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Supplementary Materials for

An automated DNA computing platform for rapid etiological diagnostics

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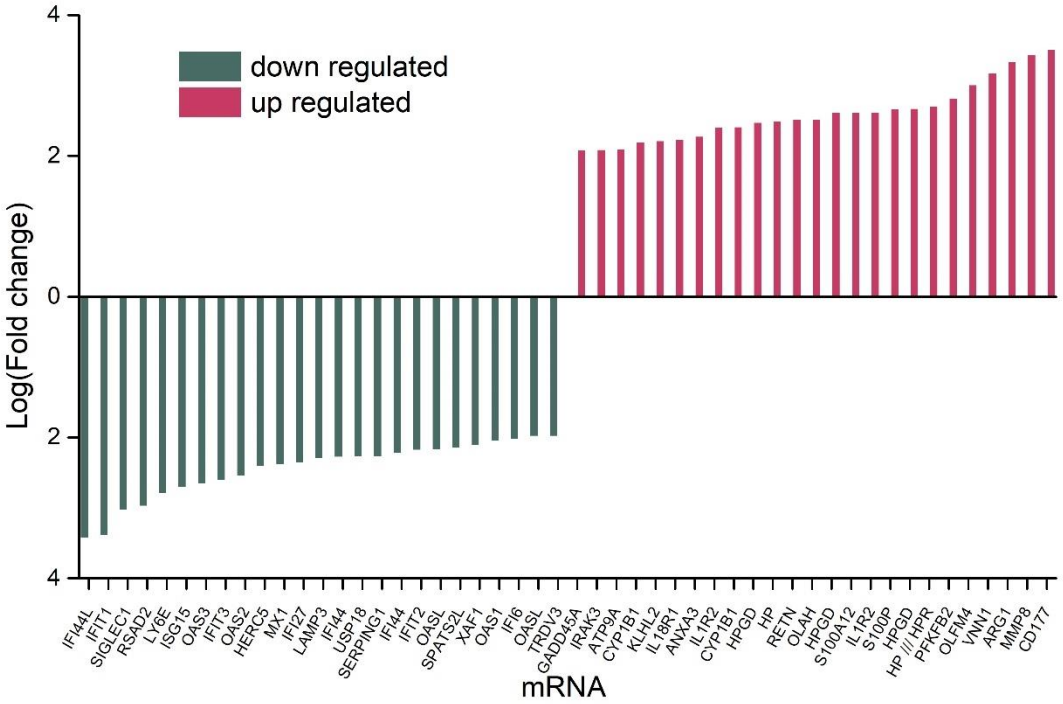

Fig. S1. Expression fold changes of mRNAs in the bacterial infection samples compared to those of viral infection samples. The original data were obtained from the NCBI GSE63990 database.

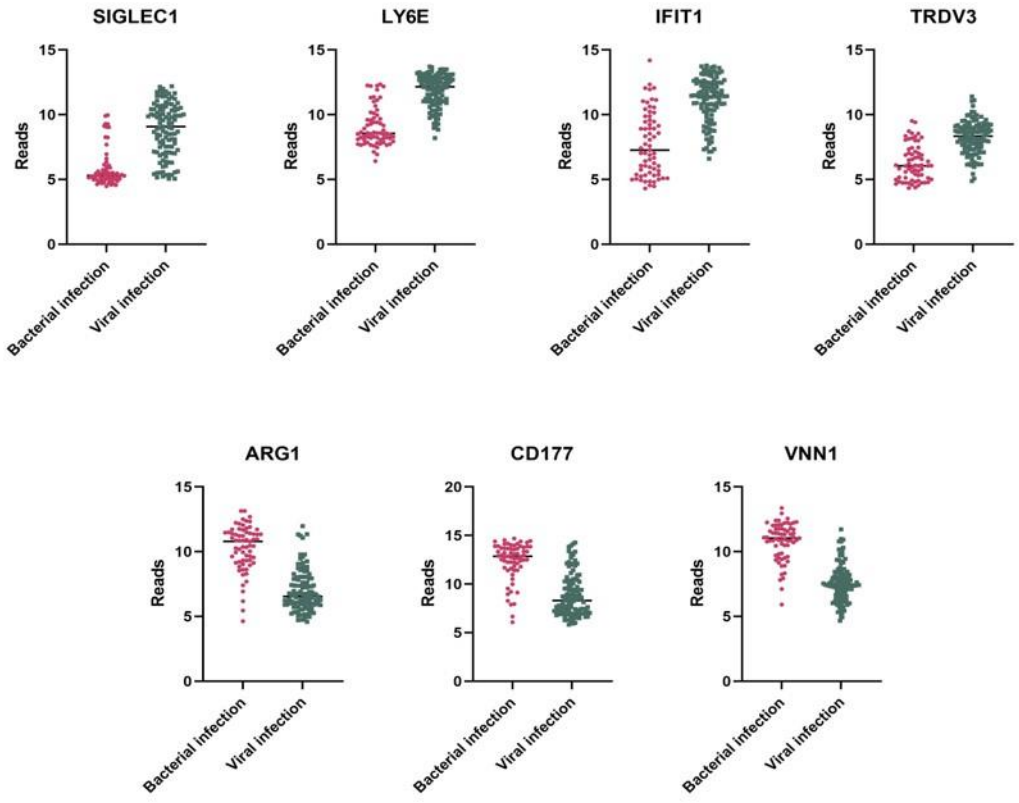

Fig. S2. Expression levels of seven selected mRNAs between bacterial and viral infection samples (values from the mRNA-seq reads in the GEO database). Horizontal lines indicate median values of the groups.

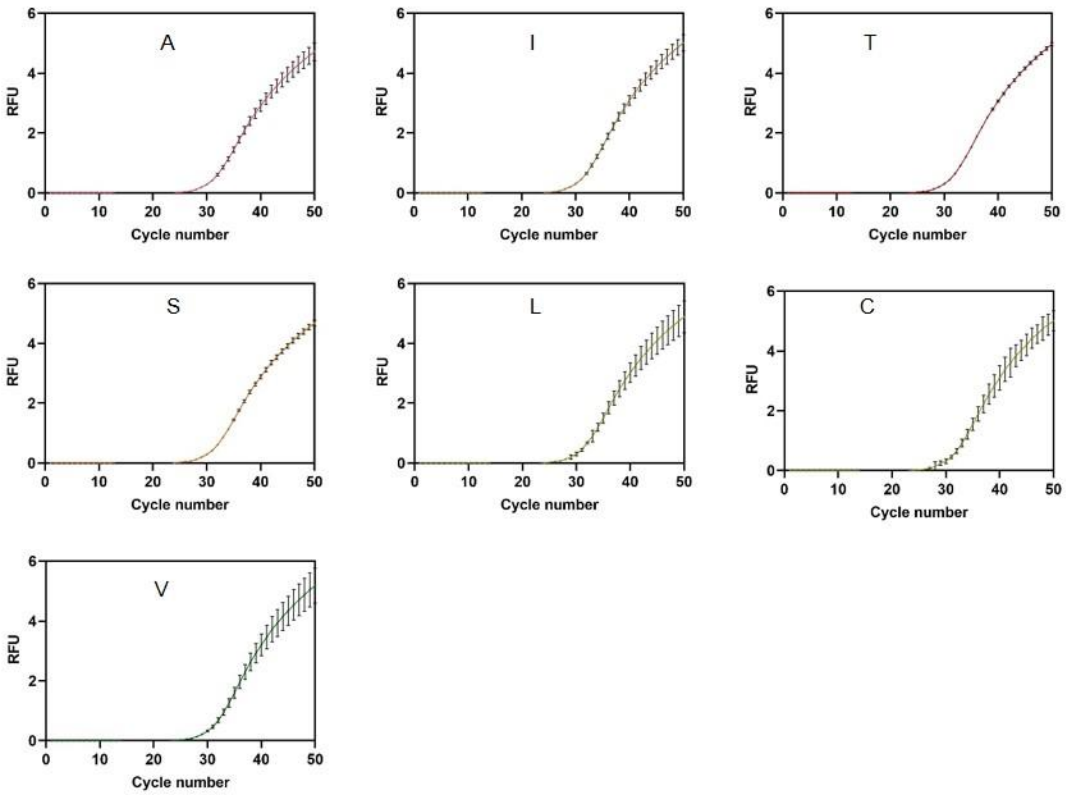

Fig. S3. LATE-PCR amplification efficiency for the seven selected mRNAs post-transcription. Labels of PCR traces were simplified by using the first letter of corresponding mRNA candidates. 1.0 pM of each synthetic cDNA was spiked to seven separate PCR tubes and incubated with the corresponding primers, enzymes and reporting probes (TaqMan) for LATE-PCR (LATE-PCR for 50 cycle; see detailed information in Table S5). Data are presented as the mean ± s.d.; n = 3 independent experiments.

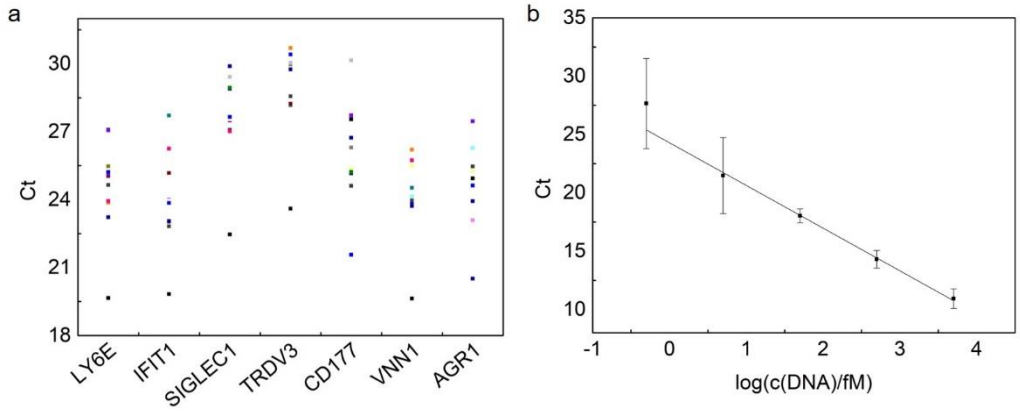

Fig. S4. Quantitation of target mRNAs in clinical samples. **a.** CT values of target mRNAs by q-PCR quantitation in 10 randomly selected blood samples with ARIs caused by bacteria (5 samples) and virus (5 samples). **b.** The calibration curve (R^2=0.99) between CT values with the concentration of cDNA by q-PCR (see Table S5). This result indicates that the concentrations of mRNAs are variable, but always below 50 fM in the clinical samples.

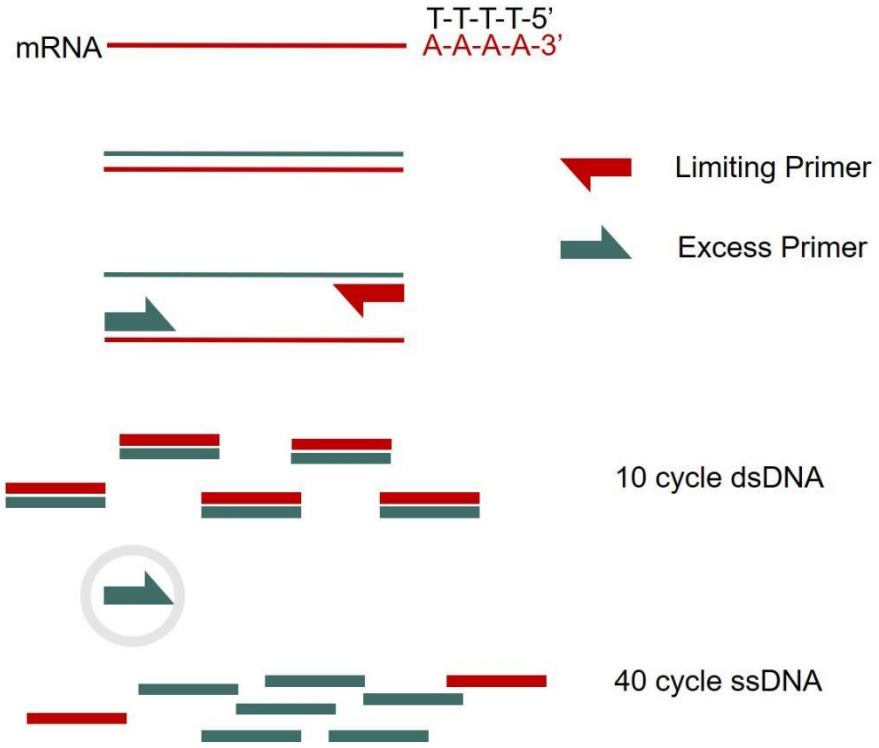

Fig. S5. Scheme of reverse transcription and linear-after-the-exponential (LATE-PCR) processes.

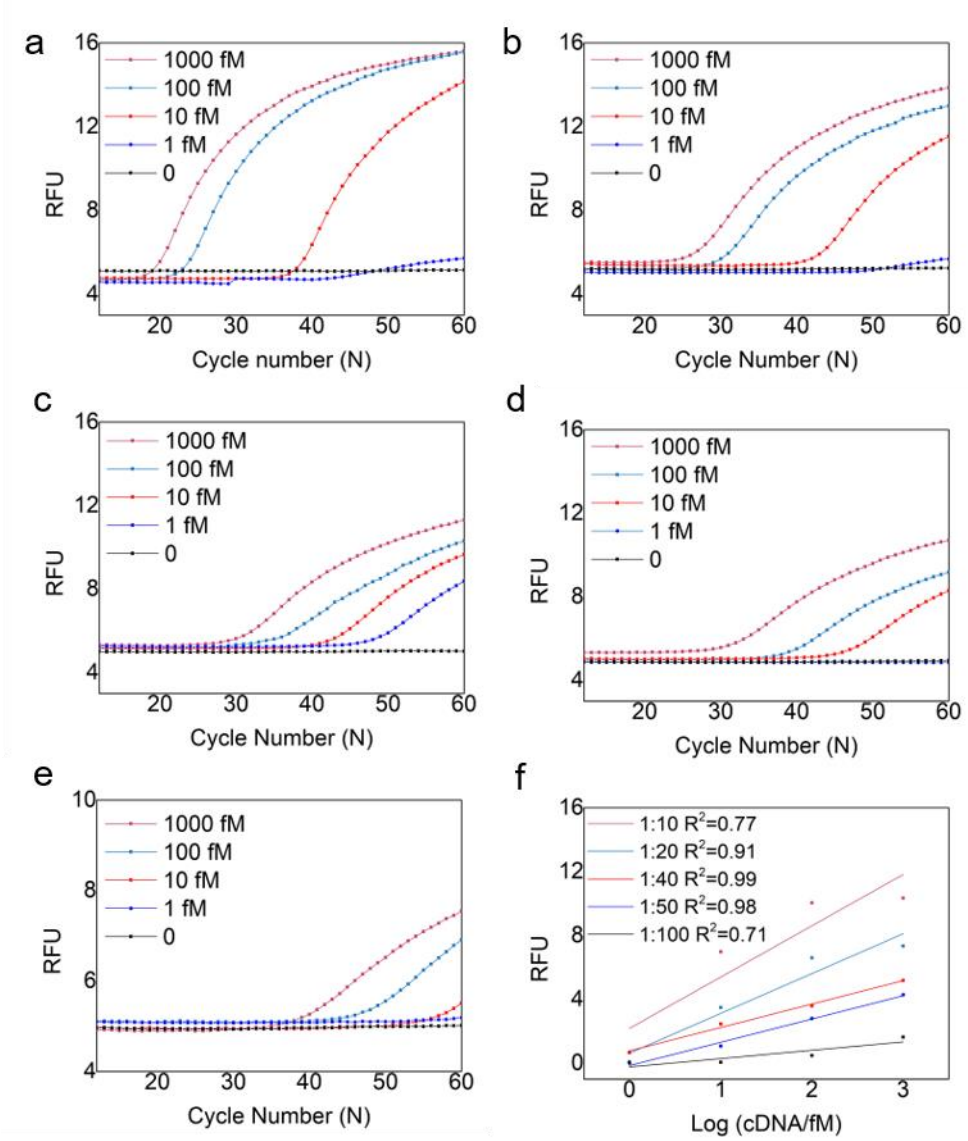

Fig. S6. Optimization of forward (F) and reverse primer (R) ratios (**a.** F: R=1:10; **b.** F: R=1:20; **c.** F: R=1:40; **d.** F: R=1:50; **e.** F: R=1:100) in LATE-PCR. The concentrations of ssDNA products were analyzed by TaqMan reporting probes. **f.** Plot of logarithmic initial concentrations of mRNA templates *vs.* fluorescence readings of LATE-PCR at cycle = 50. Taking together the final product amounts and linearity, we selected F: R=1:40 as the optimum conditions for amplification.

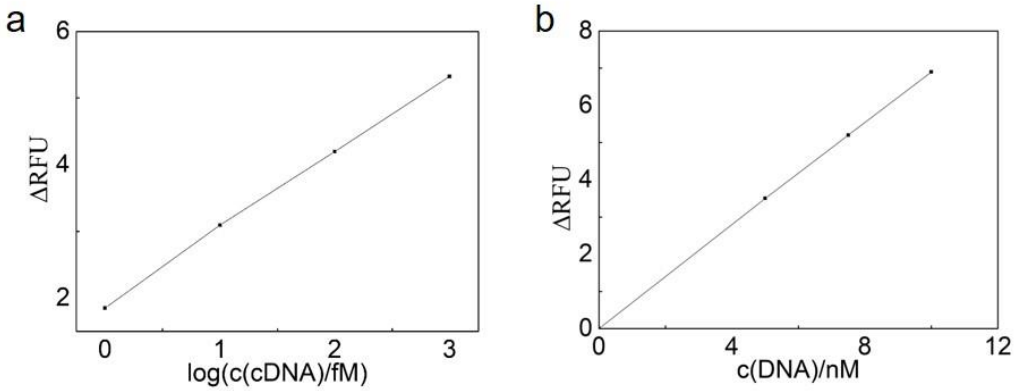

Fig. S7. Quantification of final product post-LATE-PCR. a. cDNA amplicon of SIGLEC1 after LATE-PCR (50 cycle) *vs*. fluorescence change (ΔRFU, quantified by using duplex A reporter) were quantified by duplex A reporter (see Table S5). **b.** Synthetic DNA with known concentrations *vs*. fluorescence change (ΔRFU, quantified by using duplex A reporter). Results showed that cDNA inputs from 0-1000 fM can be amplified to higher concentrations of ssDNA (≥ 1 nM) by LATE-PCR.

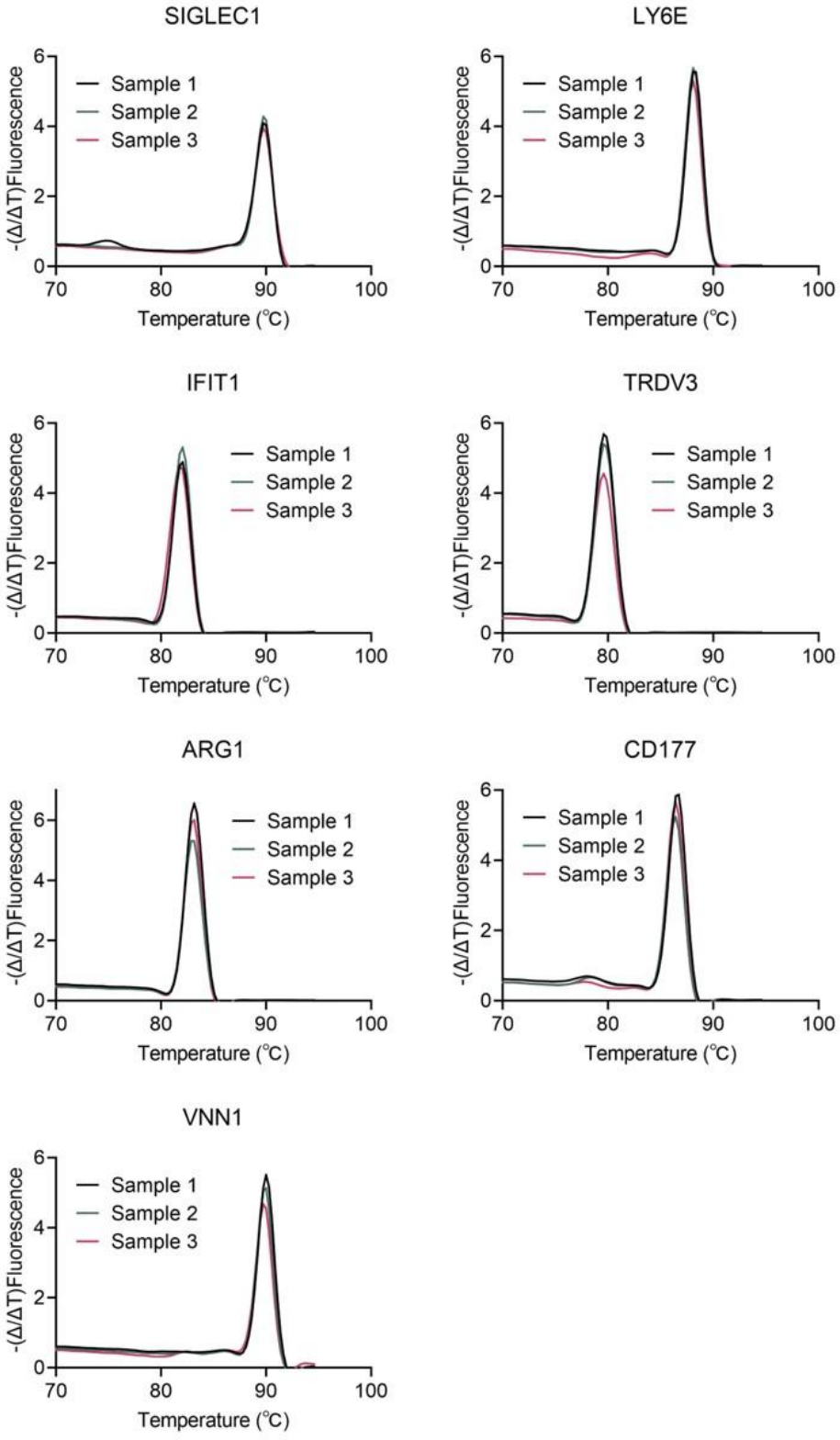

Fig. S8. Verification of primer specificity by q-PCR in three clinical blood samples (qPCR for 40 cycle; see Table S5). Peaks in the melting curve indicate the melting temperature (Tm) of target mRNA.

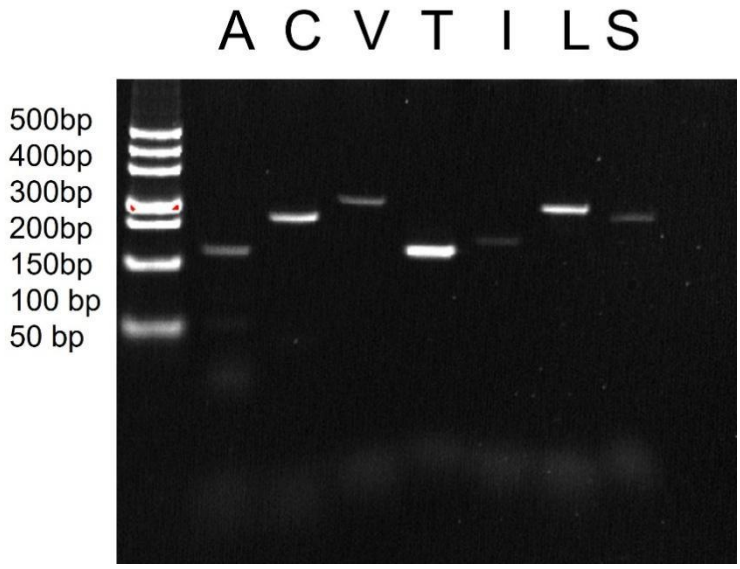

Fig. S9. Polyacrylamide gel electrophoresis results obtained from q-PCR with the designed primer pairs (see Table S5). (A: ARG1, C: CD177, V: VNN1, T: TRDV3, I: IFIT1, L: LY6E, S: SLGLEC1).

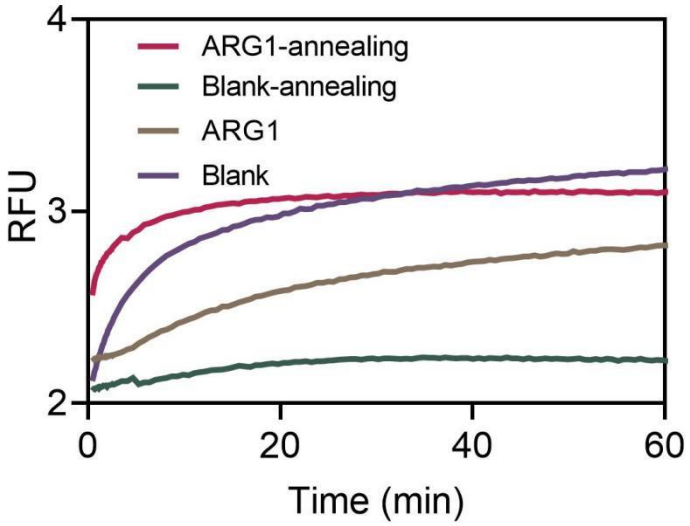

Fig. S10. Effect of thermal annealing to improve reporting efficiency of mRNA with intensive secondary structures post-reverse transcription. Thermal annealing was performed by heating the reactants (10 nM ARG1 cDNA) to 70°C for 10 s and then cooling them to 25°C at a rate of -1°C/10 s together with A reporter.

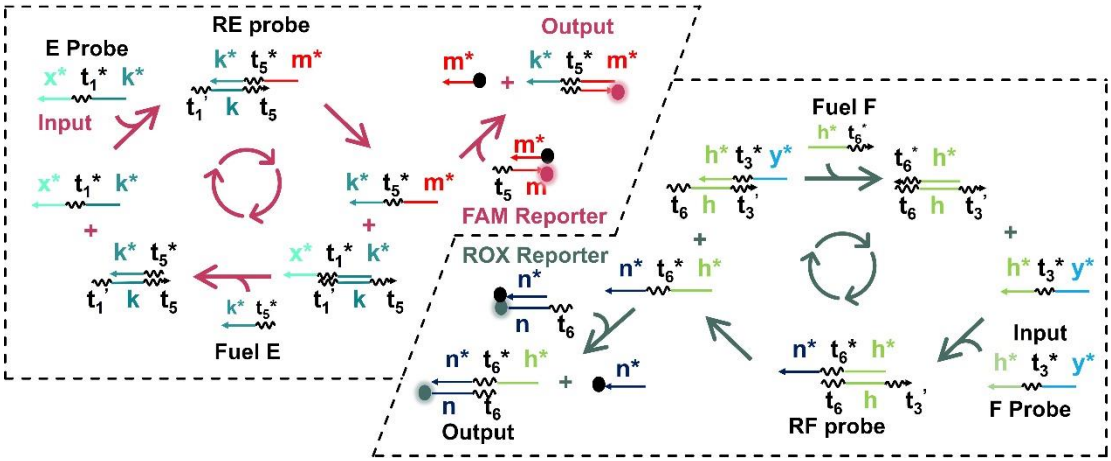

Fig. S11. Scheme of the amplification step for sensitivity improvement. (Two distinct fluorescent reporters with FAM and ROX fluorophores were used to report the corresponding outputs associated with positive and negative weights after the subtraction operation. A catalytic entropy-driven amplification restores the offset signals and improves the reporting sensitivity.)

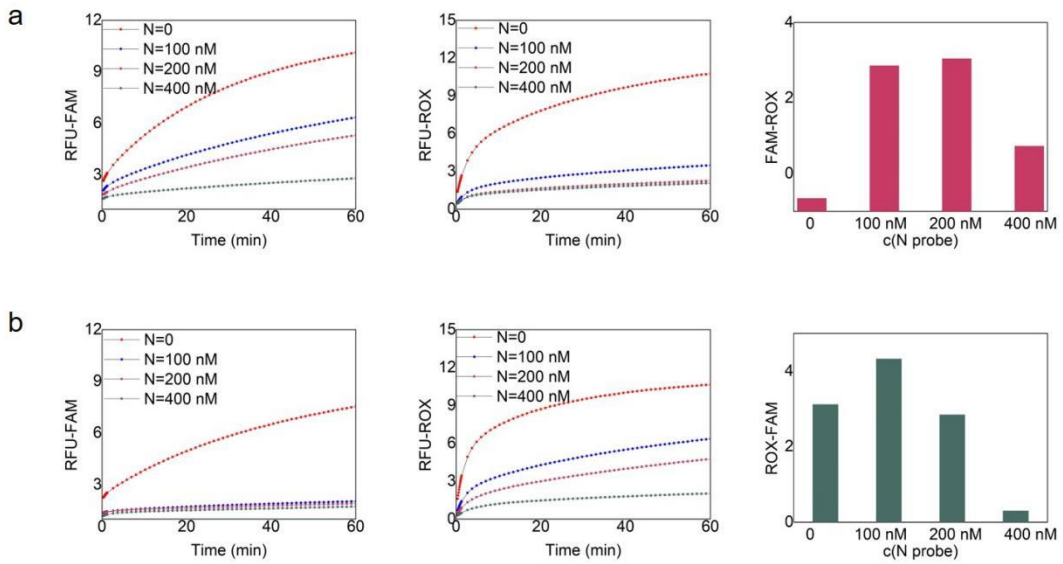

Fig. S12. Optimization of N probe concentrations for more sensitive reporting in the subtraction step. **a.** c (E probe) =30 nN; c (F probe) =20 nM; **b.** c (E probe) =20 nN, c (F probe) =30 nM. (Restoration probe E/F:100 nM; Fuel E/F: 200 nM; FAM/ROX reporter 200 nM).

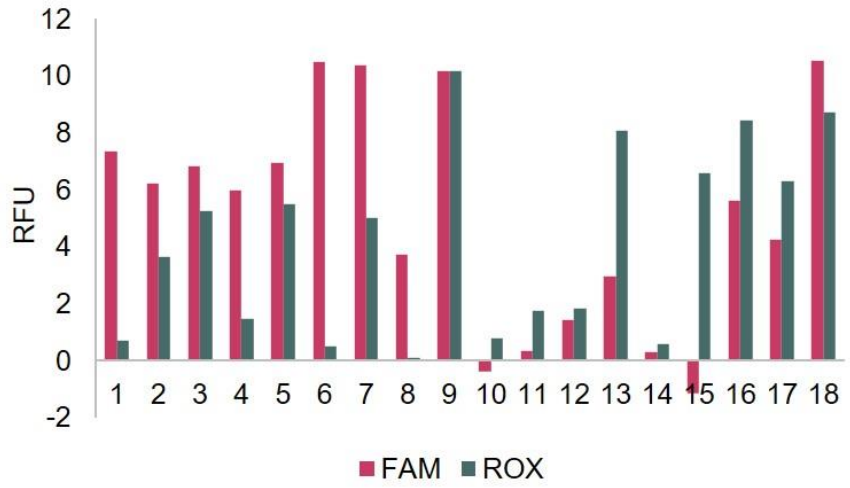

Fig. S13. Fluorescence readings of 18 synthetic samples. (Bacterial infection samples: No. 1-9; Viral infection samples: No. 10-18). (RFU(FAM)) - (RFU(ROX)) > 0 is considered bacterial infection, whereas (RFU(FAM))- (RFU(ROX)) < 0 is considered viral infection.

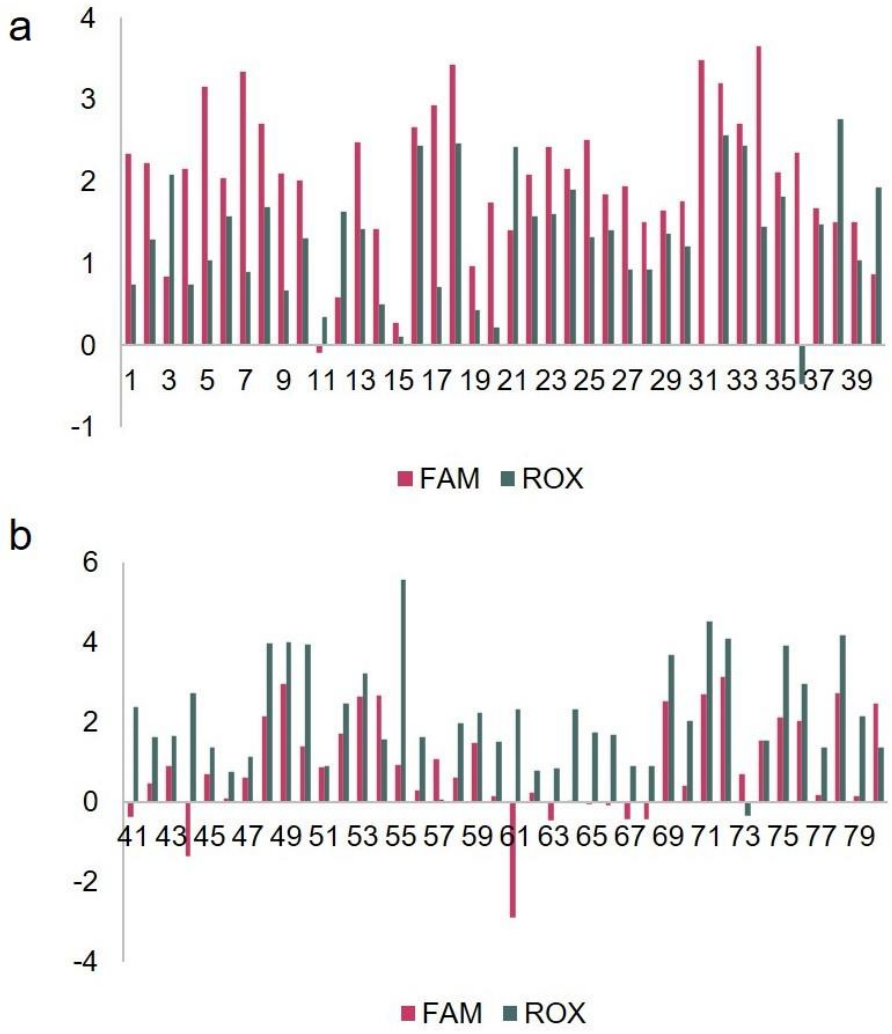

Fig. S14. Fluorescence readings of 80 clinical blood samples. **a.** Fluorescence readings of bacterial infection samples (No. 1-40) from both FAM and ROX reporting. **b.** Fluorescence readings of viral infection samples (No. 41-80) from both FAM and ROX reporting. (RFU(FAM)) - (RFU(ROX)) > 0 is considered bacterial infection, whereas (RFU(FAM)) -(RFU(ROX)) < 0 is considered viral infection.

a

Synthetic Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
True Type	B	B	B	B	B	B	B	B	B	V	V	V	V	V	V	V	V	V
Predicted Type	B	B	B	B	B	B	B	B	B	V	V	V	V	V	V	V	V	B

b

Clinical Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	34	36	37	38	39	40
True Type	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Predicted Type	B	B	V	B	B	B	B	B	B	B	V	V	B	B	B	B	B	B	B	B	V	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	V	B	V

Clinical Samples	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
True Type	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
Predicted Type	V	V	V	V	V	V	V	V	V	V	V	V	V	B	V	V	B	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	B	B	V	V	V	V	V	B

Fig. S15. Classification results of the DNA computation-based diagnostic system with 18 synthetic and 80 clinical samples. a. Ture and predicted types of the DNA computation-based diagnostic system with 18 synthetic samples. b. Ture and predicted types of the DNA computation-based diagnostic system with 80 clinical samples. (B: Bacterial infection; V: Viral infection)

Table S1. Sensitivity, specificity, and the area under the ROC curve (AUC) of single mRNA and the panel (7 mRNA with trained model) for the etiological diagnosis of ARI.

	Sensitivity	**Specificity**	**AUC**
Panel	89.6	91.2	94.2
ARG1	88.1	85.0	90.6
CD177	82.1	84.1	86.8
IFIT1	82.1	75.2	86.0
TRDV3	74.6	83.2	85.1
LY6E	72.1	92.9	91.0
SIGLEC1	80.6	86.7	88.9
VNN1	91.0	84.1	92.8

Table S2. Concentrations of 7 cDNAs in the synthetic samples (1: 1.0 pM; 0: 0 pM）

Sample	SIGLEC1	LY6E	IFIT1	TRDV3	VNN1	CD177	ARG1
1	1	0	0	0	1	1	1
2	1	1	0	0	1	1	1
3	1	1	0	1	1	1	1
4	1	0	1	0	1	1	0
5	1	0	0	0	1	0	1
6	0	0	0	0	1	1	1
7	1	0	1	0	1	1	1
8	0	1	0	1	1	1	1
9	1	1	1	0	1	1	1
10	1	1	1	1	1	0	0
11	1	1	1	1	0	1	0
12	1	1	1	0	0	1	1
13	1	1	0	0	1	0	0
14	1	1	0	0	0	1	0
15	1	1	1	1	0	0	0
16	1	1	1	1	1	0	1
17	1	1	1	1	0	1	1
18	1	1	1	1	1	1	0

Table S3. Clinical sample related information.

Sample No.	Pathogen detected	NEU%	LYM%	MON%	CRP
1	Parainfluenza 1,2,3	85.60	7.30	6.20	0.640
2	Influenza B virus	76.90	14.30	8.50	17.420
3	Parainfluenza 1,2,3	54.20	35.30	7.50	35.130
4	Influenza B virus; Parainfluenza 1,2,3	68.00	23.50	8.10	0.830
5	Parainfluenza 1,2,3	68.90	21.10	7.60	7.480
6	Epstein-Barr virus	80.10	13.50	5.60	0.840
7	Influenza B virus; Parainfluenza	62.30	28.50	6.20	11.050
8	Influenza B virus	57.90	30.20	10.50	0.920
9	Influenza B virus	44.30	44.60	7.30	0.960
10	Influenza B virus	55.10	36.10	6.40	1.210
11	Influenza B virus	70.90	20.90	6.40	1.430
12	Influenza B virus	65.00	24.80	5.80	1.500
13	Influenza B virus	75.00	21.00	3.50	0.850
14	Parainfluenza 1,2,3	66.20	26.40	6.20	0.840
15	Influenza B virus	59.70	30.60	8.40	94.470
16	Parainfluenza 1,2,3	68.20	15.60	15.80	0.850
17	Influenza B virus	67.60	23.60	6.80	0.940
18	Parainfluenza virus	68.50	26.10	4.60	0.670
19	Parainfluenza virus	43.50	47.80	5.90	3.520
20	Parainfluenza virus	66.80	28.10	4.30	15.430
21	Parainfluenza virus	61.70	29.50	6.40	19.170
22	Parainfluenza virus	81.80	11.30	5.90	1.430

23	Parainfluenza virus	58.40	35.70	5.10	2.570
24	Parainfluenza virus	81.80	10.60	7.50	0.870
25	Parainfluenza virus	56.40	36.40	5.30	0.880
26	Parainfluenza virus	80.80	8.40	9.50	1.240
27	Parainfluenza virus	50.50	43.00	5.50	1.300
28	Parainfluenza virus	64.40	28.50	6.20	1.210
29	Parainfluenza virus	65.10	25.30	8.30	1.540
30	Parainfluenza virus	61.50	25.20	9.60	0.660
31	Influenza B virus	54.40	37.20	6.10	9.080
32	Influenza B virus	60.30	33.40	5.20	1.510
33	Parainfluenza virus	70.10	23.20	5.00	6.380
34	Parainfluenza virus	55.80	33.40	7.10	1.510
35	Parainfluenza virus	54.00	32.80	5.60	67.780
36	Parainfluenza virus	63	14	8	7.390
37	Parainfluenza virus	59.10	29.10	6.70	8.120
38	Influenza B virus	61.20	31.40	6.20	0.670
39	Influenza B virus	64.00	23.30	9.50	0.700
40	Influenza B virus	74.10	17.90	6.90	0.830
41	Legionella pneumophila serotype I -IgM	59.90	31.00	7.10	4.110
42	Mycoplasma pneumoniae IgM	75.70	15.50	6.90	0.550
43	Legionella pneumophila serotype I -IgM	64.60	28.40	6.20	9.860
44	Legionella pneumophila serotype I -IgM；Mycoplasma pneumoniae IgM	57.10	32.10	6.80	5.900

45	Legionella pneumophila serotype I IgM	72.80	13.60	13.00	4.970
46	Mycoplasma pneumoniae；Gram-negative coccus (+)；Gram-positive cocci (+)	80.70	12.40	5.20	24.570
47	Legionella pneumophila; Mycoplasma pneumoniae	65.1	21.70	7.50	3.310
48	Legionella pneumophila	80.60	13.10	5.60	32.250
49	Legionella pneumophila	85.10	7.70	7.00	51.320
50	Mycoplasma pneumoniae	70.30	21.70	6.90	6.990
51	Legionella pneumophila	66.50	26.60	5.10	8.870
52	Legionella pneumophila	67.40	21.70	8.00	9.550
53	Mycoplasma pneumoniae	76.50	9.00	13.30	19.780
54	Legionella pneumophila	65.40	25.00	9.10	15.300
55	Mycoplasma pneumoniae	69.80	22.00	6.80	28.910
56	Mycoplasma pneumonia; Legionella pneumophila	86.20	9.30	4.40	0.920
57	Mycoplasma pneumoniae	71.60	19.80	7.30	38.840
58	Mycoplasma pneumoniae	53.60	34.20	7.40	0.950
59	Mycoplasma pneumoniae	40.80	38.70	13.80	3.450
60	Mycoplasma pneumoniae	63.60	28.70	5.70	32.250
61	Mycoplasma pneumoniae	70.90	22.40	5.20	1.420
62	Mycoplasma pneumoniae	59.40	30.50	8.10	8.870
63	Legionella pneumophila	64.20	27.60	6.40	2.140
64	Legionella pneumophila	59.20	34.60	4.60	2.560
65	Mycoplasma pneumoniae	74.30	20.10	4.30	13.860
66	Mycoplasma pneumoniae	81.00	10.10	8.50	51.320
67	Mycoplasma pneumoniae	75.10	16.20	7.80	96.500

68	Mycoplasma pneumoniae	59.20	32.80	6.80	15.430
69	Mycoplasma pneumoniae	90.50	4.00	3.80	1.710
70	Legionella pneumophila	65.80	26.40	5.90	17.420
71	Legionella pneumophila	72.80	20.80	4.60	3.190
72	Legionella pneumophila	60.60	30.10	6.80	3.960
73	Legionella pneumophila	52.70	35.70	10.20	38.840
74	Legionella pneumophila	68.80	21.10	9.00	4.970
75	Legionella pneumophila	73.30	18.20	6.10	3.190
76	Legionella pneumophila	53.90	32.20	8.50	96.500
77	Legionella pneumophila	67.30	21.30	9.30	2.520
78	Legionella pneumophila	83.70	10.70	4.70	0.700
79	Legionella pneumophila	70.60	18.50	9.10	21.000
80	Legionella pneumophila	75.60	15.60	6.20	4.700

Table S4. Comparison between DNA computational method and existing methods for ARI diagnosis

Diagnostic Method	**Diagnostic Accuracy**	**Result analysis requirements**	**Time required for diagnosis**	**Instrumentation requirements**	**Cost**
Blood routine	<65%	Manual and Automated	1 hour	Minimal	$
CRP/PCT	<75%	Manual and Automated	6 hours	Minimal	$$
q-PCR	~85%	Manual	6-12 hours	Minimal	$$
Sequencing	>90%	Manual and Automated	1 day	High	$$$
DNA microarray	88%	Manual and Automated	12 hours	High	$$$
Our platform	86%	Automated	4 hours	Minimal	$$

Table S5. Detailed reaction conditions used in this paper

RNA extraction		
Total RNA was extracted from human blood using the Qiagen PAXgene Blood RNA Kit according to the manufacturer's protocol. Specifically, a lysis reagent is added to each blood sample first. Then the lysate is separated by adding chloroform and centrifugation. The sample is then applied to a spin column where the total RNAs, including mRNAs, can be eluted in a small volume of nuclease-free water.		
RT-qPCR quantification		
Total RNA was extracted from each blood sample with Qiagen Research kits. The isolated RNA was first reversely transcribed to cDNA using procedures described below. Then, the acquired cDNAs were amplified by using the qPCR Mix (Takara) according to the procedures in the instructions.		
components & concentrations	Components	Concentrations (20 μL)
	2×PCR Master Mix	10 μL
	Forward Primer	0.25 μM
	Reverse Primer	0.25 μM
	cDNA	2 μL
	RNase-free water	Up to 20 μL
Protocol	95°C 5 min → 40 cycles of 95°C 5 sec, 55°C 30 sec	
Reverse transcription		
We used a reverse transcription kit (Takara) to convert RNAs into cDNAs under the conditions and protocol shown in the table below:		
components & concentrations	Components	Concentrations (10 μL)
	5× gDNA Eraser Buffer	2.0 μL
	gDNA Eraser	1.0 μL
	Total RNA	100 ng
	RNase Free dH_2O	Up to 10 μL
Protocol	42°C for 2 min → 4°C	
	PrimeScript RT Enzyme Mix I	1.0 μL
	RT Primer Mix	1.0 μL
	5 x PrimeScript Buffer	4.0 μL
	RNase Free dH_2O	4.0 μL
Protocol	37°C for 15 min → 85°C 5 sec → 4°C	
LATE-PCR		
Reaction components & concentrations	Components	Concentrations (20 μL)
	2×Hotstart PCR Master Mix	10 μL
	Excess primer	1000 nM
	Limiting primer	25 nM
	cDNA	As required
	PCR-grade water	Up to 20 μL
	TaqMan probes	As required
PCR protocols	95°C 5 min →	

	10 cycles of 95°C 10 sec, 55°C 10 sec and 72°C 20 sec →40 cycles of 95°C 10 sec, 50°C 10 sec and 72°C 20 sec → 72°C 10 min → 4°C hold	
Automation detection		
	Operation	Time
Step 1	RNA extraction	30 min
Step 2	RT	30 min
Step 3	LATE-PCR	60 min
Step 4	DNA computation	120 min
Probe preparation		
Probe name	Concentration	
A Probe S-1-top	24 μM	
A Probe S-1-bottom	20 μM	
A Probe S-2-top	24 μM	
A Probe S-2-bottom	20 μM	
A Probe S-3-top	24 μM	
A Probe S-3-bottom	20 μM	
A Probe S-4-top	24 μM	
A Probe S-4-bottom	20 μM	
A Probe L-1-top	24 μM	
A Probe L-1-bottom	20 μM	
A Probe L-2-top	24 μM	
A Probe L-2-bottom	20 μM	
A Probe L-3-top	24 μM	
A Probe L-3-bottom	20 μM	
A Probe L-4-top	24 μM	
A Probe L-4-bottom	20 μM	
A Probe T-1-top	24 μM	
A Probe T-1-bottom	20 μM	
A Probe I-1-top	24 μM	
A Probe I-1-bottom	20 μM	
A Probe V-1-top	24 μM	
A Probe V-1-bottom	20 μM	
A Probe V-2-top	24 μM	
A Probe V-2-bottom	20 μM	
A Probe V-3-top	24 μM	
A Probe V-3-bottom	20 μM	
A Probe V-4-top	24 μM	
A Probe V-4-bottom	20 μM	
A Probe V-5-top	24 μM	
A Probe V-5-bottom	20 μM	
A Probe C-1-top	24 μM	
A Probe C-1-bottom	20 μM	
A Probe C-2-top	24 μM	
A Probe C-2-bottom	20 μM	
A Probe C-3-top	24 μM	
A Probe C-3-bottom	20 μM	

A Probe C-4-top	24 μM	
A Probe C-4-bottom	20 μM	
A Probe A-1-top	24 μM	
A Probe A-1-bottom	20 μM	
B probe E top	24 μM	
B probe E bottom	20 μM	
B probe F top	24 μM	
B probe F bottom	20 μM	
N probe top	24 μM	
N probe bottom	20 μM	
Restoration probe E top	24 μM	
Restoration probe E bottom	20 μM	
Restoration probe F top	24 μM	
Restoration probe F bottom	20 μM	
Reporter top	24 μM	
Reporter bottom	20 μM	
Fluorescence kinetic experiment		
Probe name	Concentration	Reaction condition
A Probe S-1	25 nM	Buffer: 1×TE buffer with 12.5 mM Mg^{2+} Temperature: 37°C
A Probe S-2	25 nM	
A Probe S-3	25 nM	
A Probe S-4	25 nM	
A Probe L-1	25 nM	
A Probe L-2	25 nM	
A Probe L-3	25 nM	
A Probe L-4	25 nM	
A Probe T-1	25 nM	
A Probe I-1	25 nM	
A Probe V-1	25 nM	
A Probe V-2	25 nM	
A Probe V-3	25 nM	
A Probe V-4	25 nM	
A Probe V-5	25 nM	
A Probe C-1	25 nM	
A Probe C-2	25 nM	
A Probe C-3	25 nM	
A Probe C-4	25 nM	
A Probe A-1	25 nM	
B probe 1	100 nM	
B probe 2	100 nM	
N probe	200 nM	
Restoration probe E	100 nM	
Restoration probe F	100 nM	
Fuel E	200 nM	

Fuel F	200 nM	
FAM reporter	200 nM	
ROX reporter	200 nM	

Table S6. All DNA sequences

Name Sequence	**Sequence (5'→3')**	**Label**
Primer-SIGLEC1-F	TGCCTCTACCTCCACCTACTT	/
Primer-SIGLEC1-R	GCCCATGCTCTGCTTACAAA	/
Primer-LY6E-F	ATCTGTACTGCCTGAAGCC	/
Primer-LY6E-R	GGAAGCCACACCAACATTGAC	/
Primer-TRDV3-F	TGCTCTGCACTTACGACACT	/
Primer-TRDV3-R	GCACCTTCTGATCTGCTGTT	/
Primer-IFIT1-F	ATGGGCCTTGCTGAAGTGTG	/
Primer-IFIT1-R	TCAGGGTTTTCAGGGTCCAC	/
Primer-VNN1-F	AAGCCATGCGATACCAGTGA	/
Primer-VNN1-R	TGCCAAAACTTCCAAAGGTGG	/
Primer-CD177-F	CCACACCAGACAAATCGG	/
Primer-CD177-R	GCAGGAAGGGCAAACCACTC	/
Primer-ARG1-F	TGGACAGACTAGGAATTGGCA	/
Primer-ARG1-R	CCAGTCCGTCAACATCAAAACT	/
A Probe S-1-top	TAGAGGTGGGGGCCTGCTACA	/
A Probe S-1-bottom	GCCTCCTTCTCCAGGTGTAGCAGGCCCC CACCTCTACCTCACTCACCTCATCACTA	/
A Probe S-2-top	TAGAGGTGGTGGGCCTCCTGC	/
A Probe S-2-bottom	GGCCCAACAGCAGGAGCAGGAGGCCCA CCACCTCTACCTCACTCACCTCATCACTA	/
A Probe S-3-top	TAGAGGTCCAGCAGCTGCTCT	/
A Probe S-3-bottom	GCAGTCCCAGGACCCAGAGCAGCTGCT GGACCTCTACCTCACTCACCTCATCACTA	/
A Probe S-4-top	TAGAGGGGGTCAGAGCCCTGC	/
A Probe S-4-bottom	ACTGATGCAGGCGGTGCAGGGCTCTGAC CCCCTCTACCTCACTCACCTCATCACTA	/
A Probe L-1-top	TAGAGGCATCTGCTCCGACCA	/
A Probe L-1-bottom	ACGCAGTAGTTGTCCTGGTCGGAGCAGA TGCCTCTACCTCACTCACCTCATCACTA	/
A Probe L-2-top	TAGAGGGACTGTGTCTGCTAG	/
A Probe L-2-bottom	TTCCCAATGCCGGCACTAGCAGACACAG TCCCTCTACCTCACTCACCTCATCACTA	/
A Probe L-3-top	TAGAGGTCTCGTGACATTTGG	/
A Probe L-3-bottom	TTGCTCAGGCTGTGGCCAAATGTCACGA GACCTCTACCTCACTCACCTCATCACTA	/
A Probe L-4-top	TAGAGGGACCTGTTCCCCGGC	/
A Probe L-4-bottom	TCTGGGATGGGGCAGGCCGGGGAACAG GTCCCTCTACCTCACTCACCTCATCACTA	/
A Probe T-1-top	TAGAGGTCTGGTACCGGATAA	/
A Probe T-1-bottom	AGGAATAATCTGGCCTTATCCGGTACCA GACCTCTACCTCACTCACCTCATCACTA	/
A Probe I-1-top	TAGAGGTTATGAACGGGCCAA	/
A Probe I-1-bottom	TTTTCAAAGCAGGCCTTGGCCCGTTCAT AACCTCTACCTCACTCACCTCATCACTA	/
A Probe V-1-top	GATTGTGACTTTCAAGGGTGT	/
A Probe V-1-bottom	ATCTATCCACCTCCACCTCTACACCCTTG AAAGTCACAATCTCAGGCTCCTTGGGT	/

A Probe V-2-top	AAATCAATTCAATGTGGGTGT	/
A Probe V-2-bottom	ATCTATCCACCTCCACCTCTACACCCACA TTGAATTGATTTTCACCCATGAAAAGG	/
A Probe V-3-top	CTACCATAAGCAAAAGGGTGT	/
A Probe V-3-bottom	ATCTATCCACCTCCACCTCTACACCCTTT TGCTTATGGTAGCGTGCCACCAGTTTT	/
A Probe V-4-top	ATTTGATTCTCAAGGGGGTGT	/
A Probe V-4-bottom	ATCTATCCACCTCCACCTCTACACCCCCT TGAGAATCAAATACCACATCAGTGTTG	/
A Probe V-5-top	TGGCCGTTACCAATAGGGTGT	/
A Probe V-5-bottom	ATCTATCCACCTCCACCTCTACACCCTAT TGGTAACGGCCATCAGGGGGACACTGA	/
A Probe C-1-top	GAAGCGTGATGTGCAGGGTGT	/
A Probe C-1-bottom	ATCTATCCACCTCCACCTCTACACCCTGC ACATCACGCTTCTCACGCGCAGAGAAG	/
A Probe C-2-top	GCATGAGGGAGGTGGGGGTGT	/
A Probe C-2-bottom	ATCTATCCACCTCCACCTCTACACCCCCA CCTCCCTCATGCTGAGAGGCAGGAGGC	/
A Probe C-3-top	GTCTCTCACTTGGGGGGGTGT	/
A Probe C-3-bottom	ATCTATCCACCTCCACCTCTACACCCCCC CAAGTGAGAGACTCCAGGCCCTCAGCC	/
A Probe C-4-top	GGCCCCAGCGCTGTGGGGTGT	/
A Probe C-4-bottom	ATCTATCCACCTCCACCTCTACACCCCAC AGCGCTGGGGCCAGTGCCAGCCCCACC	/
A Probe A-1-top	CTAGGAAGAAAGAAAGGGTGT	/
A Probe A-1-bottom	ATCTATCCACCTCCACCTCTACACCCTTT CTTTCTTCCTAGTAGATAGCTGAGTGT	/
B probe E top	GGGTGTAGAGGTGGAGGTGGATAGAT	/
B probe E bottom	ACAACCACTTACTTCTTCATCTATCCACC TCCACCTCT	/
B probe F top	TAGTGATGAGGTGAGTGAGGTAGAGG	/
B probe F bottom	CCTCACTCACCTCATCACTACTATCATCA CACATCTAT	/
N probe top	CTATCATCACACATCTATACAACCACTTA CTTCTTC	/
N probe bottom	GGATAGATGAAGAAGTAAGTGGTTGTAT AGATGTGTGATGATAGTAGTGATG	/
Restoration probe E top	ATAGATGAAGAAGTAAGTGGTTGTTGAG AT	/
Restoration probe E bottom	CATCCAATCACATACATCTCAACAACCA CTTACTTCTTC	/
Restoration probe F top	TACATAATAGATGTGTGATGATAGTAGTG A	/
Restoration probe F bottom	CTATCATCACACATCTATTATGTACATAAC ACAATCACA	/
Fuel E	TCTCAACAACCACTTACTTCTTC	/
Fuel F	CTATCATCACACATCTATTATGT	/
Final FAM reporter top	CATCCAATCACATAC	5’-BHQ1

Final FAM reporter bottom	TGAGATGTATGTGATTGGATG	3’-FAM
Final ROX reporter top	CATAACACAATCACA	3’-BHQ2
Final ROX reporter bottom	TGTGATTGTGTTATGTACATA	5’-ROX
B Reporter FAM (a)	GGATAGATGAAGAAGTAAGTGGTTGT	3’-FAM
B Reporter FAM (b)	ACAACCACTTACTTCTTC	5’-BHQ1
B Reporter ROX (a)	ATAGATGTGTGATGATAGTAGTGATG	5’-ROX
B Reporter ROX (b)	CTATCATCACACATCTAT	3’-BHQ2
A Reporter FAM (a)	GGGTGTAGAGGTGGAGGTGG	3’-FAM
A Reporter FAM (b)	CCACCTCCACCTCT	5’-BHQ1
A Reporter ROX (a)	TGAGGTGAGTGAGGTAGAGG	5’-ROX
A Reporter ROX (b)	CCTCACTCACCTCA	3’-BHQ2