Supporting Information

Homogeneous and Universal Transduction of Various Nucleic Acids to Off-Shelf Device Based on Programmable Toehold Switch Sensing

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Methods

Chemicals and materials.

The Bayer Contour Next Blood Glucose Test Strips and Bayer Contour Next Blood Glucose Monitoring System used for the tests were bought from Amazon.com. All the reagents used to build the sensor plasmid were purchased from Takara (Dalian, China). NASBA Kits were obtained from Life Sciences (Petersburg, FL, USA). DNase/RNase-Free Water and Bacteria DNA Kit were from Tiangen (Beijing, China). Bst 2.0 DNA polymerase, 10 × Isothermal buffer (10 × Iso), Deoxynucleotide (dNTP) Solution Mix, RNase Inhibitor, Murine, HiScribe™ T7 Quick High Yield, RNA Synthesis Kit and PURExpress® In Vitro Protein Synthesis Kit were purchased from New England Biolabs (Ipswich, MA, U.S.A.). The oligonucleotides used in this paper were synthesized by Sangon Biotech (Shanghai, China), and the sequences are presented in Table S1. All oligonucleotides were

stored in H_2O at -20 °C. The concentrations of the DNA suspensions were determined by measuring the absorbance at 260 nm using the DeNovix DS-11+ FX spectrophotometer (DeNovix Inc., Wilmington, DE, USA). Unless otherwise indicated, all regents were of analytical grade. Buffers used here were: 1 × Iso Buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 4 mM $MgSO_4$, 0.1% Tween 20, pH 8.8).

Construction of Sensing Plasmid.

Toehold switch constructs were amplified from DNA templates [1] (Addgene, plasmid number: 75006) and ligated to the *invertase* reporter gene [2] via PCR with the sites of restriction enzyme *Bgl*II and *Xho*I on the 5' and 3' ends. The amplicons were inserted into pET-21a vector backbone which was digested prior to assembly using *Bgl*II and *Xho*I.

Standard cell-free reactions and glucose generation.

Cell-free reactions [3] in the presence or absence of 24% analystes consisted of 40% Solution A and 30% Solution B, 0.5% RNase Inhibitor, and 0.5 nM expression plasmid. The mixtures were incubated at 37°C for 2 hr, stopped by placing the tubes on ice. After cell-free reactions, 1M sucrose was mixed with the products in a 1:2 volume ratio, and incubated at different temperatures to generate glucose.

NASBA amplifications.

Zika virus is chosen as the model target because it is a flavivirus transmitted by mosquitoes and can cause severe fetal microcephaly and Guillain–Barre ´ syndrome.[4] Zika template RNAs used for NASBA reaction were synthetized from trigger plasmid [1] (Addgene, plasmid number: 75008) using RNA Synthesis Kit and diluted to corresponding concentrations. NASBA reaction mixtures containing 3.35 µL Reaction buffer, 1.65 µL Nucleotide Mix, 0.05 µL RNase inhibitor, 0.2 µL primers mixtures including 12.5 µM of each primer, 0.25 µL nuclease free water, and 2 µL different concentrations of template RNAs were assembled on ice and heated at 65°C for 2 min, followed by a 10 min incubation at 41°C. Then, 2.5 µL Enzyme Mix was added to initiate the NASBA reaction (for a final volume of 10 µL), and the mixtures were incubated at 41°C for 2 hr.

Preparation of analytes based on four-way transduction.

Different concentrations of the T2 or LAMP amplicons were hybridized with 167 nM BM and 208 nM TH in 1 × Iso Buffer via a standard annealing process, in which mixtures were heated at 95°C for 5 min, cooling down to 37°C at a rate of 0.1°C/s.

LAMP Reactions.

Enterobacter sakazakii is chosen as the model target because it is regarded as an emerging opportunistic human pathogen that causes neonatal meningitis, bacteremia, and enterocolitis.[5] Primers mixture was prepared ahead, containing 20 µM FIP and 10 µM BIP, 5 µM each B3 and F3. LAMP reaction mixtures including 2 µL of different concentrations of *ompA* synthetic DNA template, 2 µL primers mixture, 2.5 µL of 4 mM dNTPs, 0.5 µL of 100 mM $MgCl_2$, 5 µL of 5 M betaine in a total volume of 23 µL 1 × Iso Buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8) were annealed to 95 °C for 2 min, followed by chilling on ice for another 5 min. Then 2 µL (8U) of Bst 2.0 DNA polymerase were added to the above mixtures to initiate the LAMP reactions. The reactions were incubated at 63 °C for 90 min, followed by heating to 80 °C for 20 min to denature the polymerase.

***Enterobacter sakazakii* Preparation and Processing.**

Enterobacter sakazakii (CMCC 45401) supplied by the National Center for Medical Culture Collection (CMCC) was used as a model organism to demonstrate the efficacy of the sensor. The strain was stored in 16% (v/v) glycerol solution at −80 °C until used. The bacterial strain was grown in Luria-Bertani broth (LB: 10 g NaCl, 10 g tryptone, 5 g yeast extract, and H_2O to 1, 000 mL) medium at 37 °C for 24 hr. The inoculation assay was prepared by adding 10-fold serial dilutions of the pathogen in 0.8% NaCl, ranging from 1 to 10^9 CFU mL^{-1}. The genomic DNA templates were extracted with a Bacteria DNA kit.

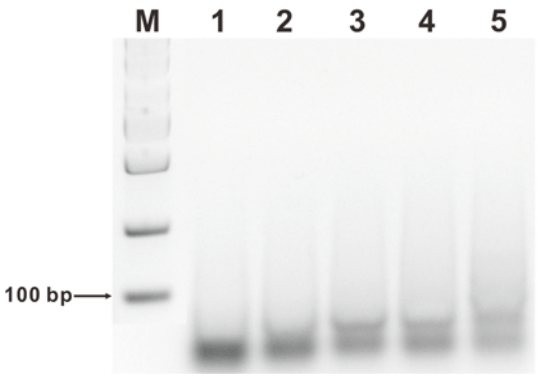

Figure S1. The 1.5% agarose gel electrophoresis of the RNA amplicons obtained by NASBA reaction using different copies of Zika template RNAs (Zika RNA). M: 2,000 bp plus DNA Marker (Beijing Zoman Biotechnology Co., L td.); Lane 1: no Zika RNA; Lane 2: 20 copies of Zika RNA; Lane 3: 200

copies of Zika RNA; Lane 4: 2,000 copies of Zika RNA; Lane 5: 20,000 copies of Zika RNA.

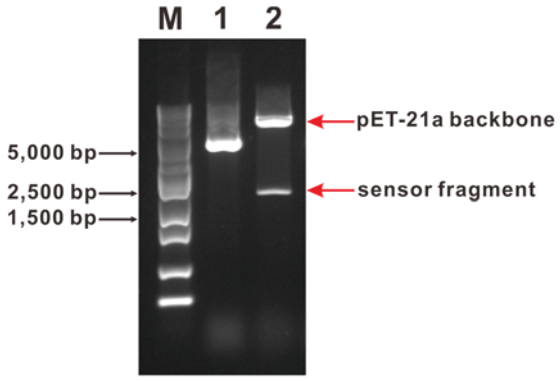

Figure S2. The 1 % agarose gel analysis of Sensing Plasmid. Lane M: 15,000 bp plus DNA Marker (Beijing Zoman Biotechnology Co., L td.); lane 1: Sensing Plasmid; lane 2: Sensing Plasmid DNA digested with *Bgl*II and *Xho*I.

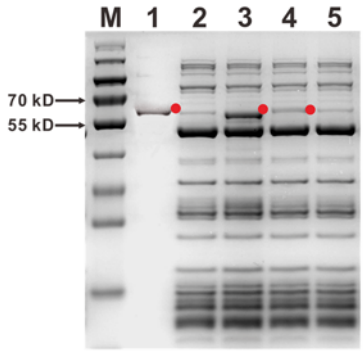

Figure S3. SDS-PAGE of invertase from different sources. Lane1: home-made purified invertase; Lane2-Lane5 are cell-free expression, Lane2: with no plasmid; Lane3: with plasmid only contains invertase gene; Lane4: with Sensing Plasmid triggered by 500 nM T1; Lane5: with only Sensing Plasmid.

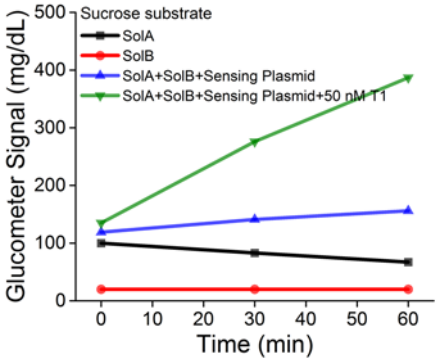

Figure S4. Glucose generation kinetic curves with different components of cell-free expression kit at 55 ºC. SolA: Solution A in cell-free expression kit; SolB: Solution B in cell-free expression kit. SolA and SolB will not induce glucose increasing in presence of sucrose, so there is no invertase in the whole of cell-free system. The steady-state glucose background in the absence of Sensing Plasmid might come from some redox reagents in the solutions. Nevertheless, because they will not bring glucose increase along with time, the accuracy of the detection won't be affected.

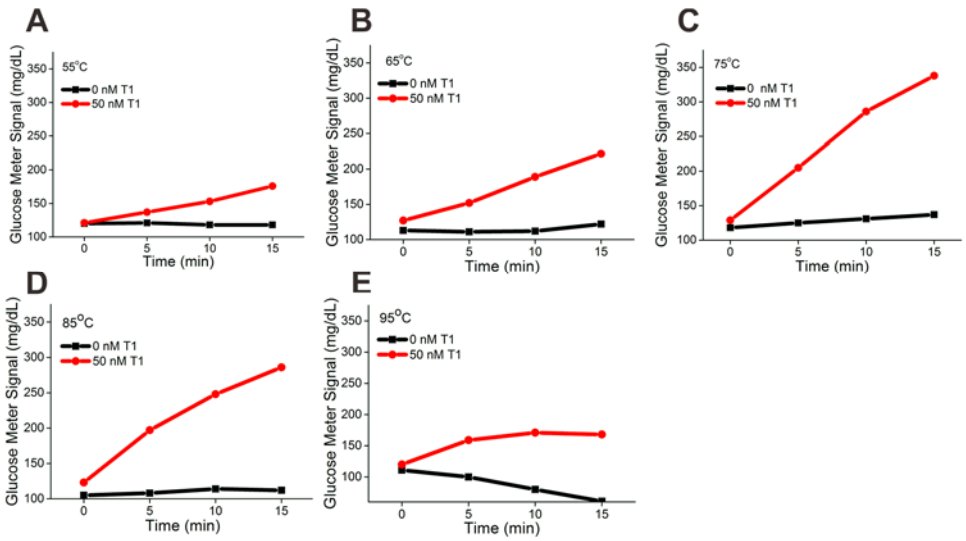

Figure S5. Different temperatures of kinetic glucose production curves with and without 50 nM T1 after cell-free system incubated at 37ºC for 2 hr. The cell-free products and 1 mM sucrose was mixed in a 2:1 volume ratio and incubated at (A) 55ºC, (B) 65ºC, (C) 75ºC, (D) 85ºC and (E) 95ºC for 15 min. The glucose yielded was measured using a glucose meter every 5 min.

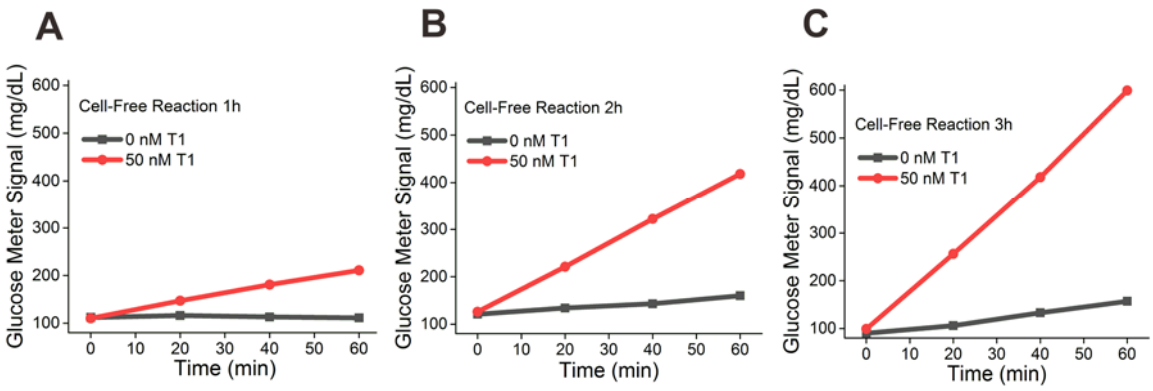

Figure S6. Optimization for the incubation time of cell-free reaction with T1. Kinetic glucose production curves after cell-free system with and without 50 nM T1 incubated at 37°C for (A) 1 hr, (B) 2 hr and (C) 3 hr.

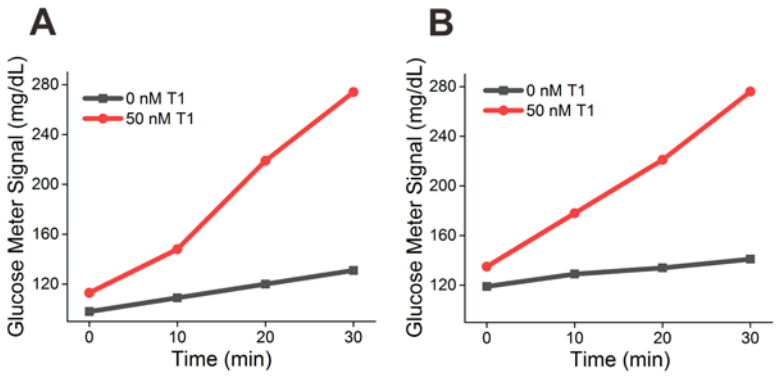

Figure S7. Optimization for the volume of sucrose substrate. The cell-free products and 1 M sucrose was mixed in a (A) 1:1 volume ratio and (B) 2:1 volume ration incubated at 55°C for 30 min. The glucose yielded was measured using a glucose meter every 10 min.

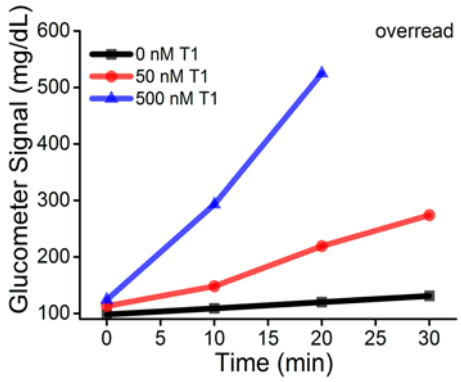

Figure S8. Glucose generation kinetic curves with and without T1. The readout of 500 nM T1 is over 600 mg/dL which is out of a PGM range.

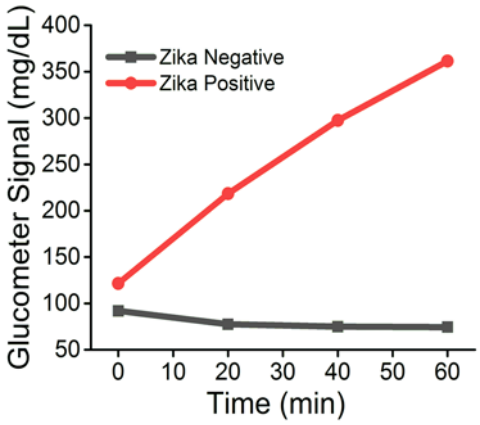

Figure S9. Glucose generation kinetic curves with and without Zika NASBA products amplified from 20,000 copies of Zika RNA.

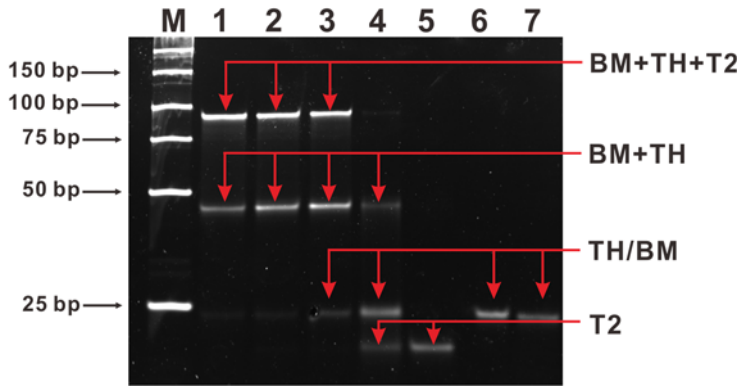

Figure S10. 10% native PAGE characterization of the formation of T2:TH:BM intermediate structure in different buffers. The concentrations of T2, TH and BM were 50 nM, 50 nM and 40 nM, respectively. Before use, complex T2:TH:BM, TH:BM, TH and BM was, in respective, prepared in different concentrations of isothermal buffer by an annealing process which included heating at 95°C for 5 min and cooling down to 37°C at a rate of 0.1°C/s. M: DNA Marker A (25-500 bp) was used as size marker; Lane 1: T2, TH and BM annealing in 1 × Iso Buffer; Lane 2: T2, TH and BM annealing in 0.56 × Iso Buffer; Lane 3: T2, TH and BM annealing in 0.33 × Iso Buffer; Lane 4: T2, TH and BM annealing in H_2O; Lane 5: 50 nM T2; Lane 6: 50 nM TH; Lane 7: 40 nM BM.

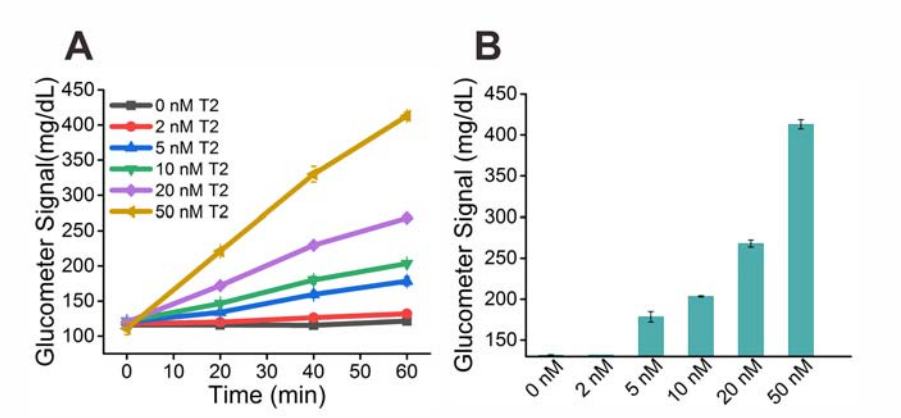

Figure S11. Proof of universality using four-way junction-based transduction to detect T2 and ompA LAMP product. (A) Glucose generation kinetic curves with and without T2. (B) Concentration dependence of T2.

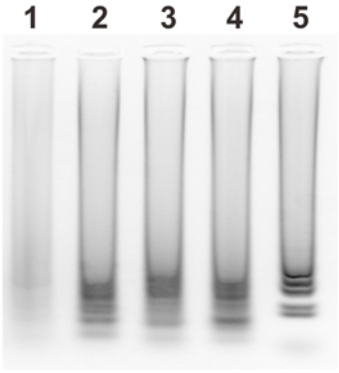

Figure S12. The 1% Agarose gel electrophoretic characterization of LAMP products amplified from target and non-target *ompA* synthetic DNA. Lane 1: no *ompA* synthetic DNA; Lane 2: 20 copies of *ompA* synthetic DNA; Lane 3: 200 copies of *ompA* synthetic DNA; Lane 4: 2,000 copies of *ompA* synthetic DNA; Lane 5: 20,000 copies of *ompA* synthetic DNA.

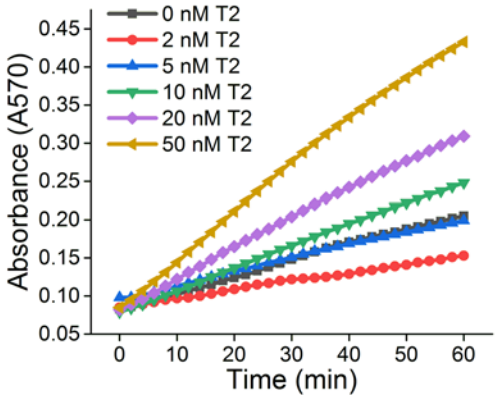

Figure S13. Kinetic absorbance curves at 570nm catalyzed by LacZ with and without T2. The cell-free expression process was operated completely according to standard method. After cell-free reactions, 14 µL of 0.9 mg/mL chlorophenol red-b-D-galactopyranoside substrate was mixed with the products. The absorbance signals were collected 2 min by Biotek Cytation™ 5 imaging multi-mode plate reader (Biotek, Winooski, U.S.A)

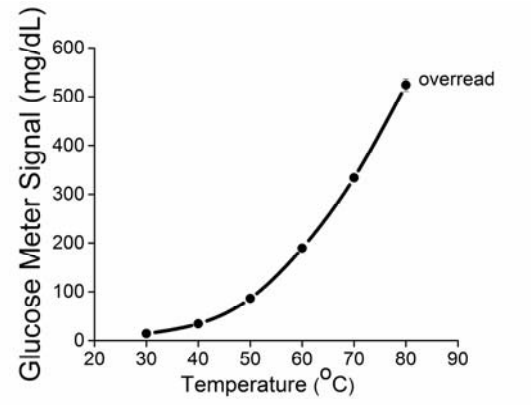

Figure S14. Temperature dependence of TmINV activity. At each temperature, equal volume of 0.16 µg thermostable invert-ase and 500 mM sucrose is incubated for 5 min, followed by measurement of glucose yielded using a PGM. At 90 °C, the readout is over 600 mg/dL which is out of a PGM range.

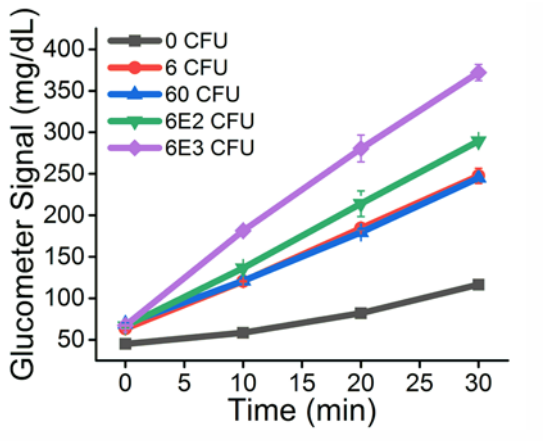

Figure S15. Glucose kinetic curves responses to both non-target and different amounts of pathogen *Enterobacter sakazaki* bacteria.

TableS1. Oligonucleotides used in this paper.

Name	Sequences(from5'to3')
toehold switches[1]	UUUCGCUCUAUUCUCAUCAGUUUCAUGUCCUGUGUCGGACUUUAGAACAGAGGAGAUAA AGAUGGACACAGGACACAACCUGGCGGCAGCGCAAAAG
T1	TCGTTAATGACACAGGACATGAAACTGATGAGAATAGAGCGAAAGTTGAGATAACGCCCAATTCA CCAAGAGCCGAAGCCACCCTGG
T2	AAAGCCAGCTGGGCGCAGGCGCGTTCGGTGGTTAC
TH	GTAACCACCGAACGCGCCTGGAGAATAGAGCGAAA
BM	GACACAGGACATGAAACTGTTCGCCCAGCTGGCTTT
NASBA Primer Sequences[1]	
Zika forward primer	AATTCTAATACGACTCACTATAGGGAGAAGGGCACAGTGGGATGATCGTTA
Zika reverse primer	CCTGTCCTCGGTTCACAATCAA
LAMP Primer Sequences	
ompA-F3	TGGTCCCAGTTCCACGATAC
ompA-B3	GTAACCCAGTTTAGCGGTCA
ompA-FIP	CCAACGTACGGGTTAACCTGGTGACGGTCCGACTCACGAA
ompA-BIP	TCGAAATGGGCTACGACTGGCTTGTACGCCCTGAGCTTTGA

Table S2. Comparation with other literature for Zika virus (ZIKV) detection

Method	Strain used	Sensitivity	Detection	Reference
NASBA	Synthetic oligonucleotides of ZIKV	20 copies of RNA (27 aM)	Development of NASBA protocol coupled with cell-free expression. Through commercial glucometers	This work
NASBA	Synthetic oligonucleotides of ZIKV	1 fM	Development of NASBA protocol coupled with cell-free expression. Through the naked eye with β-galactosidase degradation substrate.	[1]
RT-LAMP	PRVABC59	0.5 PFU	Through the naked eye with SYBR Green I	[6]
RT-LAMP	PRVABC59 (KX601168), MRS_OPY_Martinique_Pari_2015 (KU647676), H/PF/2013 (KJ776791), and MR766 (LC002520)	0.17 FFU/mL-2.3×10^{2} FFU/mL	Turbidity monitoring	[7]
RT-LAMP	MR 766 Uganda	3.3 ng/μL	Monitoring of fluorescence coupled to RT-qPCR	[8]
RT-LAMP	MR 766 Uganda (AY632535)	10^{0} ZIKV RNA copies	Development of a tape based on lateral flow assay (LFA)	[9]
LAMP	Synthetic oligonucleotides of ZIKV	1 aM	Development of LAMP protocol coupled with AC susceptometry	[10]

Table S3. Comparation with other literature for *E. sakazakii* detection

Method	Strain used	Sensitivity	Detection	Reference
LAMP	*E. sakazakii* CMCC45401	6 CFU/mL	Development of LAMP protocol coupled with cell-free expression. Through commercial glucometers	This work
Thermophilic helicase-dependent isothermal amplification （tHDA）	*C. sakazakii* ATCC 29544	10^{0-1} CFU/mL	Detecting UV366 nm light with SYBR Green I	[11]
Cross-priming amplification (CPA)	22 *E. sakazakii* strains	88±8.7892 CFU/mL	Immuno-blotting analysis through the naked eye with BESt strip	[12]
LAMP	*C. muytjensii* ATCC 51329 *C. sakazakii* ATCC 29544	10^{1} CFU/mL	Turbidity monitoring and detecting UV light with SYBR Green I	[13]
LAMP	*Cronobacter* spp. strains collected from American Type Culture Collection	9.1 fg/μL	Turbidity monitoring and detecting UV light with SYBR Green I	[14]

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