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Supporting Information

Charge-shifting Polyplex as a viral RNA Extraction Carrier for Streamlined Detection of Infectious Viruses

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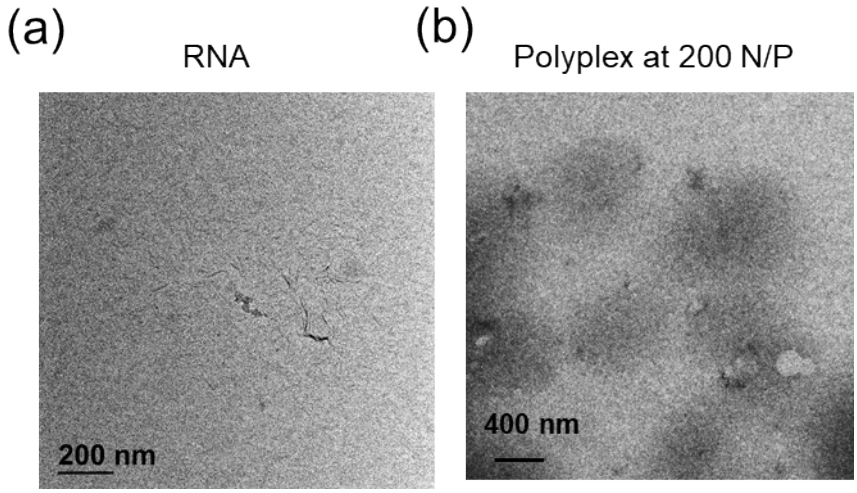


Figure S1. TEM micrograph of RNA and polyplex with the N/P ratio of 200.

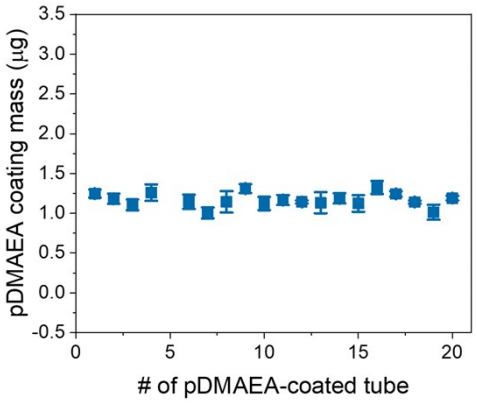
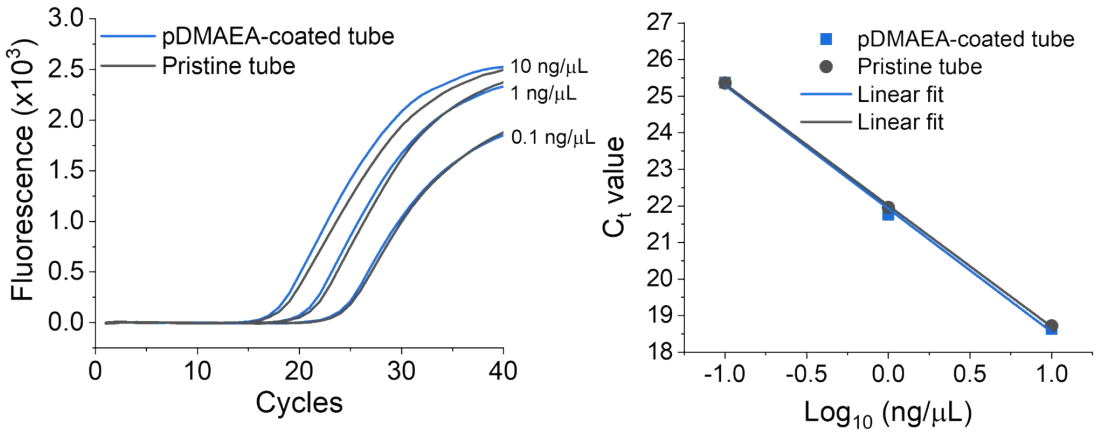


Figure S2. Quantitative analysis of pDMAEA coating on a PCR tube. pDMAEA coating mass of 20 tubes. The coating mass is determined by the concentration of pDMAEA solution obtained by introducing 20 μL of distilled water to the pDMAEA-coated tubes. The results demonstrated a high level of consistency, with a range of error of 7.2% regarding the coating mass. This level of variation indicates that the iCVD process yields highly reproducible coating thicknesses on the PCR tubes.

(a) (b)



(c)

	PCR efficiency (%)*	Slope	Y-Intercept	R^2
pDMAEA-coated tube	104.7	-3.2136	20.999	0.9989
Pristine tube	109.8	-3.1074	21.394	0.9995

Figure S3. qRT-PCR inhibitory test of pDMAEA. (a) qRT-PCR amplification curves and (b) the corresponding C_t values of template RNA (0.1 to 10 ng/μL) in pDMAEA-coated and pristine tubes. (c) Summary of qRT-PCR results using pDMAEA-coated and pristine tubes. PCR efficiency (%)* = $\{(10^{-1/\text{slope}} - 1) \times 100\}$.

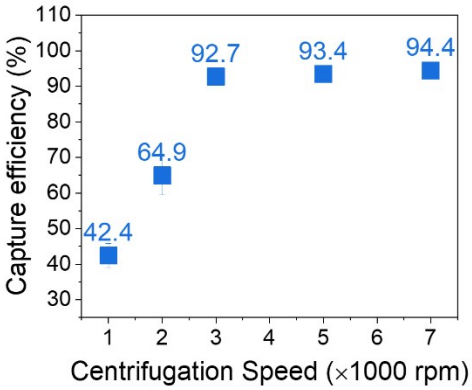


Figure S4. RNA capture efficiency of the pDMAEA-coated tube as a function of centrifuge speed (n = 3, error bar = standard deviation).

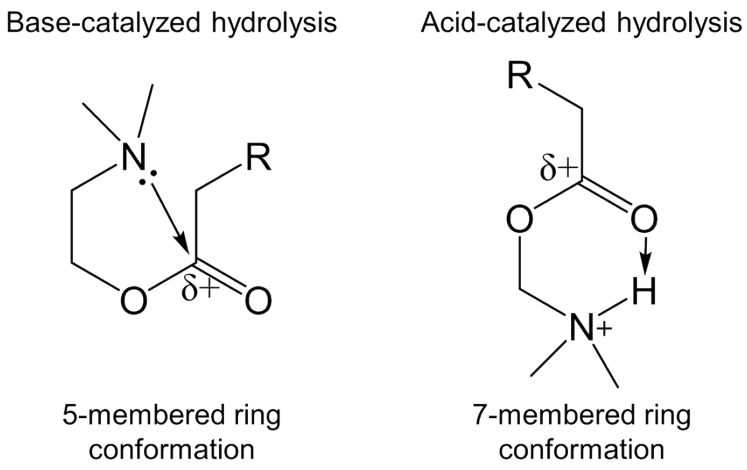


Figure S5. Self-catalyzed hydrolysis mechanism of DMAEA. 5-membered ring or 7-membered ring conformation can activate the ester moiety and make it more susceptible to nucleophilic attack by hydroxyl ions under basic and acidic conditions, respectively. [1]

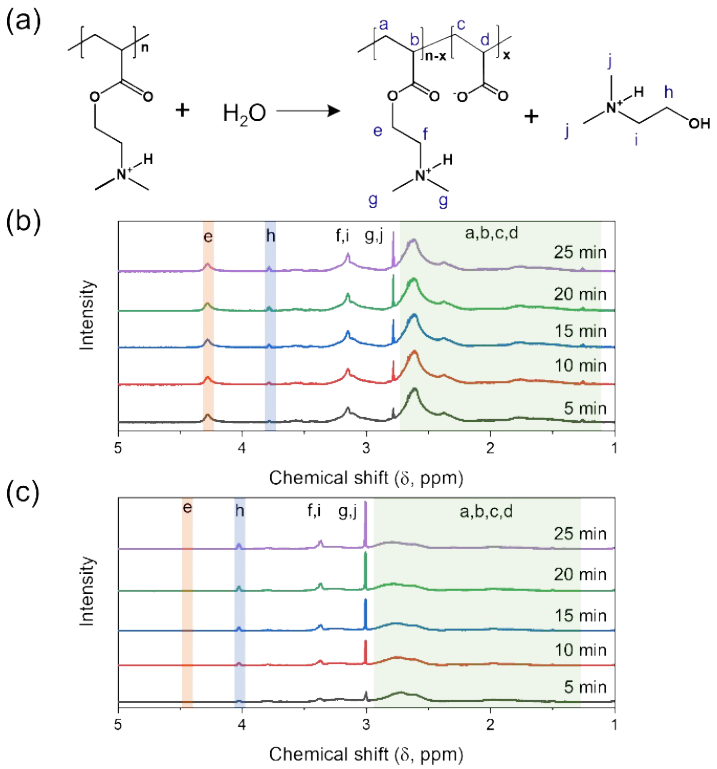


Figure S6. Real-time ^{1}H NMR analysis of pDMAEA in buffered D_2O solution at 600 MHz. (a) Hydrolysis reaction of a pDMAEA. Chemical structure of a pDMAEA is denoted by the hydrogen positions. (b, c) ^{1}H NMR spectra of pDMAEA at pH 8.0 for 25 min with 5 min intervals at (b) 25 ^{o}C and (c) 50 ^{o}C.

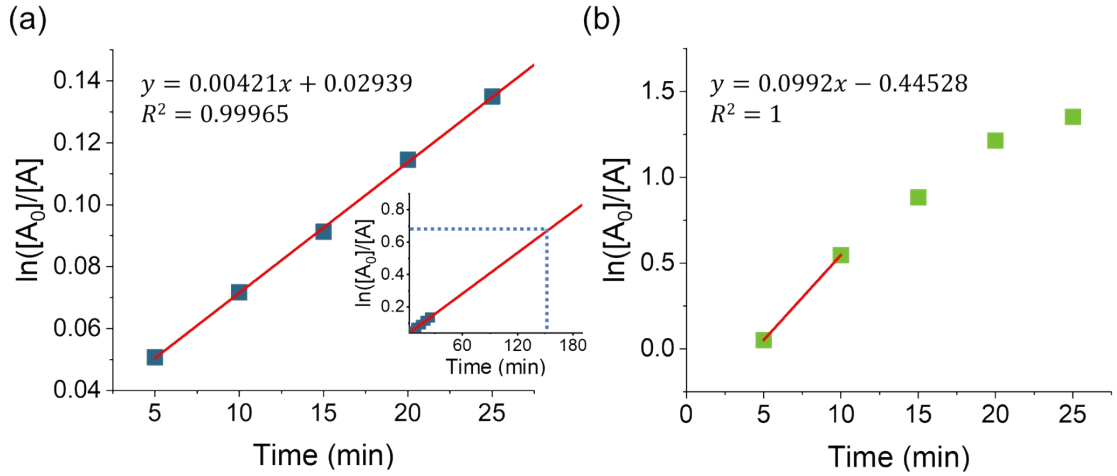


Figure S7. Linear first-order kinetic plots of the initial stages of pDMAEA hydrolysis. (a, b) Hydrolysis occurs in buffered D_2O solutions at pH 8.0 at (a) 25 °C and (b) 50 °C. Inset of (a) is extrapolate plot for the half-life of the hydrolysis at 25 °C.

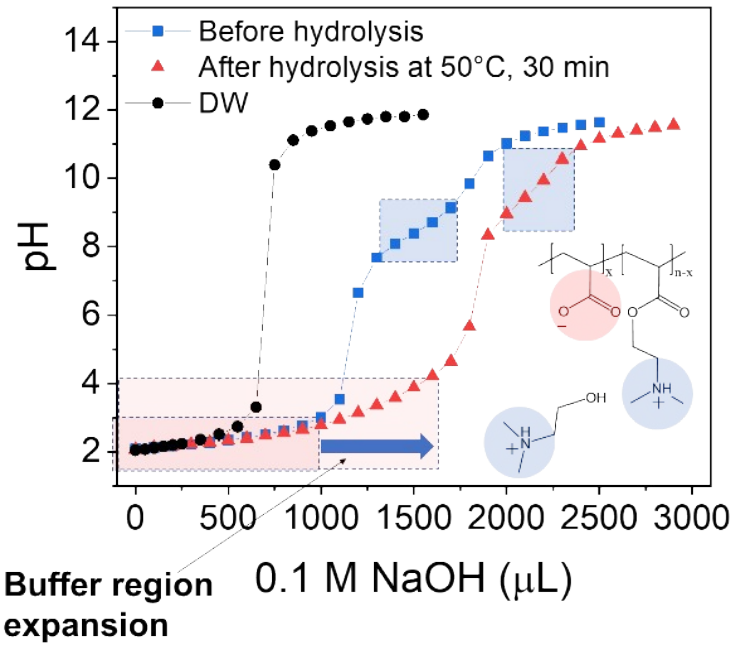


Figure S8. Potentiometric pH back-titration analysis of pDMAEA before and after hydrolysis. Blue and pink areas indicate tertiary amine group and acrylic acid group.

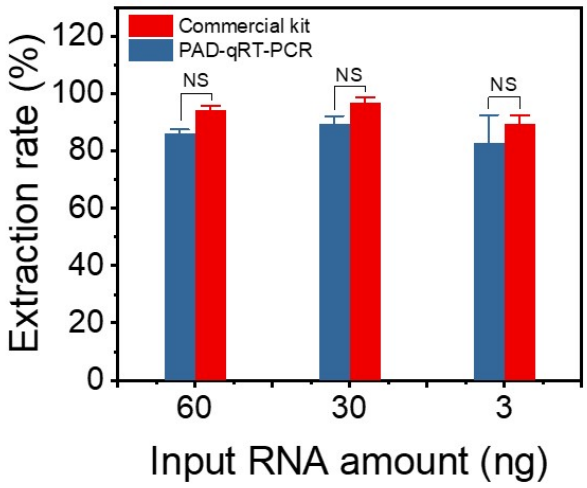


Figure S9. Comparison of extraction rates between the QIAamp Viral RNA Mini Kit (Qiagen) and PAD-qRT-PCR method at different initial RNA amounts (3 ng to 60 ng). Statistical significance is evaluated using Two sample t-test ($^{\text{Non-significance (NS)}}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$)

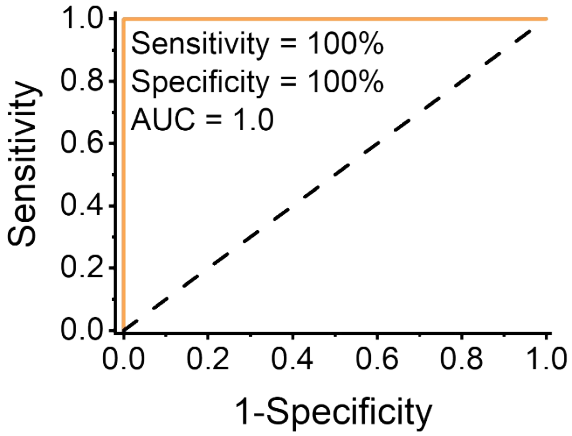


Figure S10. Receiver operating characteristic (ROC) curve generated from the clinical sample data of Figure 4e.

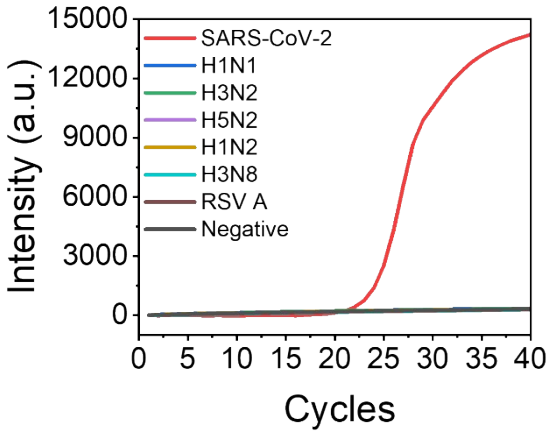


Figure S11. PAD-qRT-PCR amplification curves corresponding to Figure 5c.

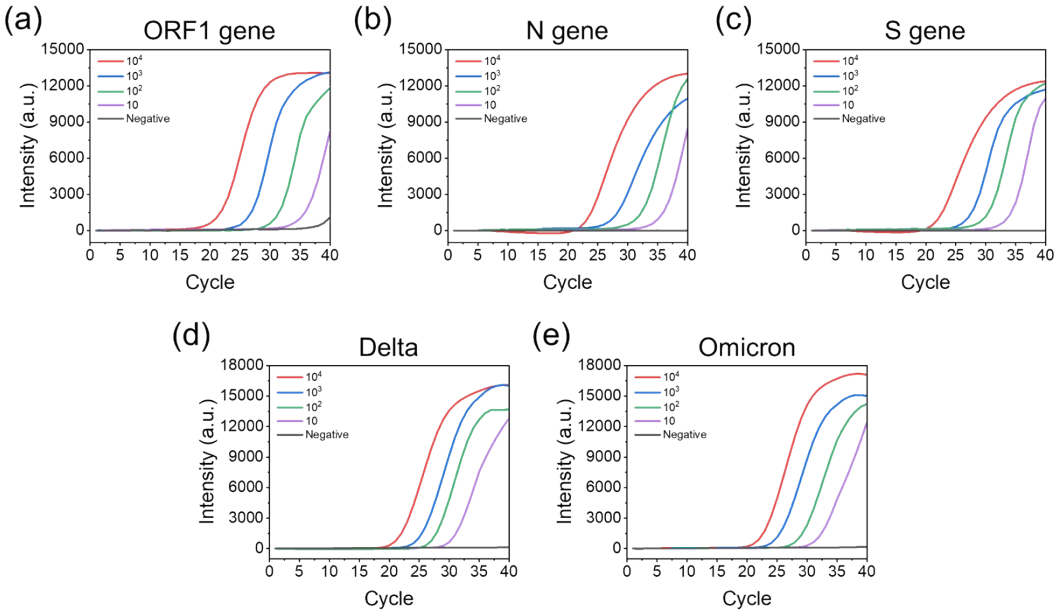


Figure S12. PAD-qRT-PCR amplification curves corresponding to Figure 5d-h.

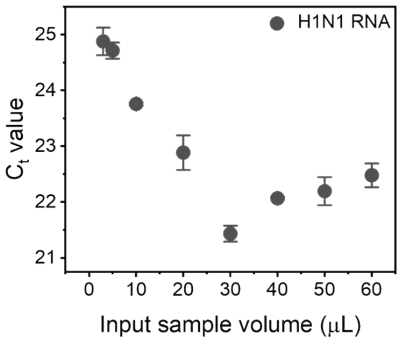


Figure S13. PAD-qRT-PCR results by varying input sample volume from 3 μL to 60 μL. Target H1N1 RNA concentration was 1 ng/μL. C_t value from 30 μL sample is the lowest.

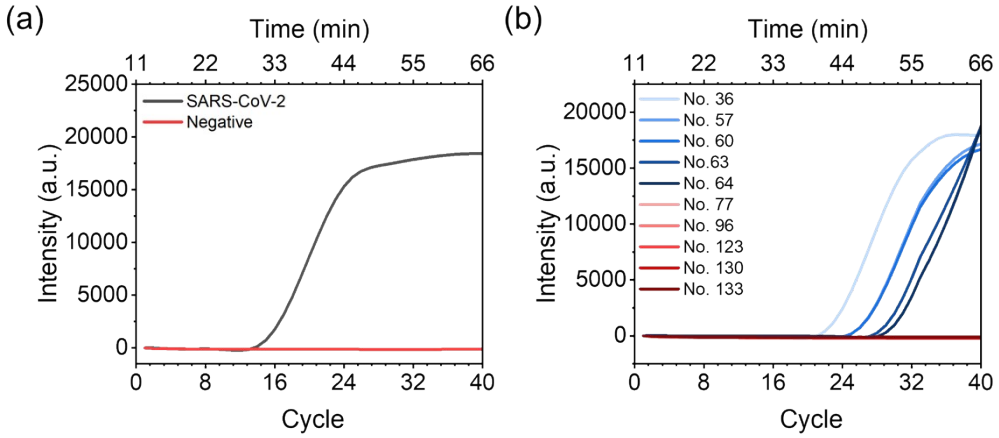


Figure S14. Compatibility of pDMAEA-coated tube with rapid qRT-PCR kit (TaqPath™ 1-step Multiplex Master Mix). (a, b) PAD-qRT-PCR results under the fast qRT-PCR condition for the detection of (a) SARS-CoV-2 (10^5 PFU/mL) and (b) clinical samples (sample numbers are referred from Table S4).

Table S1. Primer sequences used in this study.

Target	Name	Position	Sequence (5' → 3')
ORF1 gene	FP	13342–13362	CCC TGT GGG TTT TAC ACT TAA
	Probe	13377–13404	FAM[a] – CCG TCT GCG GTA TGT GGA AAG GTT ATG G – BHQ1[b]
	RP	13442–13460	ACG ATT GTG CAT CAG CTG A
N gene	FP	29125–29144	AAA TTT TGG GGA CCA GGA AC
	Probe	29222–29241	FAM – ATG TCG CGC ATT GGC ATG GA – BHQ1
	RP	29263–29282	TGG CAG CTG TGT AGG TCA AC
S gene	FP	3064–3083	ATY[c] AGR[c] GCT GCW[c] GAA ATC AG
	Probe	3201–3220	FAM – ATG AGG TGC TGA CTG AGG GA – BHQ1
	RP	3281–3300	CCW TCA TGA CAA ATD[c] GCW GG
Delta	FP	22928-22952	CCT TTT GAG AGA GAT ATT TCA ACT G
	Probe	22960-22980	FAM – TCA GGC CGG TAG CAA ACC TTG – BHQ1
	RP	23056-23079	AGT ACT ACT ACT CTG TAT GGT TGG
Omicron	FP	26503-26521	TGG CAG GTT CCA ACG GTA C
	Probe	26454-26568	FAM – AGC TCC TTG AAG AAT GGA ACC TAG – BHQ1
	RP	26579-26604	GAA GAC AAA TCC ATG TAA GGA ATA GG
H1N1	FP	49-68	TCA GGC CCC CTC AAA GCC GA
	Probe	72-93	FAM – CGC GCA GAG ACT GGA AAG TGT C – BHQ1
	RP	188-207	GGG CAC GGT GAG CGT GAA CA
IBV	FP	591-614	AGG GGA AGA CCA AAT TAC TGT TTG
	Probe	629-656	FAM – ATA ACA AAA CCC AAA TGA AGA GCC TCT A– BHQ1
	RP	676-697	CAT TAG CAG ATG AGG TGA ACT T

Table S2. Hospital results of clinical samples for influenza viruses.

Number	Virus	Sample type	C_t value
1	H1N1	Sputum	3.98
2	H1N1	Sputum	6.16
3	H1N1	Sputum	6.75
4	H1N1	Sputum	7.85
5	H1N1	NPS[a]	9.65
6	H1N1	NPS	12.17
7	H1N1	Sputum	12.62
8	H1N1	Sputum	14
9	H1N1	Sputum	14.47
10	H1N1	Sputum	15.01
11	H1N1	Sputum	20.06
12	IBV	Sputum	5.98
13	IBV	Sputum	6.79
14	IBV	Sputum	6.79
15	IBV	Sputum	7.53
16	IBV	Sputum	8.12
17	IBV	Sputum	10.98
18	IBV	Sputum	12.15
19	IBV	Sputum	13.47
20	IBV	Sputum	16.41
21	Negative	NPS	N/A
22	Negative	NPS	N/A
23	Negative	NPS	N/A
24	Negative	NPS	N/A
25	Negative	NPS	N/A
26	Negative	NPS	N/A
27	Negative	NPS	N/A
28	Negative	NPS	N/A
29	Negative	NPS	N/A
30	Negative	NPS	N/A
31	Negative	NPS	N/A
32	Negative	NPS	N/A
33	Negative	NPS	N/A
34	Negative	NPS	N/A
35	Negative	NPS	N/A

Table S3. Comparison of detection methods for SARS-CoV-2. *1 PFU/mL ~ 1000 copies/mL[6]

Tech	LOD (cp/rxn)	Sensitivity	Specificity	Assay time (min)	Compatibility	Variant detection
PAD-qRT-PCR	**~ 10***	**99.3% (144/145)**	**98.8% (79/80)**	**5 + 90 (extraction + qRT-PCR)**	**O**	**O**
SWCNT-based extraction and RT-qPCR[2]	128	N/A†	N/A	60 + 90 (extraction + qRT-PCR)	O	O
RT-LAMP Harmony COVID-19[3]	40	95% (39/41)	100% (4/4)	91 (extraction-free amplification)	X	O
SHINEv.2[4]	1000	90.5% (38/42)	100% (30/30)	90 (extraction-free amplification)	X	O
OIL-TAS + RT-qPCR or LAMP[5]	1000	100% (57/57)	100% (10/10)	30 + 30~50 (pretreatment + LAMP)	O	X

†Not available

Table S4. Hospital results of clinical samples for WT SARS-CoV-2.

Number	Sample type	C_t value			
		RdRP	E	N	AVG
1	NPS	8.3	8.7	N/A	8.50
2	NPS	9.37	11.37	N/A	10.37
3	NPS	10.14	11.57	N/A	10.86
4	NPS	10.57	11.73	N/A	11.15
5	NPS	10.59	12.03	N/A	11.31
6	NPS	12.6	10.94	10.81	11.45
7	NPS	11.69	12.27	N/A	11.98
8	NPS	11.9	12.8	N/A	12.35
9	NPS	11.56	11.9	13.8	12.42
10	NPS	12.52	11.63	13.84	12.66
11	NPS	11.13	12.88	16.52	13.51
12	NPS	15.22	15.3	12.34	14.29
13	NPS	15.36	12.78	15.09	14.41
14	NPS	14.08	14.95	N/A	14.52
15	NPS	14.35	15.33	15.45	15.04
16	NPS	14.71	15.65	N/A	15.18
17	NPS	14.7	15.81	N/A	15.26
18	NPS	14.58	15.97	N/A	15.28
19	NPS	13.35	13.69	19.05	15.36
20	NPS	14.98	15.93	N/A	15.46
21	NPS	15.28	15.85	N/A	15.57
22	NPS	15.16	16.24	N/A	15.70
23	NPS	15.24	16.25	N/A	15.75
24	NPS	16.2	16.81	N/A	16.51
25	NPS	7.56	25.52	N/A	16.54
26	NPS	15.35	16.73	19.04	17.04

Number	Sample type	C_t value			
		RdRP	E	N	AVG
27	NPS	15.47	16.6	19.48	17.18
28	NPS	18.68	15.7	18.4	17.59
29	Sputum	18.44	16.2	18.93	17.86
30	NPS	18.54	17.76	N/A	18.15
31	NPS	19.21	17.96	N/A	18.59
32	NPS	18.7	17.14	20.09	18.64
33	NPSa	19.28	17.2	20.33	18.94
34	NPS	18.97	17.63	20.6	19.07
35	NPS	18.52	19.7	N/A	19.11
36	NPS	18.81	19.31	19.34	19.15
37	NPS	19.37	19.29	N/A	19.33
38	NPS	17.96	19.05	22.5	19.84
39	NPS	20.2	20.6	N/A	20.40
40	Sputum	20.73	18.7	22.5	20.64
41	Sputum	20.81	19.04	22.15	20.67
42	NPS	21.59	19.16	21.91	20.89
43	NPS	20.61	21.88	N/A	21.25
44	Sputum	22.1	19.47	22.22	21.26
45	NPS	21.27	22.41	N/A	21.84
46	NPS	21.93	23.05	N/A	22.49
47	Sputum	23.88	20.79	23.49	22.72
48	NPS	22.37	23.07	N/A	22.72
49	NPS	23.98	23.35	26.16	24.50
50	NPS	24.58	25.26	N/A	24.92
51	NPS	25.02	23.34	27.12	25.16
52	NPS	25.11	26.1	N/A	25.61
53	NPS	25.26	26.53	N/A	25.90

Number	Sample type	C_t value			
		RdRP	E	N	AVG
54	NPS	27.3	24.34	27.02	26.22
55	NPS	26.47	26.5	N/A	26.49
56	NPS	26.8	27.56	N/A	27.18
57	NPS	27.36	27.07	N/A	27.22
58	NPS	27.15	27.38	N/A	27.27
59	NPS	26.19	27.26	33.01	28.82
60	NPS	28.73	29.27	N/A	29.00
61	NPS	28.24	28.84	30.22	29.10
62	NPS	29.19	29.06	N/A	29.13
63	NPS	29.95	28.73	29.16	29.28
64	NPS	30.17	30.04	N/A	30.11
65	NPS	31.57	31.11	32.77	31.82
66	NPS	31.43	32.7	N/A	32.07
67	Sputum	32.7	30.94	32.58	32.07
68	Sputum	33.21	30.61	33.11	32.31
69	Sputum	33.11	30.97	33.45	32.51
70	Sputum	33.11	30.97	33.45	32.51
71	NPS	33.29	33.5	33.64	33.48
72	Sputum	34.58	33.32	34.44	34.11
73	NPS	35.48	35.33	N/A	35.41
74	NPS	36.38	35.49	38.86	36.91
75	NPS	38.85	37.83	37.7	38.13
76	NPS	N/A	N/A	N/A	N/A
77	NPS	N/A	N/A	N/A	N/A
78	NPS	N/A	N/A	N/A	N/A
79	NPS	N/A	N/A	N/A	N/A
80	NPS	N/A	N/A	N/A	N/A

Number	Sample type	C_t value			
		RdRP	E	N	AVG
81	NPS	N/A	N/A	N/A	N/A
82	NPS	N/A	N/A	N/A	N/A
83	NPS	N/A	N/A	N/A	N/A
84	NPS	N/A	N/A	N/A	N/A
85	NPS	N/A	N/A	N/A	N/A
86	NPS	N/A	N/A	N/A	N/A
87	NPS	N/A	N/A	N/A	N/A
88	NPS	N/A	N/A	N/A	N/A
89	NPS	N/A	N/A	N/A	N/A
90	NPS	N/A	N/A	N/A	N/A
91	NPS	N/A	N/A	N/A	N/A
92	NPS	N/A	N/A	N/A	N/A
93	NPS	N/A	N/A	N/A	N/A
94	NPS	N/A	N/A	N/A	N/A
95	NPS	N/A	N/A	N/A	N/A
96	NPS	N/A	N/A	N/A	N/A
97	NPS	N/A	N/A	N/A	N/A
98	NPS	N/A	N/A	N/A	N/A
99	NPS	N/A	N/A	N/A	N/A
100	NPS	N/A	N/A	N/A	N/A
101	NPS	N/A	N/A	N/A	N/A
102	NPS	N/A	N/A	N/A	N/A
103	NPS	N/A	N/A	N/A	N/A
104	NPS	N/A	N/A	N/A	N/A
105	NPS	N/A	N/A	N/A	N/A
106	NPS	N/A	N/A	N/A	N/A
107	NPS	N/A	N/A	N/A	N/A

Number	Sample type	C_t value			
		RdRP	E	N	AVG
108	NPS	N/A	N/A	N/A	N/A
109	NPS	N/A	N/A	N/A	N/A
110	NPS	N/A	N/A	N/A	N/A
111	NPS	N/A	N/A	N/A	N/A
112	NPS	N/A	N/A	N/A	N/A
113	NPS	N/A	N/A	N/A	N/A
114	NPS	N/A	N/A	N/A	N/A
115	NPS	N/A	N/A	N/A	N/A
116	NPS	N/A	N/A	N/A	N/A
117	NPS	N/A	N/A	N/A	N/A
118	NPS	N/A	N/A	N/A	N/A
119	NPS	N/A	N/A	N/A	N/A
120	NPS	N/A	N/A	N/A	N/A
121	NPS	N/A	N/A	N/A	N/A
122	NPS	N/A	N/A	N/A	N/A
123	NPS	N/A	N/A	N/A	N/A
124	NPS	N/A	N/A	N/A	N/A
125	NPS	N/A	N/A	N/A	N/A
126	NPS	N/A	N/A	N/A	N/A
127	NPS	N/A	N/A	N/A	N/A
128	NPS	N/A	N/A	N/A	N/A
129	NPS	N/A	N/A	N/A	N/A
130	NPS	N/A	N/A	N/A	N/A
131	NPS	N/A	N/A	N/A	N/A
132	NPS	N/A	N/A	N/A	N/A
133	NPS	N/A	N/A	N/A	N/A
134	NPS	N/A	N/A	N/A	N/A
135	NPS	N/A	N/A	N/A	N/A

Table S5. Hospital results of clinical samples for WT and delta SARS-CoV-2.

Number	Variant	Sample type	C_t value		
			RdRP	E	AVG
1	Delta	NPS	9.81	10.64	10.23
2	Delta	NPS	10.42	10.13	10.28
3	Delta	NPS	10.28	10.53	10.41
4	Delta	NPS	10.35	10.54	10.45
5	Delta	NPS	11.59	11.89	11.74
6	Delta	NPS	12.32	12.53	12.43
7	Delta	NPS	12.89	12.88	12.89
8	Delta	NPS	13.38	13.41	13.40
9	Delta	NPS	13.83	13.65	13.74
10	Delta	NPS	13.65	13.92	13.79
11	Delta	NPS[a]	14.09	13.58	13.84
12	Delta	NPS	13.73	14.02	13.88
13	Delta	NPS	13.81	13.98	13.90
14	Delta	NPS	13.86	14.42	14.14
15	Delta	NPS	13.72	15.13	14.43
16	Delta	NPS	15.13	13.89	14.51
17	Delta	NPS	14.25	15.10	14.68
18	Delta	NPS	13.32	16.47	14.90
19	Delta	NPS	15.06	15.17	15.12
20	Delta	NPS	15.43	15.41	15.42
21	Delta	NPS	16.17	15.50	15.84
22	Delta	NPS	15.62	16.37	16.00
23	Delta	NPS	16.27	16.30	16.29
24	Delta	NPS	16.80	16.39	16.60
25	Delta	NPS	17.02	16.25	16.64
26	Delta	NPS	18.11	15.34	16.73

Number	Variant	Sample type	C_t value		
			RdRP	E	AVG
27	Delta	NPS	16.85	17.54	17.20
28	Delta	NPS	18.37	16.37	17.37
29	Delta	NPS	16.83	18.02	17.43
30	Delta	NPS	18.21	16.67	17.44
31	Delta	NPS	18.36	16.58	17.47
32	Delta	NPS	17.74	17.48	17.61
33	Delta	NPS	17.48	18.23	17.86
34	Delta	NPS	17.45	18.31	17.88
35	Delta	NPS	19.65	16.41	18.03
36	Delta	NPS	20.10	16.98	18.54
37	Delta	NPS	18.56	18.80	18.68
38	Delta	NPS	18.26	19.25	18.76
39	Delta	NPS	19.15	19.00	19.08
40	Delta	NPS	18.55	20.15	19.35
41	Delta	NPS	19.21	19.56	19.39
42	Delta	NPS	21.29	20.14	20.72
43	Delta	NPS	20.04	21.40	20.72
44	Delta	NPS	22.02	20.11	21.07
45	Delta	NPS	21.06	21.23	21.15
46	Delta	NPS	22.14	21.08	21.61
47	Delta	NPS	23.79	20.82	22.31
48	Delta	NPS	22.67	23.32	23.00
49	Delta	NPS	24.28	22.19	23.24
50	Delta	NPS	25.73	23.69	24.71
51	Delta	NPS	25.24	25.27	25.26
52	Delta	NPS	25.20	26.27	25.74

Number	Variant	Sample type	C_t value		
			RdRP	E	AVG
53	Delta	NPS	27.48	24.73	26.11
54	Delta	NPS	30.15	26.68	28.42
55	Delta	NPS	28.11	29.87	28.99
56	Delta	NPS	29.36	28.64	29.00
57	Delta	NPS	28.82	30.81	29.82
58	Delta	NPS	30.75	29.74	30.25
59	Delta	NPS	30.72	32.54	31.63
60	Delta	NPS	31.73	32.31	32.02
61	WT	NPS	9.37	11.37	10.37
62	WT	NPS	10.14	11.57	10.86
63	WT	NPS	10.57	11.73	11.15
64	WT	NPS	10.59	12.03	11.31
65	WT	NPS	11.13	12.88	12.01
66	WT	NPS	11.56	11.90	11.73
67	WT	NPS	11.69	12.27	11.98
68	WT	NPS	12.40	12.19	12.30
69	WT	NPS	12.52	11.63	12.08
70	WT	NPS	13.35	13.69	13.52

Table S6. Hospital results of clinical samples for WT and omicron SARS-CoV-2.

Number	Variant	Sample type	C_t value		
			RdRP	E	AVG
1	Omicron	NPS	14.81	14.68	14.75
2	Omicron	NPS	14.85	15.37	15.11
3	Omicron	NPS	15.24	15.96	15.60
4	Omicron	NPS	16.89	15.21	16.05
5	Omicron	NPS	16.77	16.95	16.86
6	Omicron	NPS	17.3	18.16	17.73
7	Omicron	NPS	22.08	20.77	21.43
8	Omicron	NPS	23.45	21.7	22.58
9	Omicron	NPS	23.98	24.67	24.33
10	Omicron	NPS	29.34	29.24	29.29
11	WT	NPS	9.37	11.37	10.37
12	WT	NPS	10.14	11.57	10.86
13	WT	NPS	10.57	11.73	11.15
14	WT	NPS	10.59	12.03	11.31
15	WT	NPS	11.13	12.88	12.01
16	WT	NPS	11.56	11.90	11.73
17	WT	NPS	11.69	12.27	11.98
18	WT	NPS	12.40	12.19	12.30
19	WT	NPS	12.52	11.63	12.08
20	WT	NPS	13.35	13.69	13.52

Table S7. Material cost in this assay, material cost per 1 batch (iCVD process), and cost per 100 reactions of PAD-qRT-PCR kit

Material cost			
Materials		**Amount**	**Price ($)**
2-(Dimethylamino)ethyl acrylate (DMAEA, 98%, Merck, Germany)		500 mL	324
Tert-butyl peroxide (TBPO, 98%, Merck, Darmstadt, Germany)		1 L	183
8-strip PCR tube (Bio-rad, USA)		960 ea (a tube)	120
8-strip PCR cap (Bio-rad, USA)		960 ea (a tube)	46.0
Lysis buffer	Nuclease-free water (Merck, Germany)	10 L	63.0
	EDTA, Disodium Salt, Dihydrate (MW 292.2438 g/mol, Fisher Scientific, USA)	100 g	102.
	Pierce™ TCEP-HCl (MW 286.65 gram/mol, Thermo Scientific™, USA)	10 g	200
	Proteinase K Solution (800 units/mL, NEB, USA)	800 ea	91.0
	RiboLock RNase Inhibitor (40 U/μL, Thermo Scientific™, USA)	2500 ea	85.0
Total			**1214**

Cost per iCVD 1 batch		
Materials	**Amount**	**Price ($)**
8-strip PCR tube	384 ea (a tube)	66.4
DMAEA	2 mL	1.30
TBPO	2 mL	0.366
Total	**384 ea (a tube)**	**68.1**
Cost per 100 reactions		
Components	**Amount**	**Price ($)**
pDMAEA-coated tube	100 ea (a tube)	17.7
8-strip PCR cap	960 ea (a tube)	46.0
Nuclease-free water	10 mL	0.0630
Lysis buffer {1 mM EDTA, 100 mM TCEP-HCl, 100 μL Nuclease-free water, proteinase K (0.8 U/reaction), Rnase inhibitor (10 U/reaction)}	10 mL	43.7
Total		**107**

Table S8. Estimated material and market cost for molecular diagnostics of SARS-CoV-2.

Name	Method	Material	Equipment	Cost/hundred reactions ($)
Sample Pretreatment method	Our method	pDMAEA-coated tube, lysis buffer, nuclease-free water	Mini-centrifuge	107[a]
	Silica Spin Column-based RNA kit	QIAamp Viral RNA Mini Kit	Mini-centrifuge	628[b,6]
	Magnetic bead-based RNA kit	NucleoMag® Pathogen	Magnet rack	441[b,7]

[a] Estimated material cost

[b] Approximate market cost

Table S9. Comparison of pDMAEA tube with a commercial extraction kit.

	PAD-qRT-PCR	**Commercial kit**[b]
Technology	pDMAEA-coated tube	Silica technology
Time	5 min	40 min
Cost/hundred reactions ($)	107[a]	682
Processing	Manual (1 centrifugation step)	Manual (4 centrifugation steps + 2 washing steps)
Recovery Yield	85%	90%
Main sample type	Liquid media	Liquid media
Sample amount	3~10 μL	140 μL
Elution Volume	25 μL (PCR mixture)	50 μL (distilled water)
Format	Tube	Spin columns
Applications	PCR, qPCR, real-time PCR, or other gene amplification methods	PCR, qPCR, real-time PCR, or other gene amplification methods

[a] Estimated material cost

[b] Commercial kit (QIAamp Viral RNA Mini Kit, 52904) was purchased from Qiagen

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