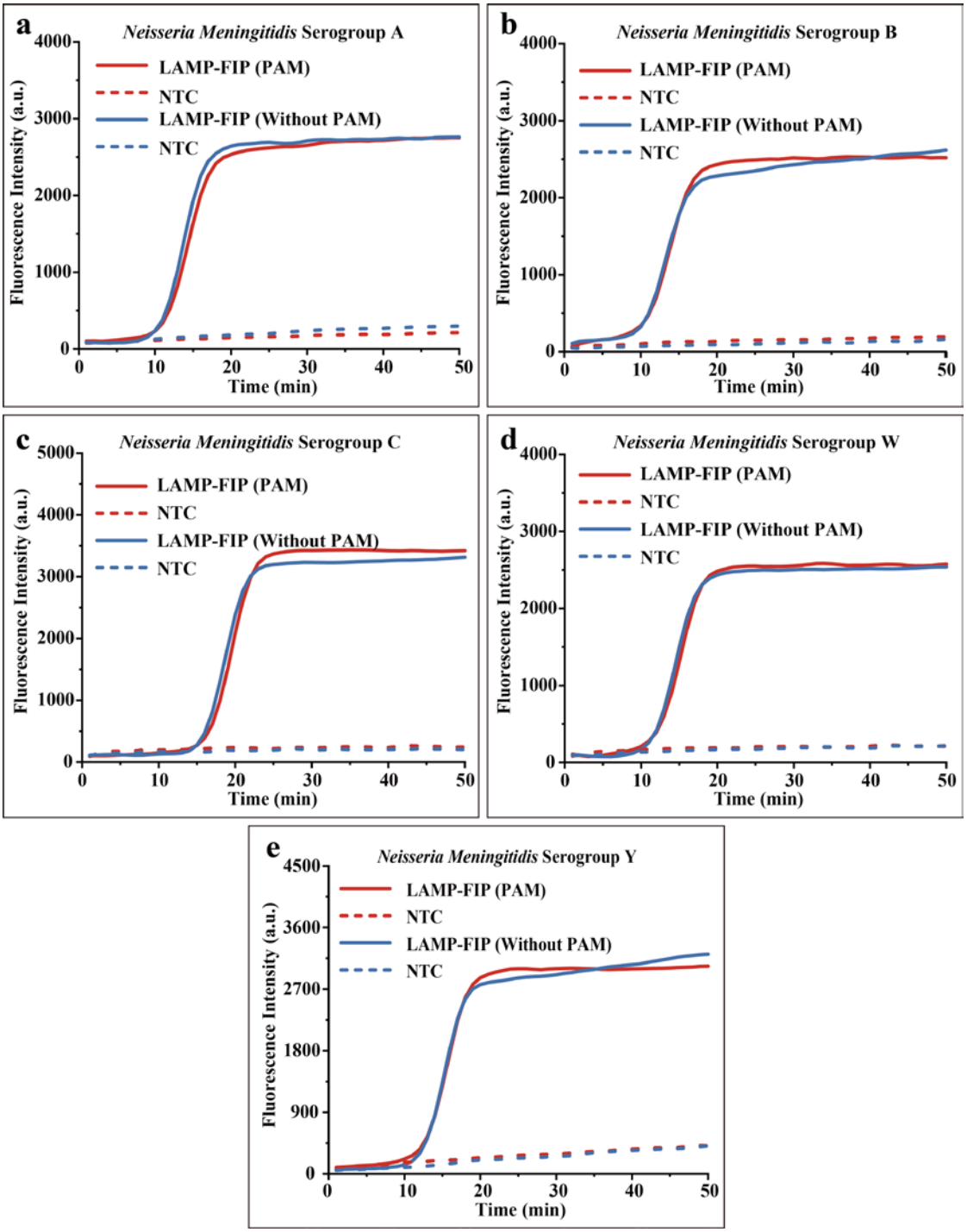

Figure S6. Test of the CON-LAMP amplification efficiency and specificity by using conventional FIPs or BIPs without PAM site and modified FIPs or BIPs with PAM site. (a) to (e) represent the LAMP amplification of *ctrA* gene for all five kinds of *N. meningitidis* serogroups (A, B, C, W, and Y) by using conventional primer without PAM site and modified primer with PAM site. NTC stands for no template control.

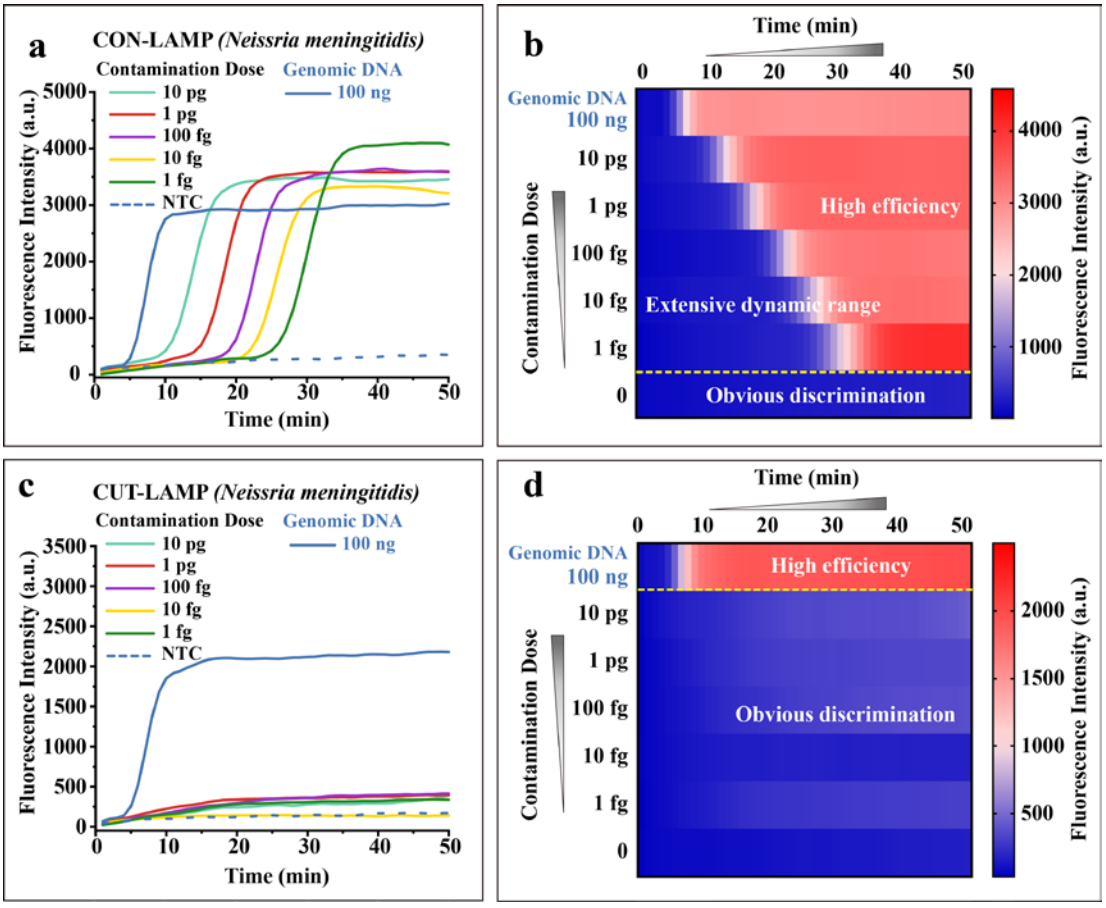

Figure S7. Contamination resistance of the LAMP reactions. (a) Contamination resistance of the CON-LAMP reaction. Genomic DNA as a positive control. (b) The dynamic range of the quantification of contamination and genomic DNA. (c) Contamination resistance of the CON-LAMP reaction. Genomic DNA as a positive control. (d) The dynamic range of the quantification of contamination and genomic DNA. All experiments are based on the amplification of the *ctrA* gene from *N. meningitidis*. NTC stands for no template control.

Supporting Tables

Table S1. Oligonucleotides used for the detection of *invA* gene of *Salmonella*.[a]

Nucleic Acids ID	Sequences (5'–3')
Primer set for CON-LAMP (*invA* gene of *Salmonella*)	
Sal-FIP	GACGACTGGTACTGATCGATAGTTTTTCAACGTTTCCTGCGG
Sal-BIP	CCGGTGAAATTATCGCCACACAAAACCCACCGCCAGG
Sal-FOP	GGCGATATTGGTGTTTATGGGG
Sal-BOP	AACGATAAACTGGACCACGG
Sal-LF	GACGAAAGAGCGTGGTAATTAAC
Sal-LB	GGGCAATTCGTTATTGGCGATAG
Primer set for CUT-LAMP (*invA* gene of *Salmonella*). Sal-BIP, Sal-FOP, Sal-FOP, Sal-BOP, Sal-LF, and Sal-LB are the same as CON-LAMP	
Sal-FIP	GACGACTGGTACTGATCGATCCAGTTTTTCAACGTTTCCTGCGG
Primer set for synthesizing sgRNA for CUT-LAMP (*invA* gene of *Salmonella*)	
sgRNA-F (Sal-FIP)	GAAATTAATACGACTCACTATAGGCGCAGGAAACGTTGAAAAACG TTTTAGAGCTAGAAATAGC
sgRNA-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAG CCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

[a]The nucleotide for generating PAM site is highlighted in yellow. sgRNA-R represents a common sgRNA reverse primer for generating sgRNA backbone.

Table S2. Oligonucleotides used for the CRIPSR/Cas9 cleavage of *EGFP*.[b]

Nucleic Acids ID	Sequences (5'–3')
Primer set for amplifying *EGFP* gene	
EGFP-F	ATGGTGAGCAAGGGCGAG
EGFP-R	TTACTTGTACAGCTCGTCCATGC
Primer set for synthesizing sgRNA for targeting *EGFP*	
sgRNA-F (*EGFP*)	GAAATTAATACGACTCACTATAGGGAAGGAGGACGGCAACATCC TGTTTTAGAGCTAGAAATAGC
sgRNA-R (*EGFP*)	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTA GCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

[b]sgRNA-R represents a common sgRNA reverse primer for generating sgRNA backbone.

Table S3. Oligonucleotides used for the detection of serogroups A, B, C, W, and Y of *N. meningitidis*.[c]

Nucleic Acids ID	Sequences (5'–3')
Primer set for CON-LAMP (*sacB* gene of *N. meningitidis* serogroup A)	
Nm-A-FIP	GAACTCTAATCTGAACCAAAATTGAGAGTTGACATGAAACTCAGCAC AG
Nm-A-BIP	CCTACAGCTAACAGATATTCTAGAAAACGAATAGTTTCGTATGCCTTC
Nm-A-FOP	CGTAAATGAAATTTGGACAG
Nm-A-BOP	TTATGATCTTCTTCATAGGGTA
Nm-A-LF	ATAGATGAACTTAAAGTTCT
Nm-A-LB	GGAAGCACTCTATTAAAAATAATC
Primer set for CUT-LAMP (*sacB* gene of *N. meningitidis* serogroup A) Nm-A-BIP, Nm-A-FOP, Nm-A-BOP, Nm-A-LF, and Nm-A-LB are the same as CON-LAMP	
Nm-A-FIP (PAM)	GAACTCTAATCTGAACCAAAATTGAGAGTTCCGACATGAAACTCAGC ACAG
Primer set for synthesizing sgRNA for CUT-LAMP (*sacB* gene of *N. meningitidis* serogroup A)	
sgRNA-F (Nm-A-FIP)	GAAATTAATACGACTCACTATAGGCTGTGCTGAGTTTCATGTGTTTTA GAGCTAGAAATAGC
sgRNA-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCC TTATTTTAACTTGCTATTTCTAGCTCTAAAAC
Primer set for CON-LAMP (*siaD* gene of *N. meningitidis* serogroup B)	
Nm-B-FIP	GGCCAGGCCTATAATTCCTTCCTTTTCTAATTGAGCCCCT
Nm-B-BIP	CACCCTCAACCCAATGTCTTTCTCATTTCAGTGTTTTCCACC
Nm-B-FOP	AAACCCTCGGCTGGTAG
Nm-B-BOP	CTTAATAATCTCTAAGTGTTCTTG
Nm-B-LB	GGAGAGTTAATTATTAACTTAATTCAA
Primer set for CUT-LAMP (*siaD* gene of *N. meningitidis* serogroup B) Nm-B-FIP, Nm-B-FOP, Nm-B-BOP, and Nm-B-LB are the same as CON-LAMP	
Nm-B-BIP (PAM)	CACCCTCAACCCAATGTCCTTTCTCATTTCAGTGTTTTCCACC
Primer set for synthesizing sgRNA for CUT-LAMP (*siaD* gene of *N. meningitidis* serogroup B)	
sgRNA-F (Nm-B-BIP)	GAAATTAATACGACTCACTATAGGAAAACACTGAAATGAGAAGTTTT AGAGCTAGAAATAGC
sgRNA-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCC TTATTTTAACTTGCTATTTCTAGCTCTAAAAC
Primer set for CON-LAMP (*siaD* gene of *N. meningitidis* serogroup C)	
Nm-C-FIP	CCTACTACCCAATGTCTGTCAATTTTGTTGGGCTGTATGGTG
Nm-C-BIP	AGTCGATGTCAGTCCAATAATTCCTGTAGTGATTAATGAACCCCCT
Nm-C-FOP	TGCTCTTCAATTAAAGCGG
Nm-C-BOP	GGTAACAATTAATCCCCGTCT
Nm-C-LF	GGGCAAATCGTGATTG
Nm-C-LB	GGGTTGTTAAATAAATTAGTGG
Primer set for CUT-LAMP (*siaD* gene of *N. meningitidis* serogroup C) Nm-C-BIP, Nm-C-FOP, Nm-C-BOP, Nm-C-LF, and Nm-C-LB are the same as CON-LAMP	
Nm-C-FIP (PAM)	CCTACTACCCAATGTCTGTCAATTCCATTGTTGGGCTGTATGGTG
Primer set for synthesizing sgRNA for CUT-LAMP (*siaD* gene of *N. meningitidis* serogroup C)	
sgRNA-F (Nm-C-FIP)	GAAATTAATACGACTCACTATAGGCACCATACAGCCCAACAAGTTTTA GAGCTAGAAATAGC
sgRNA-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCC TTATTTTAACTTGCTATTTCTAGCTCTAAAAC
Primer set for CON-LAMP (*synG* gene of *N. meningitidis* serogroup W)	
Nm-W-FIP	CTCACTTTCTGATGTCATGATCAGGTTATTCAAAGGTGAATCTTCCGA

Nm-W-BIP	GGAAGGCATGGTGTATGATATTCCGTTACTGTAATCATTCGCTCC
Nm-W-FOP	GACAATAAGTTACAAAACCGTATC
Nm-W-BOP	TCACCAGTTTTAAAAACACAACC
Nm-W-LB	TCTGTATTTTCATAAATTTCCTGC
Primer set for CUT-LAMP (*synG* gene of *N. meningitidis* serogroup W) Nm-W-BIP, Nm-W-FOP, Nm-W-BOP, and Nm-W-LB are the same as CON-LAMP	
Nm-W-FIP (PAM)	CTCACTTTCTGATGTCATGATCAGGCCTTATTCAAAGGTGAATCTTCCGA
Primer set for synthesizing sgRNA for CUT-LAMP (*synG* gene of *N. meningitidis* serogroup W)	
sgRNA-F (Nm-W-FIP)	GAAATTAATACGACTCACTATAGGAAGATTCACCTTTGAATAGTTTTAGAGCTAGAAATAGC
sgRNA-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
Primer set for CON-LAMP (*synF* gene of *N. meningitidis* serogroup Y)	
Nm-Y-BIP	CACTGCCCACTATAAGCATGTTTTGAGTTGAAGAGGATGAGTGA
Nm-Y-FIP	CGGGTTTGAAGAATTGTTGATGGTGACATTCCAGAAAATGTTAG
Nm-Y-FOP	TGTCAAAACCTCCAGC
Nm-Y-BOP	CGCTAAACGATACATTTCCA
Nm-Y-LF	GAATAAAAAGGAATATTTCGGC
Nm-Y-LB	TCTTTATTATCTGAAGAAGATAGC
Primer set for CUT-LAMP (*synF* gene of *N. meningitidis* serogroup Y) Nm-Y-FIP, Nm-Y-FOP, Nm-Y-BOP, Nm-Y-LF, and Nm-Y-LB are the same as CON-LAMP	
Nm-Y-BIP (PAM)	CACTGCCCACTATAAGCATGTTTTGCCAGTTGAAGAGGATGAGTGA
Primer set for synthesizing sgRNA for CUT-LAMP (*synF* gene of *N. meningitidis* serogroup Y)	
sgRNA-F (Nm-Y-BIP)	GAAATTAATACGACTCACTATAGGTCACTCATCCTCTTCAACGTTTTAGAGCTAGAAATAGC
sgRNA-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

[c]The nucleotide for generating PAM sites are highlighted in yellow. sgRNA-R represents a common sgRNA reverse primer for generating sgRNA backbone.

Table S4. Oligonucleotides used for the detection of *ctrA* gene of *N. meningitidis*.[d]

Nucleic Acids ID	Sequences (5'–3')
Primer set for CON-LAMP (*ctrA* gene of *N. meningitidis*)	
Nm-*ctrA*-FIP	CAAACACACCACGCGCATCAGATCTGAAGCCATTGGCCGTA
Nm-*ctrA*-BIP	TGTTCCGCTATACGCCATTGGTACTGCCATAACCTTGAGCAA
Nm-*ctrA*-FOP	AGCYAGAGGCTTATCGCTT
Nm-*ctrA*-BOP	ATACCGTTGGAATCTCTGCC
Nm-*ctrA*-LF	CGATCTTGCAAACCGCCC
Nm-*ctrA*-LB	GCAGAACGTCAGGATAAATGGA
Primer set for CUT-LAMP (*ctrA* gene of *N. meningitidis*) **Nm-*ctrA*-BIP, Nm-*ctrA*-FOP, Nm-*ctrA*-FOP, Nm-*ctrA*-BOP, Nm-*ctrA*-LF, and Nm-*ctrA*-LB are the same as CON-LAMP**	
Nm-*ctrA*-FIP (PAM)	CAAACACACCACGCGCATCAGACCTCTGAAGCCATTGGCCGTA
Primer set for synthesizing sgRNA for CUT-LAMP (*ctrA* gene of *N. meningitidis*)	
sgRNA-F-*ctrA*-FIP (PAM)	GAAATTAATACGACTCACTATAGGGTACGGCCAATGGCTTCAGGTTTT AGAGCTAGAAATAGC
sgRNA-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCC TTATTTTAACTTGCTATTTCTAGCTCTAAAAC

[d]The nucleotide for generating PAM site is highlighted in yellow. sgRNA-R represents a common sgRNA reverse primer for generating sgRNA backbone.

Table S5. Oligonucleotides used for the detection of *Zika* virus.[e]

Nucleic Acids ID	Sequences (5'–3')
Primer set for CON-LAMP (*Zika* virus)	
Zika-MR766-FIP	GGCGGCATTTCAAATGGCCAGCTCGCTGGAGCTCTAGAGG
Zika-MR766-BIP	TATTCCTTGTGCACTGCGGCATGACTGTTCCATGCAGTGTT
Zika-MR766-FOP	GAAGGAGCCGTTCACACG
Zika-MR766-BOP	CCTGCATACTGCACCTCC
Zika-MR766-LF	CCTTTGCACCATCCATCTCAG
Zika-MR766-LB	TTCACATTCACCAAGGTCCCA
Primer set for CUT-LAMP (*Zika* virus) ***Zika*-MR766-FIP, *Zika*-MR766-FOP, *Zika*-MR766-BOP, *Zika*-MR766-LF, and *Zika*-MR766-LB are the same as CON-LAMP**	
Zika-BIP-PAM	TATTCCTTGTGCACTGCGGCACCTGACTGTTCCATGCAGTGTT
Primer set for synthesizing sgRNA for CUT-LAMP (*Zika* virus)	
sgRNA-F (*Zika* BIP)	GAAATTAATACGACTCACTATAGGGAACACTGCATGGAACAGTCG TTTTAGAGCTAGAAATAGC
sgRNA-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTA GCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

[e]The nucleotide for generating PAM site is highlighted in yellow. sgRNA-R represents a common sgRNA reverse primer for generating sgRNA backbone.

Table S6. Oligonucleotides used for the CRISPR/Cas12a detection system.

Nucleic Acids ID	Sequences (5'–3')
Template for synthesizing crRNA for targeting *invA* gene of *Salmonella*	
Salmonella-invA-crRNA-1	GCCCTTAATACGACTCACTATAGGGAATTTCTACTGTTGTAGATCTG CGGTACTGTTAATTACCACGC
Salmonella-invA-crRNA-2	GCGTGGTAATTAACAGTACCGCAGATCTACAACAGTAGAAATTCCCT ATAGTGAGTCGTATTAAGGGC
Fluorescent probe used in CRISPR/Cas12a system	
Probe	FAM-TTTGCTGCTGAGGAA-BHQ
Primer set for CON-LAMP (*ermC* gene of *MRSA*)	
ermC FIP	CCAGACGTTTGTTTACGGTATAACTCTCATAGACGAAGAAAGTGGAG
ermC BIP	TGGCGGAAAAAAACTTAAAATCGTTGTATTGTTACTTAAGTGGACAT
ermC FOP	GTCTATAAAGGCTCTCAGAGG
ermC BOP	GCTATTTCTCTTGTTGTAGCTATC
Gene sequence of *ermC*	
ermC (2069~2365), GenBank accession NO. V01278	AATCGTCAATTCCTGCATGTTTTAAGGAATTGTTAAATTGATTTTTTG TAAATATTTTCTTGTATTCTTTGTTAACCCATTTCATAACGAAATAAT TATACTTTTGTTTATCTTTGTGTGATATTCTTGATTTTTTTCTACTTAA TCTGATAAGTGAGCTATTCACTTTAGGTTTAGGATGAAAATATTCTC TTGGAACCATACTTAATATAGAAATATCAACTTCTGCCATTAAAAGT AATGCCAATGAGCGTTTTGTATTTAATAATCTTTTAGCAAACCCGTA TTCCACGATTA