Supporting Information for

A CRISPR/Cas12 trans-cleavage reporter enabling label-free colorimetric detection of SARS-CoV-2 and its variants

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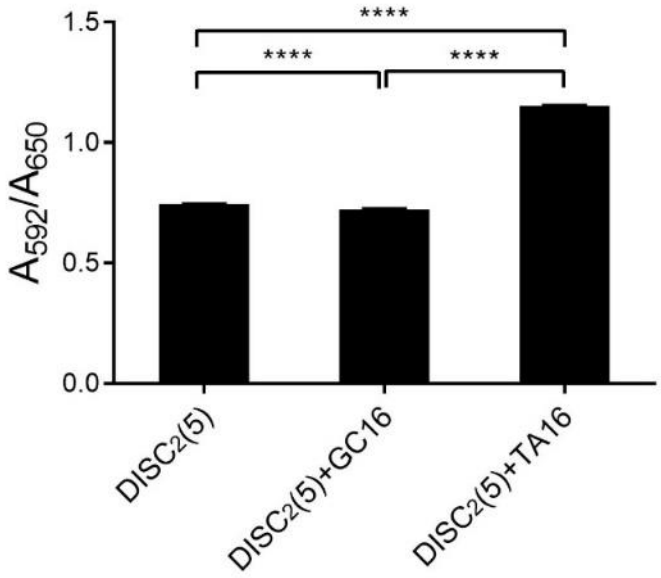


Figure S1. Plot of A_{592}/A_{650} values versus the samples of $DISC_2(5)$ with different DNA sequences. The final concentrations of TA16, GC16, and $DISC_2(5)$ were 4 μM, 4 μM, and 50 μM, respectively. Error bars were calculated from triplicate experiments. Two-tailed unpaired t-test with Welch's correction was used to analyze the statistical significance between samples ($n = 3$; ****$P < 0.0001$).

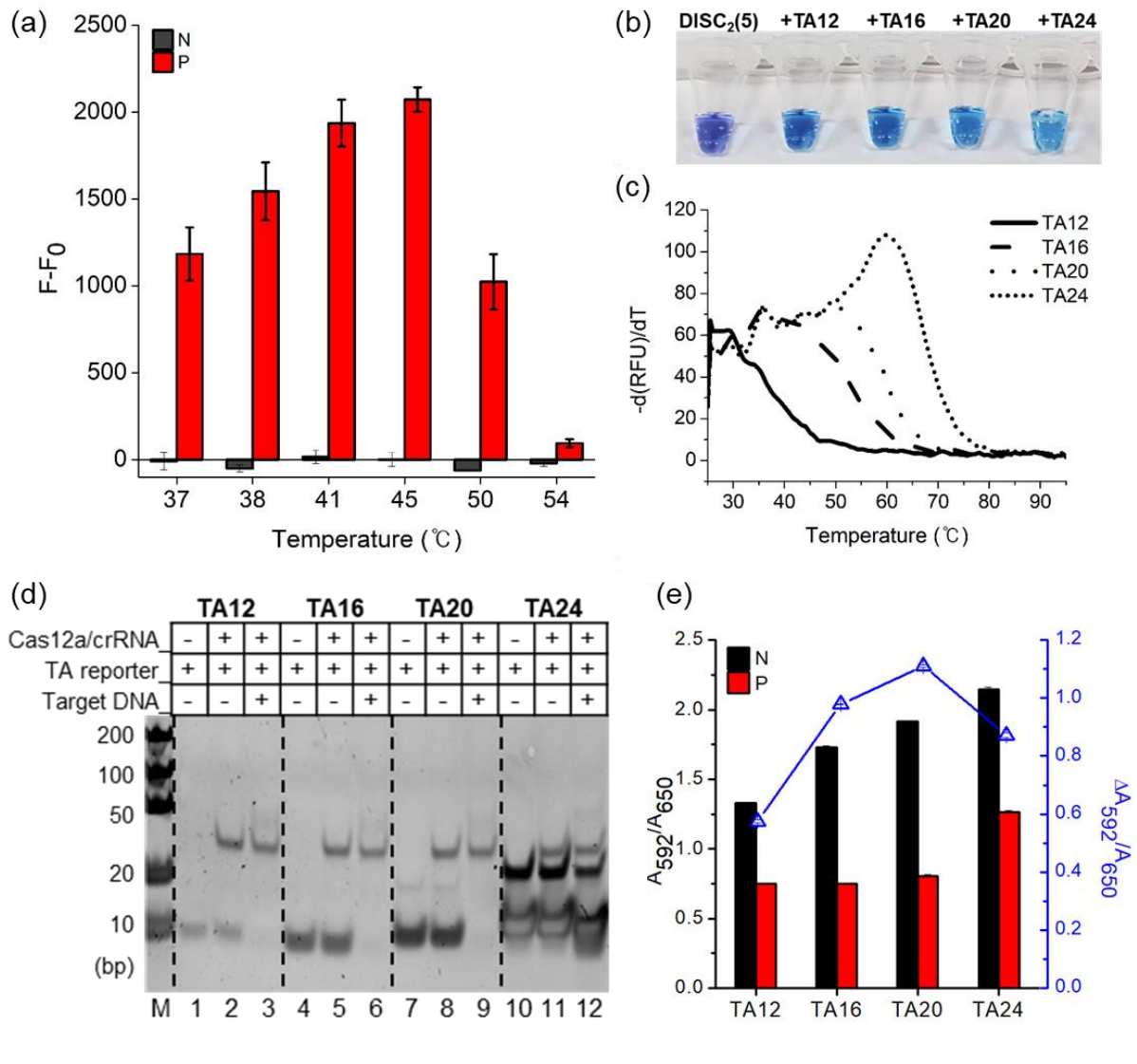
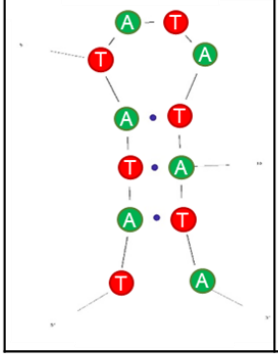
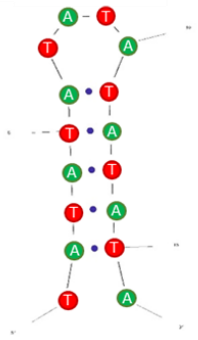
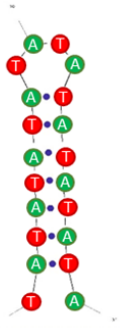
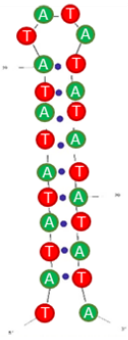


Figure S2. Optimization of TA repeat sequences. (a) Plot of fluorescence signal change ($F-F_0$) versus reaction temperature in the absence (N) or presence (P) of target DNA. $F-F_0$ was obtained by deducting the initial fluorescence intensity (F_0) from the fluorescence intensity after Cas12a reaction of 1 h (F). The final concentrations of target DNA, Cas12a/crRNA, and F-Q reporter (FAM-BHQ1) were 100 pM, 50 nM, and 200 nM, respectively. Error bars were calculated from triplicate experiments. (b) Photograph of $DISC_2(5)$ in the absence or presence of TA repeat sequences with different lengths at room temperature. The final concentrations of TA repeat sequences and $DISC_2(5)$ were 4 μM and 50 μM, respectively. (c) Melting curve analysis of TA repeat sequences with different lengths. Melting temperatures (T_m) of TA12,

TA16, TA20, and TA24 were 25.5, 35.5, 50.5, and 59.5 ℃, respectively. (d) PAGE analysis result of TADICA reaction (45 ºC and 30 min) with various TA repeat sequences. The final concentrations of target DNA, Cas12a/crRNA, and TA repeat sequences were 5 nM, 50 nM, and 200 nM, respectively. (e) Plot of A_{592}/A_{650} (left) and $\Delta(A_{592}/A_{650})$ (right) versus TA repeat sequences with different lengths, obtained from the TADICA reaction at 45 ºC for 2 h in the absence (N) or presence (P) of target DNA. The final concentrations of TA repeat sequences, $DISC_2(5)$, Cas12a/crRNA, and target DNA are 4 μM, 20 μM, 50 nM, and 5 nM, respectively. Error bars were calculated from triplicate experiments.

(a)

TA12	TA16	TA20	TA24
			

(b)

		TA12	TA16	TA20	TA24
Hairpin	ΔG (kcal.mole^{-1})	1.1	0.2	-0.7	-1.6
	T_m (℃)	31.5	43.4	49.2	52.7
Self-dimer	ΔG (kcal.mole^{-1})	-13.14	-18.01	-22.88	-27.75
	T_m (℃)	26.5	36.8	43.1	47.5

Figure S3. Computational analysis of TA repeat sequences using Oligo Analyzer 3.1. (a) Hairpin structure of TA repeat sequences. (b) Free energy change (ΔG) and T_m of hairpin structure and self-dimer. Parameters were set to match the TADICA reaction conditions: Target type = DNA, [Oligo] = 4 μM, [Na^+] = 50 mM, [Mg^{2+}] = 10 mM, [dNTP] = 0 mM, and temperature = 45 ℃.

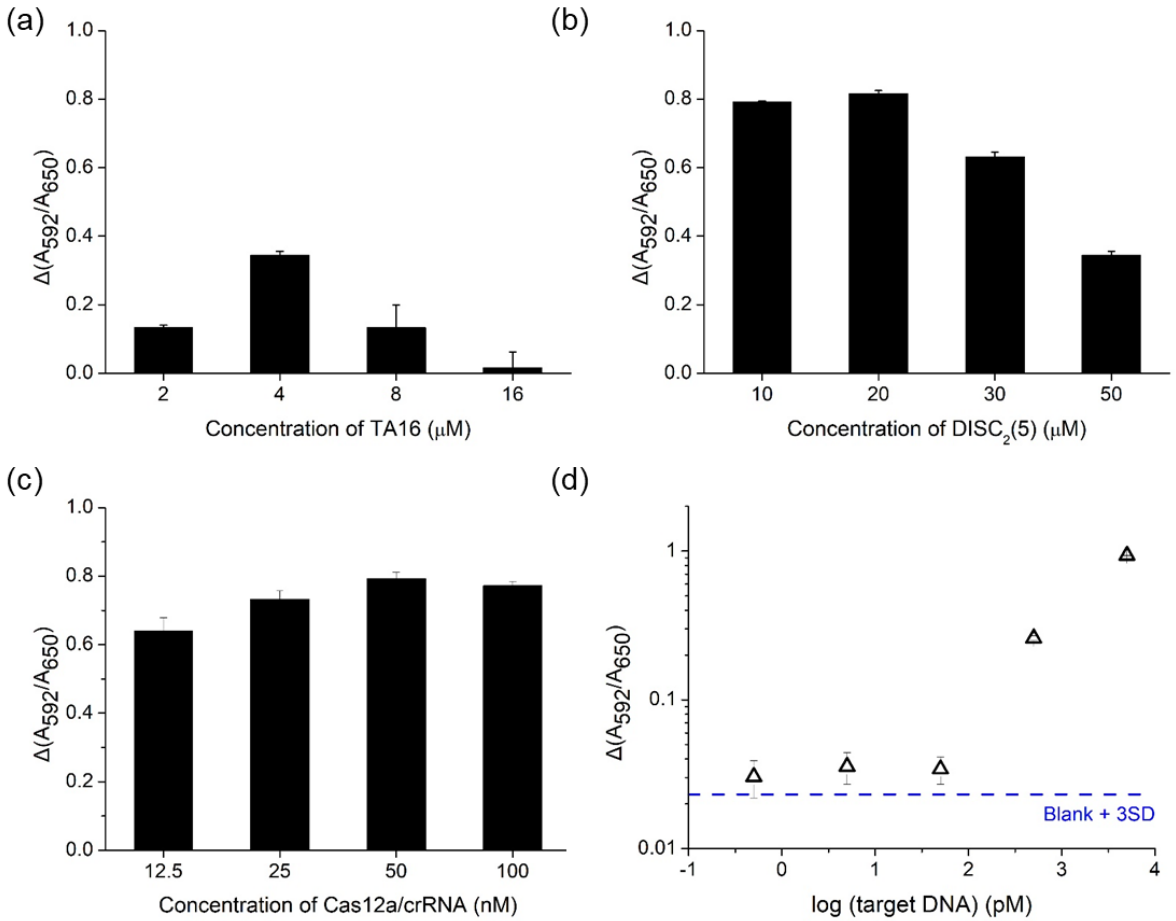


Figure S4. Optimization and sensitivity of the TADICA reaction. The TADICA reaction was conducted at 45 ℃ for 2 h with different reaction conditions. (a) Plot of $\Delta(A_{592}/A_{650})$ versus concentration of TA16. The final concentrations of target DNA, Cas12a/crRNA, and $DISC_2(5)$ were 5 nM, 50 nM, and 50 μM, respectively. (b) Plot of $\Delta(A_{592}/A_{650})$ versus concentration of $DISC_2(5)$. The final concentrations of target DNA, Cas12a/crRNA, and TA repeat sequences were 5 nM, 50 nM, and 4 μM, respectively. (c) Plot of $\Delta(A_{592}/A_{650})$ versus concentration of Cas12a/crRNA. The final concentrations of target DNA, $DISC_2(5)$, and TA repeat sequences were 5 nM, 20 μM, and 4 μM, respectively. (d) Plot of $\Delta(A_{592}/A_{650})$ versus concentration of target DNA. The final concentrations of TA repeat sequences, $DISC_2(5)$, and Cas12a/crRNA were 4 μM, 20 μM, and 50 nM, respectively. Error bars were calculated from triplicate experiments.

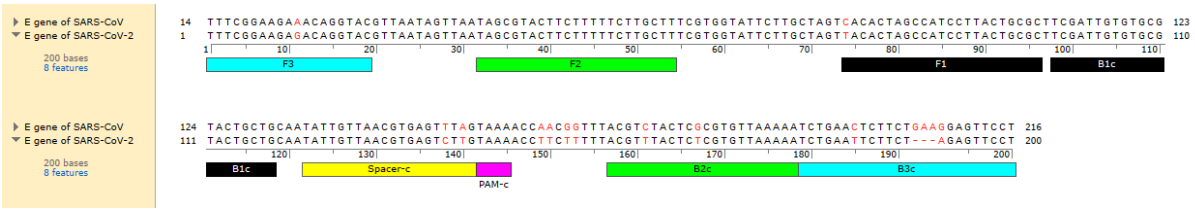


Figure S5. Alignment of SARS-CoV (NC_004718) and SARS-CoV-2 (MN908947.3) E gene for LAMP primer design. The alignment was conducted by SnapGene.

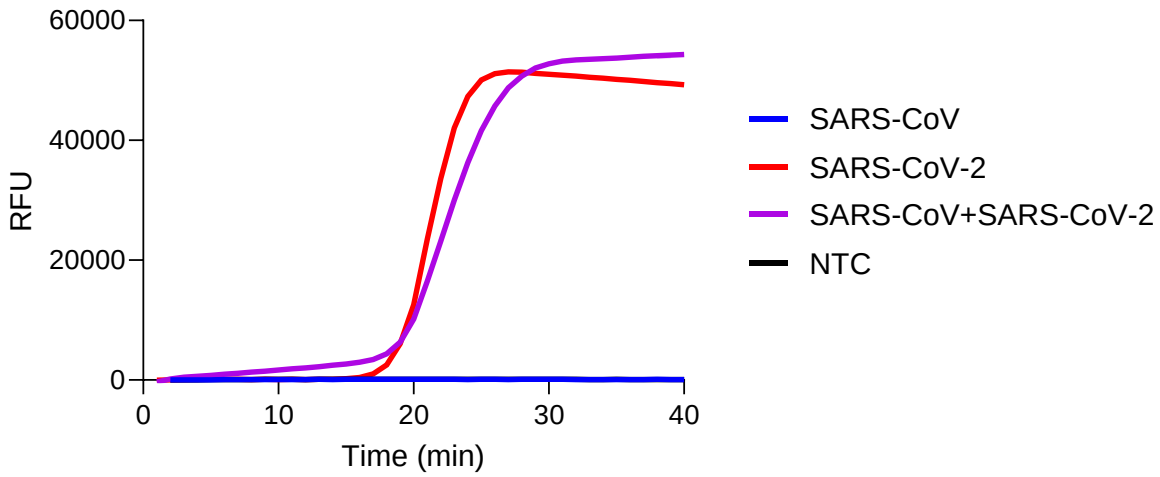


Figure S6. Real-time fluorescence curves of the LAMP reaction using SARS-CoV-2 E gene primer. Concentrations of SARS-CoV and SARS-CoV-2 RNA were 40,000 copies/μL.

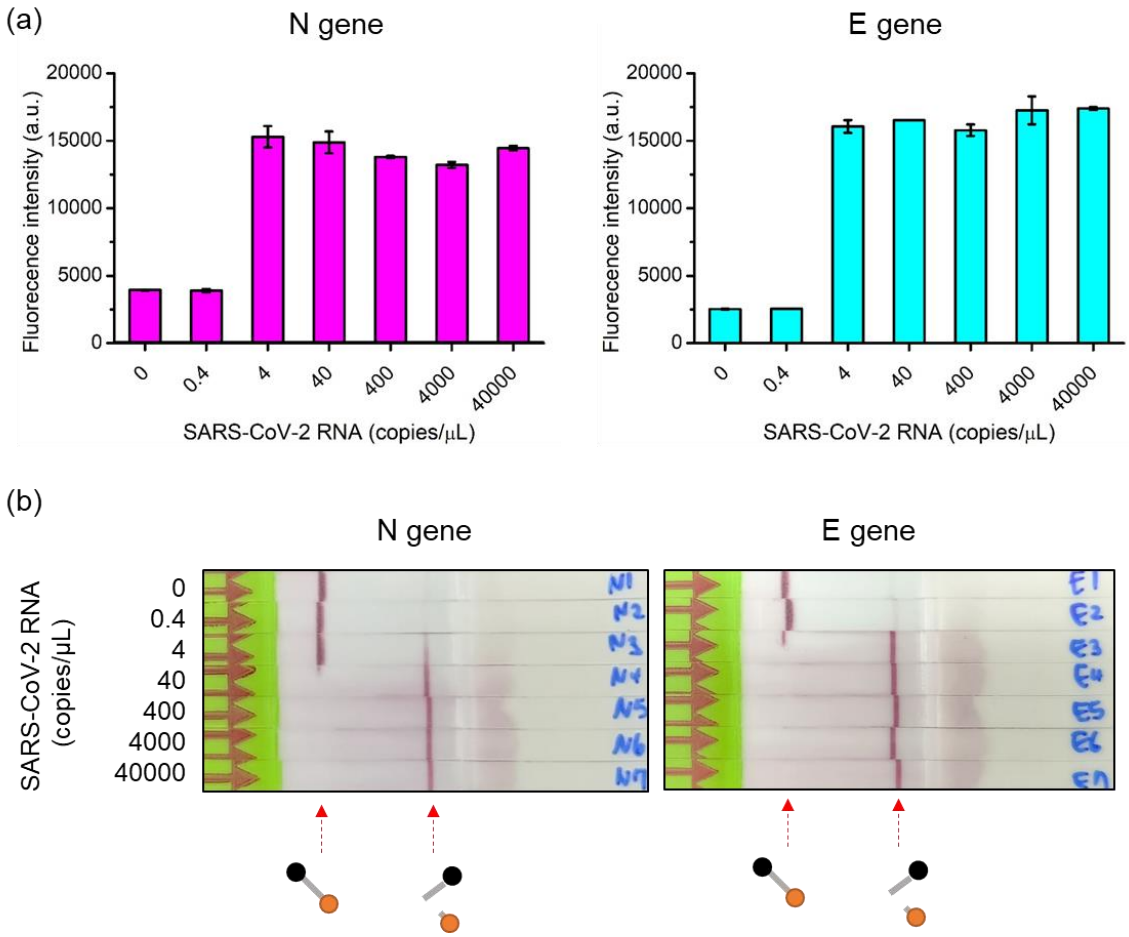


Figure S7. CRISPR/Cas-based SARS-CoV-2 assays with conventional dual-labeled DNA reporters in conjunction with LAMP. (a) Plot of fluorescence intensity versus various concentrations of SARS-CoV-2 RNA. Error bars were calculated from triplicate experiments. (b) Photographs of LFA strips after assaying various concentrations of SARS-CoV-2 RNAs.

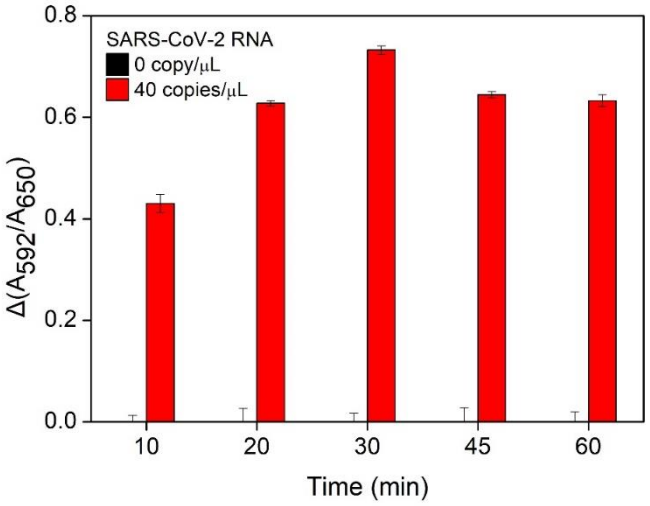


Figure S8. Reaction time optimization of the LAMP-TADICA assay. Plot of $\Delta(A_{592}/A_{650})$ versus TADICA reaction time with LAMP products. Error bars were calculated from triplicate experiments.

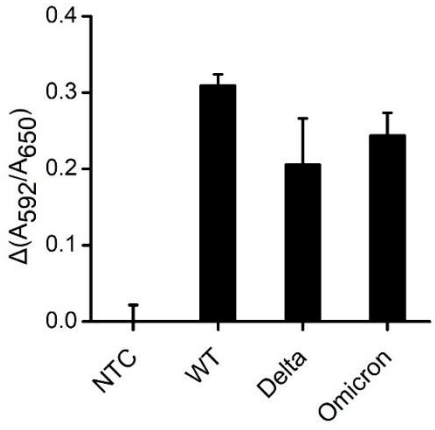


Figure S9. Plot of $\Delta(A_{592}/A_{650})$ versus SARS-CoV-2 variants after the RPA-TADICA reaction with the universal crRNA. The final concentrations of Cas12a/crRNA, TA repeat sequences, $DISC_2(5)$, and SARS-CoV-2 RNA were 50 nM, 4 μM, 20 μM, and 5 copies/μL, respectively. Error bars were calculated from triplicate experiments.

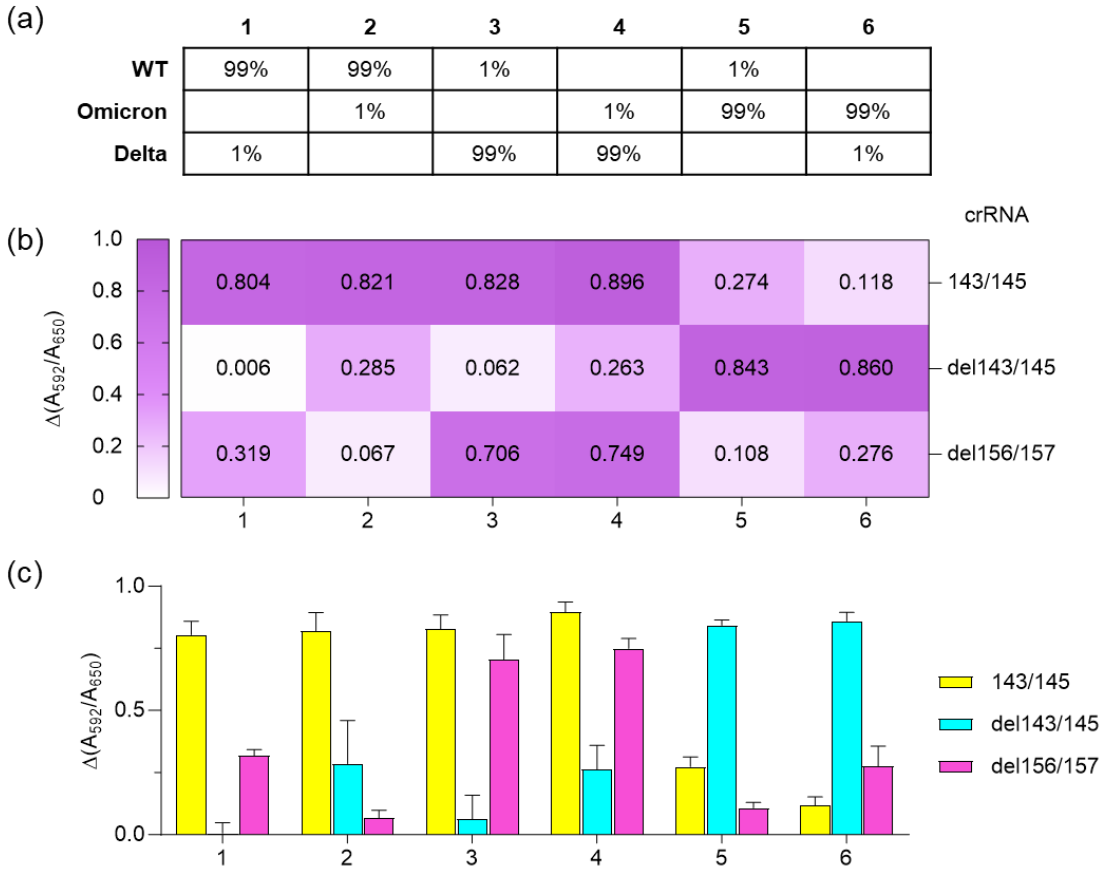
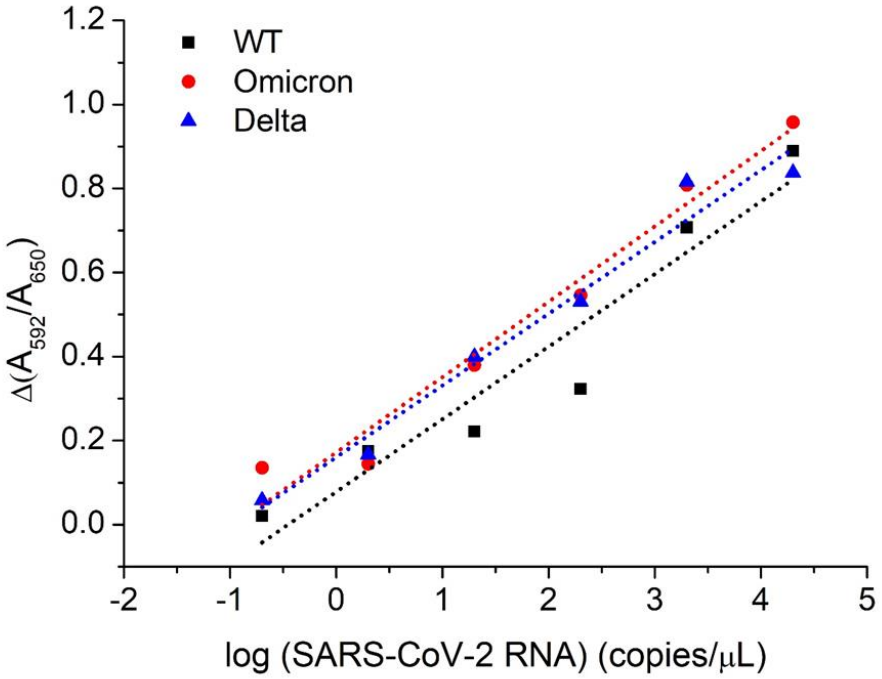


Figure S10. RPA-TADICA assay for mixed SARS-CoV-2 variant detection at significantly different loads: 200 copies/μL and 20,000 copies/μL. (a) Mixed ratio of three SARS-CoV-2 gRNAs (1%: 200 copies/μL; 99%: 20,000 copies/μL) across the samples. (b) Heatmap and (c) plot of the $\Delta(A_{592}/A_{650})$ values after RPA-TADICA reaction of the samples with target-specific crRNAs (143/145, del143/145, and del156/157). Error bars were calculated from triplicate experiments.



	Linear equation	R^2
WT	$\Delta(A_{592}/A_{650})=0.17271\log C_{target}+0.07839$	0.90299
Omicron	$\Delta(A_{592}/A_{650})=0.17929\log C_{target}+0.17219$	0.95947
Delta	$\Delta(A_{592}/A_{650})=0.17086\log C_{target}+0.17086$	0.96441

Figure S11. Linear relationships between $\Delta(A_{592}/A_{650})$ and the logarithm of WT, delta, and omicron SARS-CoV-2 RNA concentrations (C_{target}) after the RPA-TADICA reactions with the crRNAs targeting 143/145, del156/157, and del143/145, respectively.

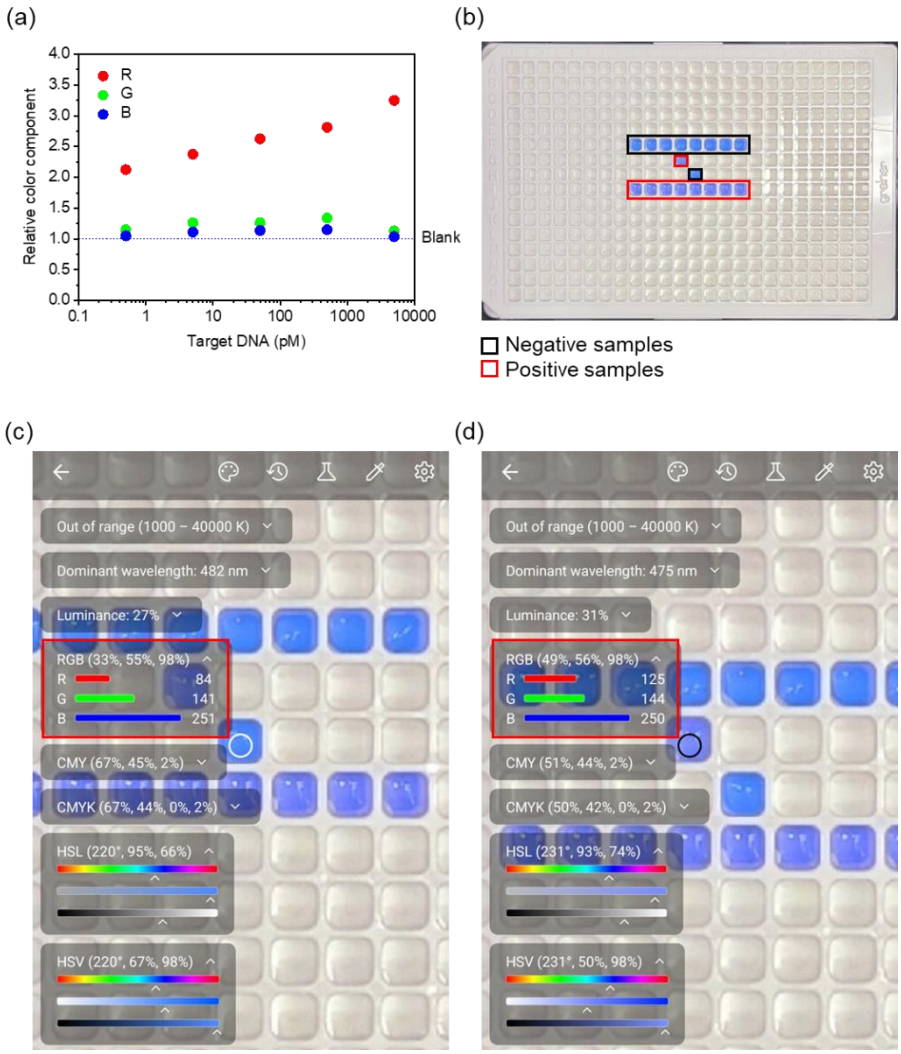


Figure S12. Smartphone-based analysis of the TADICA assay results. (a) Relative RGB values of the TADICA reaction solutions with synthetic target ssDNAs ranging from 0.5 pM to 5 nM. The relative color component was determined by dividing the RGB values of the sample by those of the blank. (b-d) Smartphone-based diagnosis of twenty COVID-19 patients using the LAMP-TADICA assay. (b) Image of the LAMP-TADICA reaction solutions with 20 clinical samples. (c-d) Representative images analyzing wells containing negative (c) and positive (d) samples.

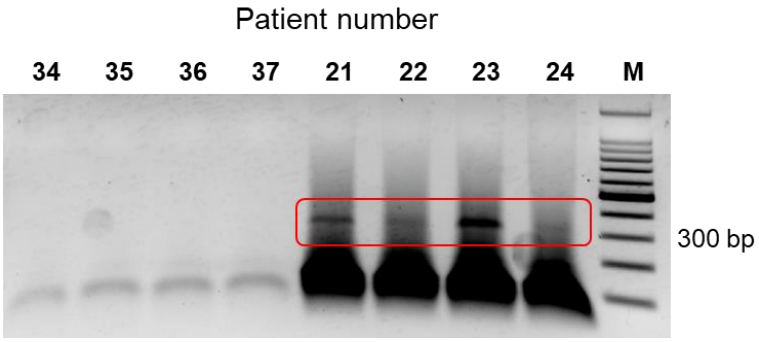


Figure S13. PAGE analysis result of RPA product with selected clinical samples (M: 100 bp DNA ladder). The RPA product band (299 bp) was observed only from positive clinical samples.

Table S1. Oligonucleotide sequences used in this work.

Classification	Name	Sequence (5' → 3')[a-c]
TA repeat sequences	TA12	TAT ATA TAT ATA
	TA16	TAT ATA TAT ATA TAT A
	TA20	TAT ATA TAT ATA TAT ATA TA
	TA24	TAT ATA TAT ATA TAT ATA TAT ATA
GC repeat sequences	GC16	GCG CGC GCG CGC GCG C
F-Q reporter	FAM-BHQ1 reporter	FAM-TTA TT-BHQ1
	ROX-BHQ2 reporter	ROX-CCC CCC CC-BHQ2
LFA reporter	FAM-biotin reporter	FAM-TTA TTA TT-biotin
Synthetic target DNA	Target DNA	AGC GCT GGG GGC AAA TTG TGC AAT TTG CGG
crRNA for synthetic target DNA	crRNA	/AltR1/rUrA rArUrU rUrCrU rArCrU rCrUrU rGrUrA rGrArU rArArU rUrGrC rArCrA rArUrU rUrGrC rCrCrC rCrA/AltR2/
crRNA for SARS-CoV-2 LAMP products	crRNA (N)	/AltR1/rUrA rArUrU rUrCrU rArCrU rCrUrU rGrUrA rGrArU rUrArA rUrCrA rGrUrU rCrCrU rUrGrU rCrUrG rArU/AltR2/
	crRNA (E)	/AltR1/rUrA rArUrU rUrCrU rArCrU rCrUrU rGrUrA rGrArU rCrArA rGrArC rUrCrA rCrGrU rUrArA rCrArA rUrA/AltR2/
qRT-PCR primer for SARS-CoV-2 N gene	N FP	GCC AAA AGG CTT CTA CGC A
	N RP	TTT GGC CTT GTT GTT GTT GG
RT-LAMP primer for SARS-CoV-2 N gene	N F3	ACA ATG TAA CAC AAG CTT TCG
	N B3	GTA GGT CAA CCA CGT TCC
	N LF	TGG TCC CCA AAA TTT CCT
	N LB	ATT GGC ATG GAA GTC ACA

Classification	Name	Sequence (5' → 3')[a-c]
	N FIP	TCA GTT CCT TGT CTG ATT AGT TCC TAG ACG TGG TCC AGA ACA A
	N BIP	TGG CCG CAA ATT GCA CAA TTG TGT GAC TTC CAT GCC AA
RT-LAMP primer for SARS-CoV-2 E gene	E F3	TTT CGG AAG AGA CAG GTA C
	E B3	AGG AAC TCT AGA AGA ATT CAG A
	E LF	ACT AGC AAG AAT ACC ACG
	E LB	TCT TGT AAA ACC TTC TTT
	E FIP	CGC AGT AAG GAT GGC TAG TGT AGC GTA CTT CTT TTT CTT GCT T
	E BIP	TCG ATT GTG TGC GTA CTG CTG TTT TTA ACA CGA GAG TAA ACG T
RT-RPA primer for SARS-CoV-2 S gene	S FP	HTR TCT CTG GGA CCA ATG GTA CTA AGA GGT TT
	S RP	AAA AGT GCA ATT ATT CGC ACT AGA ATA AAC TC
	S RT	AGG CTG AGA GAC ATA TTC
crRNA for SARS-CoV-2 RPA products	crRNA-universal	/AltR1/rUrArA rUrUrU rCrUrA rCrUrC rUrUrG rUrArG rArUrG rUrArC rUrArC rUrUrU rArGrA rUrUrC rGrArArG/AltR2/
	crRNA-143/145	/AlTR1/rUrArA rUrUrU rCrUrA rCrUrC rUrUrG rUrArG rArUrG rGrUrG rUrUrU rArUrU rArCrC rArCrA rArArArA/AlTR2/
	crRNA-del143/145	/AlTR1/rUrArA rUrUrU rCrUrA rCrUrC rUrUrG rUrArG rArUrG rArCrC rArCrA rArArA rArCrA rArCrA rArArArG/AlTR2/
	crRNA-del156/157	/AlTR1/rUrArA rUrUrU rCrUrA rCrUrC rUrUrG rUrArG rArUrG rArUrG rGrArA rArGrU rGrGrA rGrUrU rUrArUrU/AlTR2/

[a] Sequences written in red represent complementary sequences between target DNA and crRNA.

[b] Abbreviations: FAM, carboxyfluorescein; BHQ-1, black hole quencher-1.

[c] 'rA' represents RNA oligonucleotides.

Table S2. Diagnostic results of 119 clinical samples using LAMP-TADICA and qRT-PCR.

Patient #	Sample type	LAMP-TADICA			qRT-PCR		Match
		$\Delta(A_{592}/A_{650})$	SD	Diagnosis[(a)]	C_t	Diagnosis[(b)]	
1	NPS	1.21	0.10	Positive	29.40	Positive	O
2	NPS	1.21	0.10	Positive	23.80	Positive	O
3	NPS	1.17	0.09	Positive	33.01	Positive	O
4	NPS	1.12	0.07	Positive	22.80	Positive	O
5	NPS	1.12	0.17	Positive	22.50	Positive	O
6	NPS	1.11	0.19	Positive	31.20	Positive	O
7	NPS	1.09	0.08	Positive	30.10	Positive	O
8	NPS	0.98	0.20	Positive	28.30	Positive	O
9	NPS	0.97	0.05	Positive	11.30	Positive	O
10	NPS	0.94	0.05	Positive	13.10	Positive	O
11	NPS	0.88	0.14	Positive	28.31	Positive	O
12	NPS	0.87	0.11	Positive	28.30	Positive	O
13	NPS	0.86	0.09	Positive	21.20	Positive	O
14	NPS	0.83	0.07	Positive	13.80	Positive	O
15	NPS	0.82	0.12	Positive	18.40	Positive	O
16	NPS	0.82	0.11	Positive	30.10	Positive	O
17	NPS	0.80	0.05	Positive	28.31	Positive	O
18	NPS	0.80	0.10	Positive	32.10	Positive	O
19	NPS	0.80	0.06	Positive	32.10	Positive	O
20	NS	0.78	0.01	Positive	8.90	Positive	O
21	NS	0.77	0.02	Positive	8.30	Positive	O
22	NS	0.77	0.01	Positive	20.09	Positive	O
23	NS	0.77	0.01	Positive	13.10	Positive	O
24	NS	0.77	0.01	Positive	31.10	Positive	O
25	NS	0.77	0.01	Positive	10.81	Positive	O
26	NS	0.76	0.01	Positive	20.60	Positive	O
27	NPS	0.76	0.10	Positive	35.80	Positive	O
28	NS	0.75	0.02	Positive	22.40	Positive	O
29	NPS	0.75	0.07	Positive	26.90	Positive	O
30	NS	0.74	0.01	Positive	25.20	Positive	O
31	NS	0.74	0.02	Positive	25.70	Positive	O
32	NPS	0.74	0.01	Positive	19.05	Positive	O
33	NPS	0.73	0.02	Positive	34.50	Positive	O
34	NPS	0.71	0.02	Positive	11.30	Positive	O
35	NPS	0.70	0.04	Positive	25.40	Positive	O
36	NPS	0.69	0.08	Positive	29.16	Positive	O
37	NPS	0.68	0.07	Positive	23.10	Positive	O
38	NPS	0.68	0.10	Positive	12.50	Positive	O
39	NPS	0.67	0.00	Positive	28.12	Positive	O
40	NPS	0.65	0.01	Positive	28.30	Positive	O
41	NPS	0.63	0.04	Positive	28.31	Positive	O
42	NPS	0.60	0.01	Positive	33.40	Positive	O
43	NPS	0.60	0.06	Positive	26.30	Positive	O
44	NPS	0.58	0.13	Positive	29.10	Positive	O
45	NPS	0.57	0.06	Positive	17.80	Positive	O
46	NPS	0.54	0.09	Positive	11.30	Positive	O
47	NPS	0.53	0.10	Positive	28.90	Positive	O
48	NPS	0.52	0.06	Positive	26.16	Positive	O
49	NPS	0.52	0.15	Positive	21.30	Positive	O
50	NPS	0.51	0.04	Positive	25.40	Positive	O
51	NPS	0.51	0.08	Positive	23.40	Positive	O
52	NPS	0.49	0.04	Positive	13.84	Positive	O
53	NPS	0.45	0.03	Positive	23.60	Positive	O
54	NPS	0.44	0.17	Positive	30.22	Positive	O
55	NPS	0.43	0.01	Positive	25.34	Positive	O
56	NPS	0.42	0.15	Positive	33.10	Positive	O
57	NPS	0.41	0.10	Positive	14.20	Positive	O
58	NPS	0.40	0.03	Positive	17.50	Positive	O
59	NPS	0.39	0.04	Positive	34.20	Positive	O
60	NPS	0.36	0.01	Positive	28.10	Positive	O
61	OS	0.10	0.01	Negative	N/A	Negative	O
62	OS	0.07	0.02	Negative	N/A	Negative	O

Patient #	Sample type	LAMP-TADICA			qRT-PCR		Match
		$\Delta(A_{592}/A_{650})$	SD	Diagnosis[a]	C_t	Diagnosis[b]	
63	OS	0.06	0.01	Negative	N/A	Negative	O
64	NPS	0.05	0.10	Negative	N/A	Negative	O
65	OS	0.04	0.02	Negative	N/A	Negative	O
66	OS	0.01	0.03	Negative	N/A	Negative	O
67	NPS	0.01	0.03	Negative	N/A	Negative	O
68	NPS	0.01	0.12	Negative	N/A	Negative	O
69	OS	0.00	0.06	Negative	N/A	Negative	O
70	NPS	0.00	0.02	Negative	N/A	Negative	O
71	NS	0.00	0.06	Negative	N/A	Negative	O
72	OS	-0.01	0.05	Negative	N/A	Negative	O
73	OS	-0.02	0.02	Negative	N/A	Negative	O
74	NPS	-0.02	0.05	Negative	N/A	Negative	O
75	NPS	-0.03	0.13	Negative	N/A	Negative	O
76	NPS	-0.04	0.11	Negative	N/A	Negative	O
77	NPS	-0.05	0.14	Negative	N/A	Negative	O
78	NPS	-0.06	0.06	Negative	N/A	Negative	O
79	NPS	-0.06	0.10	Negative	N/A	Negative	O
80	NPS	-0.06	0.13	Negative	N/A	Negative	O
81	NPS	-0.07	0.02	Negative	N/A	Negative	O
82	NPS	-0.07	0.11	Negative	N/A	Negative	O
83	NPS	-0.07	0.09	Negative	N/A	Negative	O
84	NPS	-0.09	0.04	Negative	N/A	Negative	O
85	NPS	-0.09	0.04	Negative	N/A	Negative	O
86	NPS	-0.09	0.11	Negative	N/A	Negative	O
87	NPS	-0.09	0.02	Negative	N/A	Negative	O
88	NPS	-0.09	0.11	Negative	N/A	Negative	O
89	NPS	-0.10	0.04	Negative	N/A	Negative	O
90	NPS	-0.10	0.03	Negative	N/A	Negative	O
91	NPS	-0.13	0.12	Negative	N/A	Negative	O
92	NPS	-0.14	0.01	Negative	N/A	Negative	O
93	NPS	-0.15	0.20	Negative	N/A	Negative	O
94	NPS	-0.16	0.20	Negative	N/A	Negative	O
95	NPS	-0.17	0.08	Negative	N/A	Negative	O
96	NPS	-0.17	0.05	Negative	N/A	Negative	O
97	NPS	-0.19	0.09	Negative	N/A	Negative	O
98	NPS	-0.20	0.06	Negative	N/A	Negative	O
99	NPS	-0.21	0.08	Negative	N/A	Negative	O
100	NPS	-0.22	0.18	Negative	N/A	Negative	O
101	NPS	-0.22	0.09	Negative	N/A	Negative	O
102	NPS	-0.22	0.09	Negative	N/A	Negative	O
103	NPS	-0.23	0.02	Negative	N/A	Negative	O
104	NPS	-0.24	0.06	Negative	N/A	Negative	O
105	NPS	-0.24	0.13	Negative	N/A	Negative	O
106	NPS	-0.25	0.06	Negative	N/A	Negative	O
107	NPS	-0.25	0.03	Negative	N/A	Negative	O
108	NPS	-0.26	0.14	Negative	N/A	Negative	O
109	NPS	-0.27	0.01	Negative	N/A	Negative	O
110	NPS	-0.27	0.24	Negative	N/A	Negative	O
111	NPS	-0.29	0.04	Negative	N/A	Negative	O
112	NPS	-0.31	0.21	Negative	N/A	Negative	O
113	NPS	-0.32	0.09	Negative	N/A	Negative	O
114	NPS	-0.33	0.02	Negative	N/A	Negative	O
115	NPS	-0.34	0.11	Negative	N/A	Negative	O
116	NPS	-0.38	0.03	Negative	N/A	Negative	O
117	NPS	-0.41	0.09	Negative	N/A	Negative	O
118	NPS	-0.48	0.01	Negative	N/A	Negative	O
119	NPS	-0.69	0.22	Negative	N/A	Negative	O

[a] Samples with $\Delta(A_{592}/A_{650})$ values above the threshold value (blank + 5SD, 0.167), were diagnosed as positive.

[b] Samples with C_t values less than 40 were diagnosed as positive.

Table S3. Diagnostic results of 60 clinical samples using RPA-TADICA and qRT-PCR.

Patient #	Sample type	RPA-TADICA			qRT-PCR			Match
		$\Delta(A_{592}/A_{650})$	SD	Diagnosis[a]	C_t (RdRP)	C_t (E)	Diagnosis[b]	
1	NPS	0.34	0.02	Positive	13.30	18.84	Positive	O
2	NPS	0.29	0.01	Positive	14.39	13.30	Positive	O
3	NPS	0.28	0.01	Positive	15.99	24.65	Positive	O
4	NPS	0.27	0.02	Positive	14.46	17.97	Positive	O
5	NPS	0.27	0.01	Positive	16.19	16.19	Positive	O
6	NPS	0.25	0.01	Positive	23.98	15.95	Positive	O
7	NPS	0.24	0.01	Positive	14.00	25.47	Positive	O
8	NPS	0.24	0.05	Positive	21.46	19.58	Positive	O
9	NPS	0.24	0.00	Positive	15.38	22.62	Positive	O
10	NPS	0.23	0.02	Positive	14.60	36.70	Positive	O
11	NPS	0.23	0.01	Positive	22.01	27.09	Positive	O
12	NPS	0.23	0.07	Positive	17.48	19.33	Positive	O
13	NPS	0.22	0.01	Positive	20.86	38.59	Positive	O
14	NPS	0.20	0.02	Positive	19.33	29.85	Positive	O
15	NPS	0.14	0.06	Positive	19.58	15.38	Positive	O
16	NPS	0.13	0.00	Positive	18.84	14.60	Positive	O
17	NPS	0.11	0.01	Positive	29.85	17.48	Positive	O
18	NPS	0.10	0.02	Positive	36.70	23.98	Positive	O
19	NPS	0.09	0.00	Positive	22.62	21.46	Positive	O
20	NPS	0.09	0.02	Positive	27.09	20.86	Positive	O
21	NPS	0.08	0.04	Positive	38.59	22.01	Positive	O
22	NPS	0.06	0.01	Positive	25.47	14.00	Positive	O
23	NPS	0.05	0.03	Positive	24.65	14.39	Positive	O
24	NPS	0.05	0.03	Positive	17.97	14.46	Positive	O
25	NPS	0.03	0.01	Positive	15.95	15.99	Positive	O
26	NPS	0.02	0.03	Negative	N/A	N/A	Negative	O
27	NPS	0.02	0.02	Negative	N/A	N/A	Negative	O
28	NPS	0.02	0.00	Negative	N/A	N/A	Negative	O
29	NPS	0.02	0.02	Negative	N/A	N/A	Negative	O
30	NPS	0.02	0.01	Negative	N/A	N/A	Negative	O
31	NPS	0.02	0.04	Negative	N/A	N/A	Negative	O
32	NPS	0.02	0.03	Negative	N/A	N/A	Negative	O
33	NPS	0.02	0.01	Negative	N/A	N/A	Negative	O
34	NPS	0.01	0.00	Negative	N/A	N/A	Negative	O
35	NPS	0.01	0.01	Negative	N/A	N/A	Negative	O
36	NPS	0.01	0.00	Negative	N/A	N/A	Negative	O
37	NPS	0.01	0.02	Negative	N/A	N/A	Negative	O
38	NPS	0.01	0.01	Negative	N/A	N/A	Negative	O
39	NPS	0.00	0.00	Negative	N/A	N/A	Negative	O
40	NPS	0.00	0.00	Negative	N/A	N/A	Negative	O
41	NPS	0.00	0.02	Negative	N/A	N/A	Negative	O
42	NPS	-0.01	0.01	Negative	N/A	N/A	Negative	O
43	NPS	-0.01	0.04	Negative	N/A	N/A	Negative	O
44	NPS	-0.01	0.00	Negative	N/A	N/A	Negative	O
45	NPS	-0.01	0.01	Negative	N/A	N/A	Negative	O
46	NPS	-0.01	0.02	Negative	N/A	N/A	Negative	O
47	NPS	-0.01	0.00	Negative	N/A	N/A	Negative	O
48	NPS	-0.01	0.00	Negative	N/A	N/A	Negative	O
49	NPS	-0.02	0.00	Negative	N/A	N/A	Negative	O
50	NPS	-0.02	0.00	Negative	N/A	N/A	Negative	O

Patient #	Sample type	RPA-TADICA			qRT-PCR			Match
		$\Delta(A_{592}/A_{650})$	**SD**	**Diagnosis[(a)]**	**C_t (RdRP)**	**C_t (E)**	**Diagnosis[(b)]**	
51	NPS	-0.03	0.00	Negative	N/A	N/A	Negative	O
52	NPS	-0.03	0.02	Negative	N/A	N/A	Negative	O
53	NPS	-0.03	0.03	Negative	N/A	N/A	Negative	O
54	NPS	-0.04	0.01	Negative	N/A	N/A	Negative	O
55	NPS	-0.04	0.01	Negative	N/A	N/A	Negative	O
56	NPS	-0.04	0.02	Negative	N/A	N/A	Negative	O
57	NPS	-0.06	0.03	Negative	N/A	N/A	Negative	O
58	NPS	-0.07	0.02	Negative	N/A	N/A	Negative	O
59	NPS	-0.08	0.01	Negative	N/A	N/A	Negative	O
60	NPS	-0.09	0.01	Negative	N/A	N/A	Negative	O

[(a)] Samples with $\Delta(A_{592}/A_{650})$ values above the threshold value (blank + 5SD, 0.0276), were diagnosed as positive.

[(b)] Samples with C_t values less than 40 for both genes were diagnosed as positive.

Table S4. Diagnostic results of 20 clinical samples using RPA-TADICA with variant-specific crRNAs.

Patient #	WT crRNA		del143/145 crRNA		del156/157 crRNA		Result[a-c]
	$\Delta(A_{592}/A_{650})$	SD	$\Delta(A_{592}/A_{650})$	SD	$\Delta(A_{592}/A_{650})$	SD	
1	0.11	0.11	0.01	0.01	0.13	0.02	Delta
2	0.08	0.02	0.01	0.01	0.10	0.02	Delta
3	0.08	0.03	0.02	0.02	0.11	0.01	Delta
4	0.07	0.01	0.01	0.01	0.12	0.02	Delta
5	0.05	0.01	-0.03	0.01	0.11	0.01	Delta
6	0.05	0.04	-0.01	0.02	0.07	0.03	Delta
7	0.07	0.01	-0.05	0.07	0.11	0.01	Delta
8	0.06	0.03	-0.04	0.03	0.11	0.04	Delta
9	0.08	0.00	0.02	0.04	0.04	0.02	Delta
10	0.10	0.01	-0.02	0.04	0.05	0.02	Delta
11	0.02	0.06	0.13	0.03	0.02	0.01	Omicron
12	0.02	0.01	0.16	0.05	0.00	0.01	Omicron
13	0.03	0.01	0.10	0.04	-0.01	0.02	Omicron
14	0.02	0.02	0.07	0.02	0.00	0.03	Omicron
15	0.03	0.01	0.08	0.03	0.01	0.03	Omicron
16	0.01	0.02	0.09	0.04	-0.02	0.02	Omicron
17	0.02	0.01	0.08	0.04	-0.01	0.02	Omicron
18	0.01	0.01	0.11	0.01	-0.02	0.02	Omicron
19	-0.01	0.01	0.13	0.03	-0.02	0.02	Omicron
20	0.01	0.01	0.06	0.03	0.01	0.04	Omicron

[a] The $\Delta(A_{592}/A_{650})$ values were considered valid if they exceeded the threshold value (blank + 5SD, 0.038).

[b] Samples with valid $\Delta(A_{592}/A_{650})$ values for del156/157 crRNAs, but not for del143/145 crRNA, were identified as the delta variant.

[c] Samples with valid $\Delta(A_{592}/A_{650})$ values solely for del143/145 crRNA were identified as the omicron variant.

Table S5. Diagnostic results of 20 clinical samples using mutation-specific RT-PCR.

Patient #	C_t [(a)]					Result
	SARS-CoV-2	E484A	L452R	N679K	IPC[(b)]	
1	24.93	N/A	27.08	N/A	31.01	Delta
2	29.06	N/A	31.06	N/A	30.52	Delta
3	28.95	N/A	30.82	N/A	29.80	Delta
4	27.71	N/A	29.50	N/A	33.64	Delta
5	34.57	N/A	36.00	N/A	29.97	Delta
6	33.23	N/A	34.11	N/A	31.56	Delta
7	30.82	N/A	32.36	N/A	26.82	Delta
8	21.15	N/A	23.18	N/A	27.75	Delta
9	33.06	N/A	32.51	N/A	29.75	Delta
10	27.21	N/A	27.25	N/A	28.19	Delta
11	28.87	33.22	N/A	28.21	29.26	BA.1
12	25.64	26.27	N/A	25.13	27.14	BA.1
13	31.64	31.92	N/A	30.90	28.16	BA.1
14	29.14	29.36	N/A	22.22	22.56	BA.1
15	26.70	27.21	N/A	26.17	28.78	BA.1
16	29.70	29.96	N/A	28.86	30.03	BA.1
17	26.68	26.96	N/A	26.02	28.00	BA.1
18	28.22	28.84	37.67[(c)]	28.51	27.31	BA.1
19	34.58	34.29	N/A	33.67	29.37	BA.1
20	29.64	29.80	N/A	29.04	25.05	BA.1

[(a)] Cut-off value of each experiment is 39, which is provided by the manufacturer.

[(b)] IPC represents internal positive control targeting human genomic DNA.

[(c)] As per the manufacturer's guidelines, the result was considered N/A (not applicable) because validity is determined only when the ΔC_t between each target gene (E484A, L452R, N679K) and the SARS-CoV-2 gene is less than 5 C_t.

Table S6. Cost breakdown list for CRISPR reporter probes.

Method	Material	Cost[a-c] (US$)	Required amount for one reaction	Cost/hundred reactions (US$)	Equipment
Fluorescence	F-Q reporter	344.7 for 250 nmol	0.01 nmol	1.379	Fluorometer
LFA	F-B reporter	344.7 for 250 nmol	0.01 nmol	1.379	Lateral flow assay strip
TADICA	TA repeat sequences (TA16)	22.4 for 250 nmol	0.1 nmol	0.896	Smartphone or spectrometer
	$DISC_2(5)$	84.0 for 1 g	0.5 nmol	0.002	

[a] The cost of oligo nucleotides was quoted from IDT.

[b] The cost of $DISC_2(5)$ was quoted from Merck.

[c] The date of the quote is November 6th, 2023.

Table S7. Comparison of the LAMP/RPA-TADICA methods with previous CRISPR-based diagnostics for SARS-CoV-2.

Name	Principle	Limit of detection	Pre-amplification	Assay time (min)	Limitations	Reference
opvCRISPR	- Trans-cleavage of fluorophore-quencher reporter	5 copies/μL	RT-LAMP	45	- Require blue light emission - Dual labeling of the reporter probe	[S1]
AIOD-CRISPR	- Trans-cleavage of fluorophore-quencher reporter	3 copies/μL	RT-RPA	20		[S2]
SHERLOCK	- Trans-cleavage of fluorophore-biotin reporter and lateral flow strip	42 copies/ reaction	RT-RPA	70	- High detection limit - Dual labeling of the reporter probe	[S3]
DETECTR	- Trans-cleavage of fluorophore-biotin reporter and lateral flow strip	10 copies/μL	RT-LAMP	30~40	- Dual labeling of the reporter probe	[S4]
Not specified	- Trans-cleavage-mediated liberation of the linker DNA - Cas12a-mediated AuNP aggregation	75 copies/μL	RT-LAMP	45	- Synthesis of DNA-immobilized gold nanoparticles - High detection limit	[S5]
CLAP	- Trans-cleavage of the linker DNA	4 copies/μL	RT-LAMP	40		[S6]
Not specified	- Trans-cleavage of the surface DNA on gold nanoparticles	1 copy/reaction	RT-RPA	60	- Synthesis of DNA-immobilized gold nanoparticles - Requirement of centrifugation	[S7]
Not specified	- Trans-cleavage-mediated degradation of the linker DNA - Cas12a-mediated AuNP aggregation	1 copy/μL	RT-PCR	90	- Use of thermocycler - Synthesis of DNA-immobilized gold nanoparticles	[S8]
LAMP-TADICA	- Trans-cleavage of TA repeat sequences	4 copies/μL	RT-LAMP	70	-	This work
RPA-TADICA		2 copies/μL	RT-RPA	60		

Reference

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