

**Table S1.** Primers and probes designed in this study

RPA primers for the detection of SARS-CoV-2 and Omicron BA.1

Primer	Nucleotide sequence (5'-3')	GC(%)	Length (nt)	Amplicon size (Bp)	Position	Genome reference
RPA_N_2F	GAACGTGGTTGACCTACACAGGTGCCATCAAAT	48.5	33	166	29203–29235	OP714083.1
RPA_N_2R-Bio	BIOTIN-GGTAAGGCTTGAGTTCATCAGCCTCTTCTTT	41.2	34		29368–29335	OP714083.1
RPA_Probe_N1	FITC-ACAAAGATCCAAATTCAAAGATCAAGTCA[THF]TTGCTGAATAAGCA-C3	31.1	46	NA	29242–29287	OP714083.1
RPA_214ins_SET2_F	TCTTAGGAAATTGTGTTAAGAACATTGATGG	30.3	33	179	22072–22104	OP711808.1
RPA_214ins_SET2_R_Bio	Biotin-TATGTAAGCAAGTAAAGTTGAAACCTAGTG	31.3	32		22250–22219	OP711808.1
RPA_214INS_Probe_2	FITC-ACACGCCTATTATAGTCGTGAGCCAGAA[THF] ATCTCCCTCAGGGTTT-C3	50.0	46	NA	22128–22173	OP711808.1

PCR primers adapted with the T7 promotor sequence used for the synthesis of RNA standards

PCR_T7_N_2F	TAATACGACTCACTATAGGGAACGTGGTTGACCTACACA	43.6	39	166	29203–29222	OP714083.1
PCR_T7_N_2R	GGTAAGGCTTGAGTTCATCAG	45.5	22		29368–29347	OP714083.1
PCR_T7_214ins_SET2_F	TAATACGACTCACTATAGGGTAATTCAAAAATCTTAG	28.1	38	185	22086–22103	ON062940.1
PCR_T7_214ins_SET2_R	TATGTAAGCAAGTAAAGTT	25	20		22270–22251	ON062940.1

NA: Not applicable, THF: Tetrahydrofuran, FITC: Fluorescein isothiocyanate

**Table S2.** Consensus sequences for Omicron BA.1 and wild-type utilized for the generation of positive control (Omicron BA.1) and negative control SARS-CoV-2 (wild-type)

Name	Sequence (5'→3')	Length (nt)	Position	Reference
S_211	AAAAGTTGGATGGAAAGTGAGTTCAGAGTTATTCTAGTGCATAATTGCACTTTGAATATGTCTCT	395	21984–	OL817641.1
del+214	CAGCCTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTCAAAAATCTTAGGAAATTGTGTTAAG		22378	
ins_wildt	AATATTGATGGTTATTTAAAATATATTCTAACGACACGCCTATTAAATTAGTGCCTGATCTCCCTCAGG			
ype	GTTTTCGGCTTAGAACCATGGTAGATTGCCAATAGGTATTAACATCACTAGGTTCAAACCTTACT TGCTTACATAGAAGTTATTGACTCCTGGTATTCTTCAGGTTGGACAGCTGGTGCAGCTTAT TATGTGGGTTATCTAACCTAGGACTTTCTATTAATATAATGA			
S_211	CAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTATTCTAGTGCATAATTGCACTTTGA	394	21952–	OL822906.1
del+214	ATATGTCTCTCAGCCTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTCAAAAATCTTAGGAAATT		22345	
ins_delet	GTGTTAAGAATATTGATGGTTATTTAAAATATATTCTAACGACACGCCTATTATAGTGCCTGAGCCAG			
ed	AAGATCTCCCTCAGGGTTTCGGCTTAGAACCATGGTAGATTGCCAATAGGTATTAACATCACTAG GTTCAAACTTACTTGCTTACATAGAAGTTATTGACTCCTGGTATTCTTCAGGTTGGACAGCT GGTGCAGCTTATTATGTGGGTTATCTAACCTAGGACTTT			

**Table S3.** Samples used for cross-reactivity test

SARS-CoV-2 (N) RT-RPA- LF	Sample	Type	Cross- reactivity
	Human metapneumovirus	Clinical sample (cDNA)	No
	Influenza A virus H1N1-pandemic	Clinical sample (cDNA)	No
	Influenza C virus	Clinical sample (cDNA)	No
	Respiratory syncytial virus	Clinical sample (cDNA)	No
	Human parainfluenza virus type 1	Clinical sample (cDNA)	No
	Human parainfluenza virus type 2	Clinical sample (cDNA)	No
	Human parainfluenza virus type 3	Clinical sample (cDNA)	No
	Human parainfluenza virus type 4	Clinical sample (cDNA)	No
	Severe acute respiratory syndrome coronavirus	Bat SARS-like coronavirus isolate bat-SL-CoVZC45, genome standard (cDNA) (IDT, catalog 10006624)	No
	Middle east respiratory syndrome coronavirus	Middle East respiratory syndrome-related coronavirus isolate KNIH/002_05_2015, genome standard (cDNA) (IDT, catalog 10006623)	No
	Human coronavirus 229E	Human coronavirus 229E genome standard (ATCC-VR-740) (RNA)	No
	Human coronavirus OC43	Human coronavirus OC43 genome standard (ATCC-VR-1558) (RNA)	No
Omicron BA.1 (S) RT-RPA-LF	Alpha (B.1.1.7) VOC	Clinical sample (RNA)	No
	Delta (1.617.2) VOC	Clinical sample (RNA)	No
	Human coronavirus 229E	Human coronavirus 229E genome standard (ATCC-VR-740) (RNA)	No
	Human coronavirus OC43	Human coronavirus OC43 genome standard (ATCC-VR-1558) (RNA)	No
	Human coronavirus NL63	Clinical sample (RNA)	No
	Human coronavirus HKU1	Clinical sample (RNA)	No

**Table S4.**

Clinical samples tested using SARS-CoV-2 (N) and Omicron BA.1 (S) RT-RPA-LF

RT-RPA-LF	Kawamura children clinic <sup>a</sup>	Tohoku University Hospital <sup>b</sup>	Sendai City Institute of Public Health <sup>c</sup>	Virus Research Center, Sendai Medical Center <sup>d</sup>	Miyagi Prefectural Institute of Public Health and Environment <sup>e</sup>	Tohoku kosai Hospital <sup>f</sup>	Universidad Peruana Cayetano Heredia <sup>g</sup>	Total
SARS-CoV-2 (N)	104	50	28	5	3	-	-	190
Omicron BA.1 (S)	74	56	-	-	128	40	33	331

In total, 454 samples were tested in this study. Of these, 190 were tested for SARS-CoV-2 (N) RT-RPA-LF, 331 were tested for Omicron BA.1 (S) RT-RPA-LF, and 67 were tested for both. The institutions a,b,d,e, and f used the QIAamp Viral RNA Mini Kit. The institution e used the MagMAX CORE Nucleic Acid Purification Kit and Maxwell RSC Total Nucleic Acid Purification Kit. The institution g used a Nucleic Acid Extraction-Purification kit (Sansure Biotech, China). The institutions b, c, d, e, and f used the SARS-CoV-2 PCR protocol (32), (33).

**Table S5**

SARS-CoV-2 PCR positive (by viral load categories)					Pre- COVID-19	Total
RT-RPA	High	Moderate	Low	Very low		
Positive	44	17	15	1	0	77
Negative	0	0	3	6	104	113
Total	44	17	18	7	104	190

(a). SARS-CoV-2 (N) RT-RPA-LF results by real-time RT-PCR using viral load categories.

SARS-CoV-2 Omicron BA.1 (by viral load categories <sup>a</sup> )					Non-BA.1 VOC	Pre - COVID-19	Total
RT-RPA-LF	High	Moderate	Low	Very low			
Positive	93	39	5	0	3	3	143
Negative	5	11	16	3	82	71	188
Total	98	50	21	3	85	74	331

(b). Omicron BA.1 (S) results of real-time RT-PCR using viral load categories.

VOC: Variant of concern. Viral loads were classified as high viral load : > 9,015.7 copies/µL, moderate viral load: 385.6–9,015.7 copies/µL, low viral load: 16.5–385.5 to copies/µL, and very low viral load : < 16.5 copies/µL.

**Table S6.**

PCR primers used for the partial amplification and sequencing of the spike protein gene of the SARS-CoV-2 using the Sanger sequencing method

Primer Name	Length (nt)	Sequence: 5'→3'
SARS-CoV-2_S_38F	25	GTCAGTGTGTTAATCTTACAACCAG
SARS-CoV-2_S_1191R	25	TGCATAGACATTAGTAAAGCAGAGA
SARS-CoV-2-S-omi-1017F	20	TGAAGTTTTAACGCCACCA
SARS-CoV-2_S_2249R	24	CTGCATTCAGTTGAATCACCAACAA
SARS-CoV-2-S-omi-663R	20	CGAAAAACCCCTGAGGGAGAT
SARS-CoV-2-S-omi-486F	21	TGCGAATAATTGCACTTTGA
SARS-CoV-2_S_1583R	21	TTAGGTCCACAAACAGTTGCT
SARS-CoV-2-S-omi-1363F	26	TTGTTAGGAAGTCTAATCTCAAACC

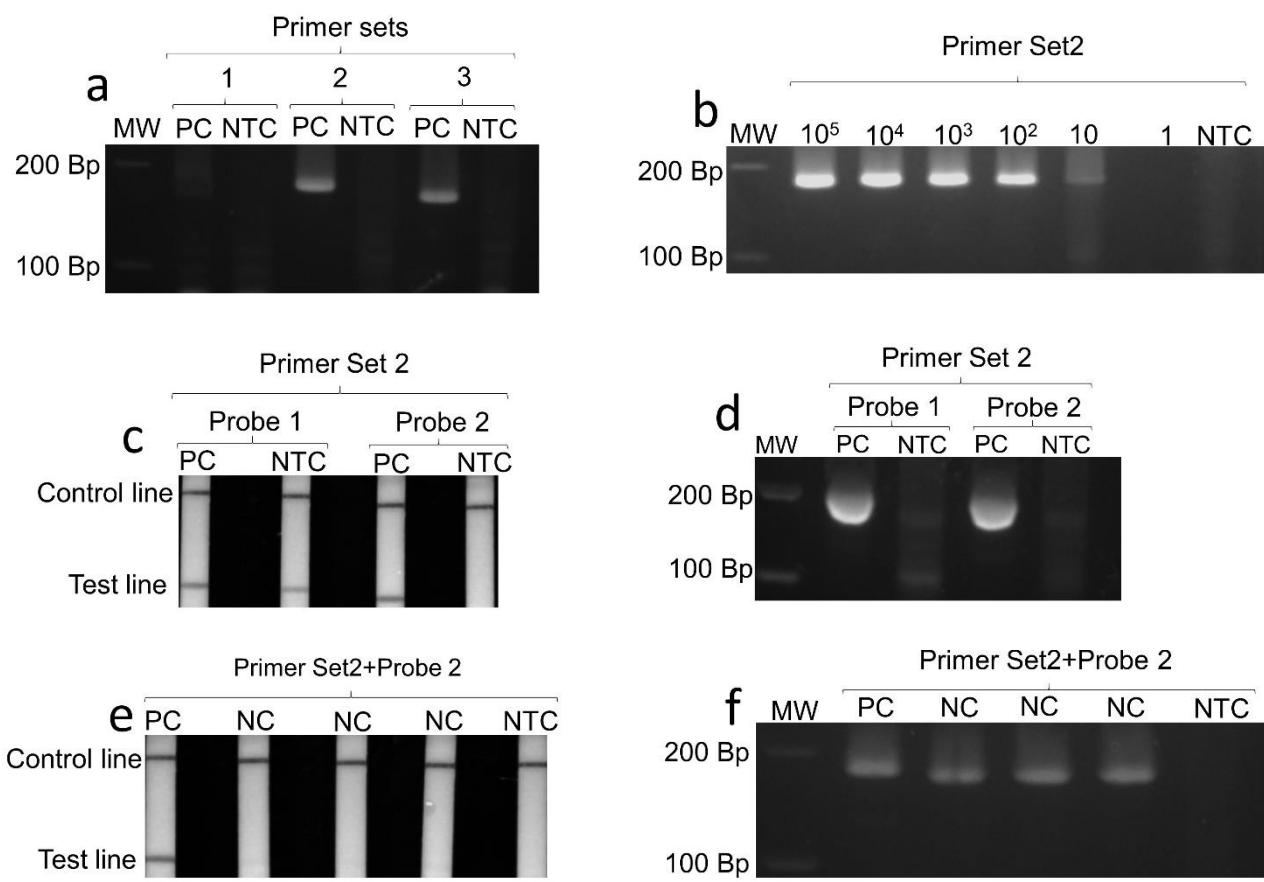
nt = nucleotides

**Table S7.**

Sensitivity of some rapid assay for SARS-CoV-2 on clinical samples under different viral load values

Test	Method	Sensitivity (%) by viral load				Target
		High	Moderate	Low	Very low	
SARS-CoV-2 (N)	RT-RPA	44/44 (100)	17/17 (100)	15/18 (83)	1/7 (14)	SARS-CoV-2 (N)
Omicron BA.1 (S)	RT-RPA	93/98 (95)	39/50 (78)	5/21 (24)	0/3 (0)	Omicron BA.1 (S)
Colorimetric RT-LAMP <sup>37</sup>	RT-LAMP	51/51 (100)	28/30 (93)	4/20 (20)	0/16 (0)	SARS-CoV-2
BD Veritor <sup>17</sup>	LFAs	13/13 (100)	10/14 (71)	0/8 (0)	1/13 (8)	SARS-CoV-2
Sofia 2 SARS Ag <sup>17</sup>	LFAs	13/13 (100)	12/14 (86)	1/8 (13)	1/13 (8)	SARS-CoV-2
BinaxNOW <sup>17</sup>	LFAs	13/13 (100)	11/14 (79)	1/8 (13)	1/13 (8)	SARS-CoV-2
Standard Q COVID-19 Ag <sup>18</sup>	LFA	4/4 (100)	18/24 (75)	5/37 (14)	0/11 (0)	SARS-CoV-2
Espline SARS-CoV-2 <sup>18</sup>	LFA	4/4 (100)	20/24 (83)	4/37 (11)	0/11 (0)	SARS-CoV-2
QuickNavi COVID19 Ag <sup>18</sup>	LFA	4/4 (100)	10/24 (42)	2/37 (5)	0/11 (0)	SARS-CoV-2

RT-LAMP: Reverse transcription loop-mediated amplification. LFA: Lateral flow antigen detection assays. N: Nucleocapsid protein gene, S: Spike protein gene. \* Test developed in this study.



**Figure S1.** Primer and probe evaluation for Omicron BA.1 (S) RT-RPA-LF. **(a)** RPA primer evaluation, amplification bands are confirmed on positive control (PC) for primers Set 2 and Set 3, **(b)** gel electrophoresis shows a detection limit of 10 copies/μL (plasmid control) for primer Set 2, **(c)** the combination of primer Set 2 and Probe 2 produced a band on PC. No band was observed for the non-template control (NTC), **(d)** gel electrophoresis confirms amplification bands for both Probes 1 and 2 at the PC, but no bands were detected at the NTC. MW: molecular weight marker. Bp: base pairs. PC: positive control, del211/ins214 plasmid control 10<sup>5</sup> copies/μL. NC: negative control, wildtype for del211/ins214 plasmid control 10<sup>5</sup> copies/μL. NTC: non-template control, nuclease-free water.