Supplementary Materials

Highly sensitive and specific diagnosis of coronavirus disease 19 (COVID-19) by reverse transcription multiple cross displacement amplification-labeled nanoparticles biosensor

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Tables

Table S1. The primers used in this study

Primers name [a]	Sequences and modifications [b]	Length [c]	Genes
F1ab-F1	5'-ACACTTAAAAACACAGTCTGTAC-3'	23 nt	
F1ab-F2	5'-AATTTAGCAAAACCAGCTACT-3'	21 nt	
F1ab -CP1	5'-GAAGCATGGGTTCGCGGAGTCGTCTGCGGTATGTGGAA-3'	38 mer	
F1ab -CP1*	5'-Dig-GAAGCATGGGTTCGCGGAGTCGTCTGCGGTATGTGGAA -3'	38 mer	
F1ab -CP2	5'-GTGCGGCACAGGCACTAGTATTATCATTGTAGATGTCAAAAGCC -3'	44 mer	
F1ab -C1	5'-GAAGCATGGGTTCGCGGAGT-3'	20 nt	F1ab
F1ab -C2	5'-GTGCGGCACAGGCACTAGTA-3'	20 nt	
F1ab -D1	5'-TGATCACAACTACAGCCAT-3'	19 nt	
F1ab -D2	5'-CTGATGTCGTATACAG-3'	16 nt	
F1ab -R1	5'-GATTGTGCATCAGCTGACT-3'	19 nt	
F1ab -R2	5'-GTTTGCGGTGTAAGTGCA-3'	18 nt	
N-F1	5'-GCTTCTACGCAGAAGGGA-3'	18 nt	
N-F2	5'-AGGCTTCTTAGAAGCCTCAG-3'	20 nt	
N-CP1	5'-CTACTGCTGCCTGGAGTTGAATTCTTCTCGTTCCTCATCACG-3'	42 mer	
N-CP1*	5'-FITC-CTACTGCTGCCTGGAGTTGAATTCTTCTCGTTCCTCATCACG-3'	42 mer	
N-CP2	5'-TGCTTGACAGATTGAACCAGCTGTGACAGTTTGGCCTTGTT-3'	41 mer	
N-C1	5'-CTACTGCTGCCTGGAGTTGAAT-3'	22 nt	N
N-C2	5'-TGCTTGACAGATTGAACCAGCT-3'	22 nt	
N-D1	5'-TCTTGAACTGTTGCGACTA-3'	19 nt	
N-D2	5'-TGAGAGCAAAATGTCTGGT-3'	19 nt	
N-R1	5'-CATTCTAGCAGGAGAAGTT-3'	19 nt	
N-R2	5'-ATGCTGCTCTTGCTTTGCT-3'	19 nt	

[a] F1ab, open reading frame 1a/b; N, nucleoprotein gene; F1ab-CP1*, 5'-labeled with Dig when used in RT-MCDA-BS assay; N-CP1*, 5'-labeled with FITC when used in RT-MCDA-BS assay.

[b] Dig, digoxigenin; FITC, fluorescein isothiocyanate.

[c] mer, monomeric unit; nt, nucleitide.

Table S2. The pathogens used in this study

Strains	Serotypes (subtypes)	No. of strains	RT-LAMP-NBS
Positive control	Unidentified	1	P
Coronavirus	HKU1	1	N
Influenza Virus A	H1N1	2	N
Influenza Virus A	H1N2	1	N
Influenza Virus A	H5	9	N
Influenza Virus A	H5N8	1	N
Influenza Virus A	H4N6	1	N
Influenza Virus A	H7N9	1	N
Influenza Virus B	Unidentified	3	N
Syncytial Virus	Unidentified	1	N
Human Adenovirus	Unidentified	2	N
Parainfluenza Virus	Unidentified	1	N
Bocavirus	Unidentified	1	N
Coxsackievirus	CAV16	1	N
Human enterovirus	EV71	1	N
Porcine circovirus	II	1	N
Swine fever virus	Unidentified	2	N
Lelysted Virus	Unidentified	2	N
Mycoplasma	Unidentified	1	N
Chlamydia	Unidentified	1	N
Lpneumophila	Unidentified	1	N
Pseudomonas aeruginosa	Unidentified	1	N
Staphylococcus aureus	Unidentified	1	N
Staphylococcus epidermidis	Unidentified	1	N
Staphylococcus saprophytics	Unidentified	1	N
Klebsiella pneumoniae	Unidentified	1	N
Neisseria meningitidis	Unidentified	1	N
Acinetobacter baumannii	Unidentified	1	N
Enterococcus faecalis	Unidentified	1	N
Enterococcus faecium	Unidentified	1	N
Shigella flexneria	Unidentified	1	N
Listeria monocytogenes	Unidentified	1	N
Staphylococcus suis	Unidentified	1	N
Bacillus cereus	Unidentified	1	N
Enterotoxigenic E. coli	Unidentified	1	N
Salmonella	Unidentified	1	N
Streptococcus pneumona	Unidentified	1	N
Vibrio parahemolyticus	Unidentified	1	N
Candida tropicalis	Unidentified	1	N

Cryptoccus neo formas	Unidentified	1	N
Candida albicans	Unidentified	1	N

Notes: Only positive control ($5x10^3$ copies each of F1ab-plasmid and np-plsmid) could be detected by the COVID-19 RT-MCDA-BS technique, indicating the extremely high selectivity of this method.

Table S3. Detection results of MCDA-BS for different types of samples

Sample number [a]	Sample type	Result of RT-PCR [b]	Ct value of RT-PCR [c]	Judgment result of MCDA-BS	Results of MCDA-BS		Patient status
					F1ab	N	
S1	Faeces	Positive	34.3	Positive	+	+	Convalescent
S2	Pharyngeal swab	Positive	36.6	Positive	+	+	Convalescent
S3	Nasal swab	Negative	—	Negative	—	—	Convalescent
S4	Faeces	Positive	35.4	Positive	+	_	Convalescent
S5	Faeces	Positive	33.7	Positive	+	+	Convalescent
S6	Faeces	Negative	—	Negative	—	—	Convalescent
S7	Faeces	Negative	—	Negative	—	—	Convalescent
S8	Faeces	Positive	36.2	Positive	+	—	Convalescent
S9	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S10	Nasal swab	Negative	—	Negative	—	—	Convalescent
S11	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S12	Anal swab	Negative	—	Negative	—	—	Convalescent
S13	Anal swab	Negative	—	Negative	—	—	Convalescent
S14	Faeces	Negative	—	Negative	—	—	Convalescent
S15	Faeces	Negative	—	Negative	—	—	Convalescent
S16	Faeces	Negative	—	Negative	—	—	Convalescent
S17	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S18	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S19	Faeces	Negative	—	Negative	—	—	Convalescent
S20	Anal swab	Negative	41.8	Positive	+	+	Convalescent
S21	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S22	Anal swab	Negative	—	Negative	—	—	Convalescent
S23	Anal swab	Negative	—	Negative	—	—	Convalescent
S24	Anal swab	Negative	41.9	Positive	—	+	Convalescent
S25	Nasal swab	Negative	—	Negative	—	—	Convalescent
S26	Nasal swab	Negative	—	Negative	—	—	Convalescent
S27	Pharyngeal swab	Positive	35.2	Positive	+	+	Convalescent
S28	Pharyngeal swab	Positive	34.3	Positive	+	+	Convalescent
S29	Nasal swab	Negative	—	Negative	—	—	Convalescent
S30	Anal swab	Negative	—	Negative	—	—	Convalescent
S31	Nasal swab	Negative	—	Negative	—	—	Convalescent
S32	Anal swab	Negative	—	Negative	—	—	Convalescent
S33	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S34	Anal swab	Negative	—	Negative	—	—	Convalescent
S35	Nasal swab	Negative	—	Negative	—	—	Convalescent
S36	Anal swab	Negative	—	Negative	—	—	Convalescent
S37	Nasal swab	Negative	—	Negative	—	—	Convalescent
S38	Anal swab	Negative	—	Negative	—	—	Convalescent

S39	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S40	Anal swab	Negative	—	Negative	—	—	Convalescent
S41	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S42	Anal swab	Negative	—	Negative	—	—	Convalescent
S43	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S44	Anal swab	Negative	—	Negative	—	—	Convalescent
S45	Nasal swab	Negative	—	Negative	—	—	Convalescent
S46	Anal swab	Negative	—	Negative	—	—	Convalescent
S47	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S48	Anal swab	Positive	33.3	Positive	+	+	Convalescent
S49	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S50	Anal swab	Positive	34.5	Positive	+	+	Convalescent
S51	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S52	Anal swab	Negative	—	Negative	—	—	Convalescent
S53	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S54	Pharyngeal swab	Negative	—	Negative	—	—	Convalescent
S55	Pharyngeal swab	Positive	30.2	Positive	+	+	Acute phase
S56	Pharyngeal swab	Positive	32.4	Positive	+	—	Acute phase
S57	Pharyngeal swab	Positive	29.4	Positive	+	+	Acute phase
S58*	Pharyngeal swab	Positive	35.9	Positive	+	+	Acute phase
S59	Pharyngeal swab	Positive	25.4	Positive	+	+	Acute phase
S60	Pharyngeal swab	Positive	26.6	Positive	+	+	Acute phase
S61	Pharyngeal swab	Positive	29.3	Positive	+	+	Acute phase
S62	Pharyngeal swab	Positive	31.3	Positive	+	—	Acute phase
S63	Pharyngeal swab	Positive	28.4	Positive	+	+	Acute phase
S64	Pharyngeal swab	Positive	29.6	Positive	+	+	Acute phase
S65	Pharyngeal swab	Positive	30	Positive	+	+	Acute phase

[a] S58: this sample was collected from a asymptomatic individual.

[b] Positive: Ct <37; Negative: Ct>40 or no Ct.

[c] '—': no Ct.

Table S4. Detection results of COVID-19 RT-MCDA-BS method for non-COVID-19 samples

Sample number	Sample type	Result of COVID-19 RT-PCR	Result of COVID-19 RT-MCDA-BS	Confirmed pathogens*
S1	Pharyngeal swab	—	—	Influenza A virus
S2	Pharyngeal swab	—	—	Influenza A virus
S3	Pharyngeal swab	—	—	Influenza A virus
S4	Pharyngeal swab	—	—	Influenza A virus
S5	Pharyngeal swab	—	—	Influenza A virus
S6	Pharyngeal swab	—	—	Influenza A virus
S7	Pharyngeal swab	—	—	Influenza A virus
S8	Pharyngeal swab	—	—	Influenza A virus
S9	Pharyngeal swab	—	—	Influenza A virus
S10	Pharyngeal swab	—	—	Influenza A virus
S11	Pharyngeal swab	—	—	Influenza A virus
S12	Pharyngeal swab	—	—	Influenza A virus
S13	Pharyngeal swab	—	—	Influenza A virus
S14	Pharyngeal swab	—	—	Influenza A virus
S15	Pharyngeal swab	—	—	Influenza A virus
S16	Pharyngeal swab	—	—	Influenza A virus
S17	Pharyngeal swab	—	—	Influenza A virus
S18	Pharyngeal swab	—	—	Others
S19	Pharyngeal swab	—	—	Others
S20	Pharyngeal swab	—	—	Others
S21	Pharyngeal swab	—	—	Others
S22	Pharyngeal swab	—	—	Others
S23	Pharyngeal swab	—	—	Others
S24	Pharyngeal swab	—	—	Others
S25	Pharyngeal swab	—	—	Others
S26	Pharyngeal swab	—	—	Others
S27	Pharyngeal swab	—	—	Others
S28	Pharyngeal swab	—	—	Others
S29	Pharyngeal swab	—	—	Others
S30	Pharyngeal swab	—	—	Others
S31	Pharyngeal swab	—	—	Others
S32	Pharyngeal swab	—	—	Others
S33	Pharyngeal swab	—	—	Others
S34	Pharyngeal swab	—	—	Others
S35	Pharyngeal swab	—	—	Others
S36	Pharyngeal swab	—	—	Others
S37	Pharyngeal swab	—	—	Others
S38	Pharyngeal swab	—	—	Others
S39	Pharyngeal swab	—	—	Others
S40	Pharyngeal swab	—	—	Others
S41	Pharyngeal swab	—	—	Others

S42	Pharyngeal swab	—	—	Others
S43	Pharyngeal swab	—	—	Others
S44	Pharyngeal swab	—	—	Others
S45	Pharyngeal swab	—	—	Others
S46	Pharyngeal swab	—	—	Others
S47	Pharyngeal swab	—	—	Others
S48	Pharyngeal swab	—	—	Others
S49	Pharyngeal swab	—	—	Others
S50	Pharyngeal swab	—	—	Others
S51	Pharyngeal swab	—	—	Others
S52	Pharyngeal swab	—	—	Others
S53	Pharyngeal swab	—	—	Others
S54	Pharyngeal swab	—	—	Others
S55	Pharyngeal swab	—	—	Others
S56	Pharyngeal swab	—	—	Others
S57	Pharyngeal swab	—	—	Others
S58	Pharyngeal swab	—	—	Others
S59	Pharyngeal swab	—	—	Others
S60	Pharyngeal swab	—	—	Others
S61	Pharyngeal swab	—	—	Others
S62	Pharyngeal swab	—	—	Others
S63	Pharyngeal swab	—	—	Others
S64	Pharyngeal swab	—	—	Others
S65	Pharyngeal swab	—	—	Others
S66	Pharyngeal swab	—	—	Others
S67	Pharyngeal swab	—	—	Others
S68	Pharyngeal swab	—	—	Others
S69	Pharyngeal swab	—	—	Others
S70	Pharyngeal swab	—	—	Others
S71	Pharyngeal swab	—	—	Others
S72	Pharyngeal swab	—	—	Others
S73	Pharyngeal swab	—	—	Others
S74	Pharyngeal swab	—	—	Others
S75	Pharyngeal swab	—	—	Others
S76	Pharyngeal swab	—	—	Others
S77	Pharyngeal swab	—	—	Others
S78	Pharyngeal swab	—	—	Others
S79	Pharyngeal swab	—	—	Others
S80	Pharyngeal swab	—	—	Others
S81	Pharyngeal swab	—	—	Others
S82	Pharyngeal swab	—	—	Others
S83	Pharyngeal swab	—	—	Others
S84	Pharyngeal swab	—	—	Others
S85	Pharyngeal swab	—	—	Others

S86	Pharyngeal swab	—	—	Others
S87	Pharyngeal swab	—	—	Others
S88	Pharyngeal swab	—	—	Others
S89	Pharyngeal swab	—	—	Others
S90	Pharyngeal swab	—	—	Others
S91	Pharyngeal swab	—	—	Others
S92	Pharyngeal swab	—	—	Others
S93	Pharyngeal swab	—	—	Others
S94	Pharyngeal swab	—	—	Others
S95	Pharyngeal swab	—	—	Others
S96	Pharyngeal swab	—	—	Others
S97	Pharyngeal swab	—	—	Others
S98	Pharyngeal swab	—	—	Others
S99	Pharyngeal swab	—	—	Others
S100	Pharyngeal swab	—	—	Others
S101	Pharyngeal swab	—	—	Others
S102	Pharyngeal swab	—	—	Others
S103	Pharyngeal swab	—	—	Others
S104	Pharyngeal swab	—	—	Others
S105	Pharyngeal swab	—	—	Others
S106	Pharyngeal swab	—	—	Others
S107	Pharyngeal swab	—	—	Others
S108	Pharyngeal swab	—	—	Others
S109	Pharyngeal swab	—	—	Others
S110	Pharyngeal swab	—	—	Others
S111	Pharyngeal swab	—	—	Others
S112	Pharyngeal swab	—	—	Others
S113	Pharyngeal swab	—	—	Others
S114	Pharyngeal swab	—	—	Others
S115	Pharyngeal swab	—	—	Others
S116	Pharyngeal swab	—	—	Others
S117	Pharyngeal swab	—	—	Others
S118	Pharyngeal swab	—	—	Others

*Note： Influenza A virus includes H1N1, H3N2, H5N1,H7 subtype et, al; Others for Parainfluenza Virus, *Mycoplasma*, *Neisseria meningitidis* et, al.

Figures

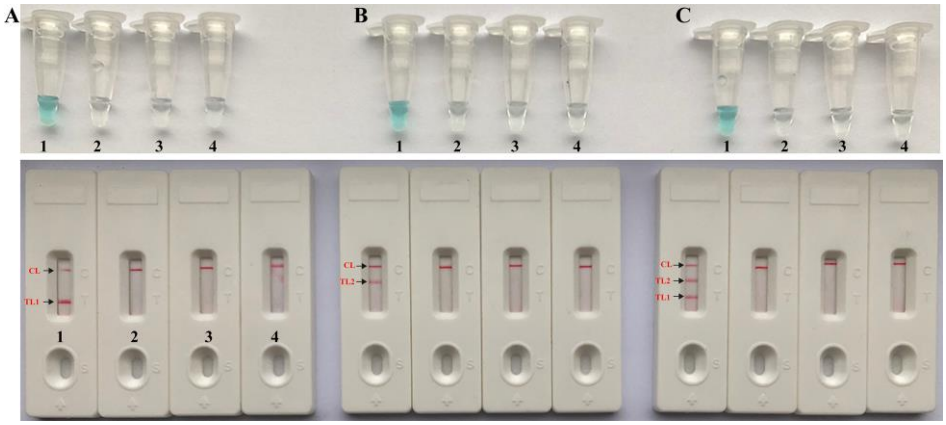

Figure S1. Confirmation and detection of F1ab- and N-RT-MCDA products

Color change of F1ab- (**A**, top row), N- (**B**, top row) and COVID-19 (**C**, top row) RT-MCDA tubes; BS applied for visual detection of F1ab- (**A**, bottom row), N- (**B**, bottom row) and COVID-19 (**C**, bottom row) RT-MCDA products. Tube A1 (BS A1), F1ab-RT-MCDA positive amplification (5×10^3 copies of F1ab-plasmids); Tube A2 (BS A2), F1ab-RT-MCDA negative amplification (H1N1); Tube A3 (BS A3), F1ab-RT-MCDA negative amplification (EV 71); Tube A4 (BS A4), Blank control (DW). Tube B1 (BS B1), N-RT-MCDA positive amplification (5×10^3 copies of N-plasmids); Tube B2 (BS B2), N-RT-MCDA negative amplification (H1N1); Tube B3 (BS B3), N-RT-MCDA negative amplification (EV 71); Tube B4 (BS B4), Blank control (DW). Tube C1 (BS C1), COVID-19 RT-MCDA positive amplification (5×10^3 copies of F1ab-plasmids and N-plasmids); Tube C2 (BS C2), COVID-19 RT-MCDA negative amplification (H1N1); Tube C3 (BS C3), COVID-19 RT-MCDA negative amplification (EV 71); Tube C4 (BS C4), Blank control (DW).

Note*: H1N1, Influenza H1N1 virus; EV 71, Human enterovirus 71; DW, Distilled water.

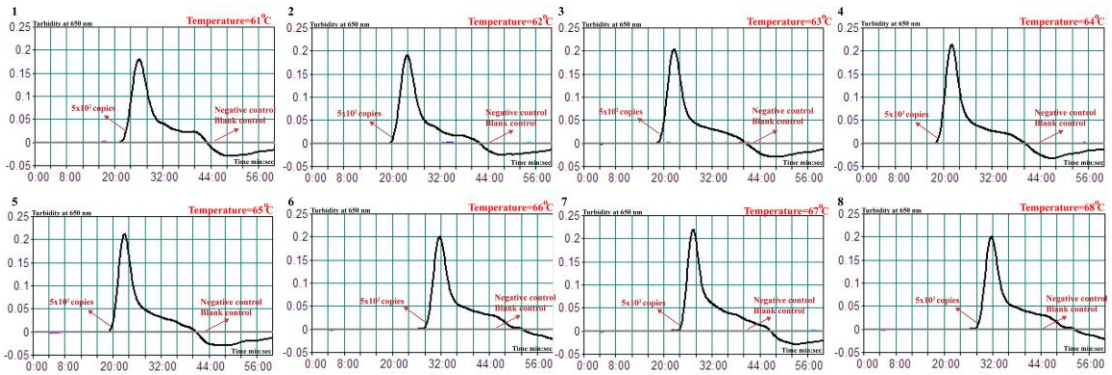

Figure S2. Optimal amplification temperature for F1ab-RT-MCDA primer set

The F1ab-RT-MCDA reactions for detection of F1ab gene of COVID-19 were monitored by real-time measurement of turbidity (LA320c). Turbidity of >0.1 was considered to be positive as the threshold value was 0.1. Eight kinetic graphs (1-8) were yielded at different temperatures (61-68°C, 1°C intervals) with target template at the level of 5×10^2 copies (F1ab-plamid) per reaction. The graphs from 63°C to 65°C showed faster amplification.

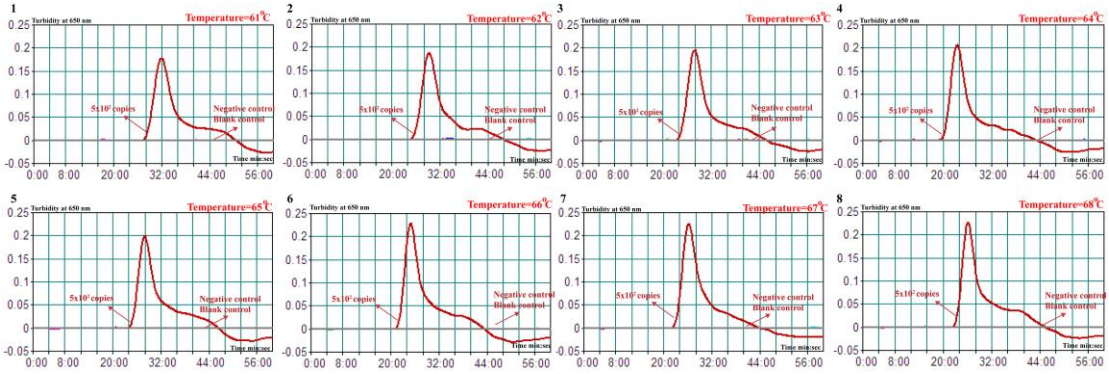

Figure S3. Optimal amplification temperature for N-RT-MCDA primer set

The N-RT-MCDA reactions for detection of N gene of COVID-19 were monitored by real-time measurement of turbidity (LA320c). Turbidity of >0.1 was considered to be positive as the threshold value was 0.1. Eight kinetic graphs (1-8) were yielded at different temperatures (61-68℃, 1ºC intervals) with target template at the level of 5×10^2 copies (N-plasmid) per reaction. The graphs from 63℃ to 65℃ showed faster amplification.

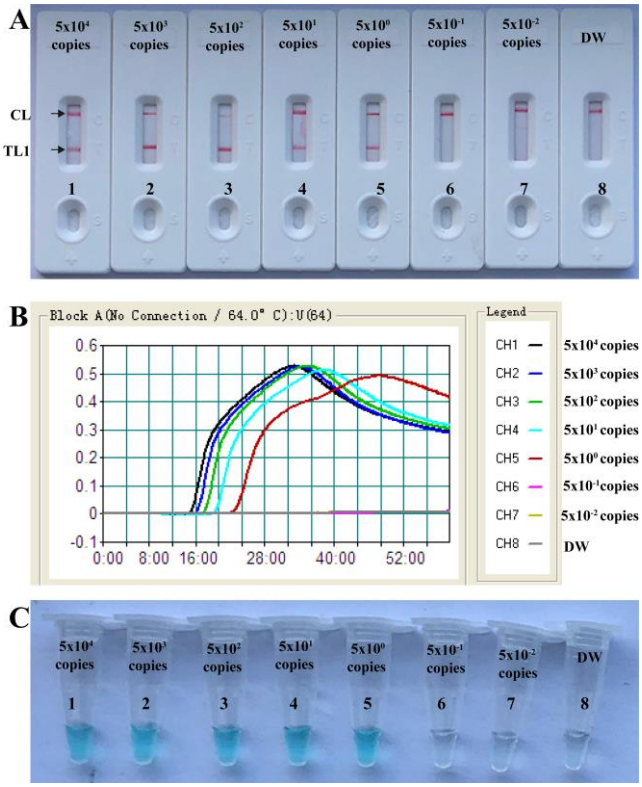

Figure S4. Analytical sensitivity of F1ab-RT-MCDA-BS assay using serially diluted F1ab-plasmid templates

A, BS applied for reporting the results; **B**, Real-time turbidity applied for reporting the results; **C**, VDR applied for reporting the results. BS (A)/Signals (B)/Tubes (C) 1-8 represented the F1ab-plasmid levels of 5×10^4, 5×10^3, 5×10^2, 5×10^1, 5×10^0, 5×10^{-1}, 5×10^{-2} copies per reaction and blank control (DW). The plasmid levels of 5×10^4 to 5×10^0 copies per reaction produced the positive reactions.

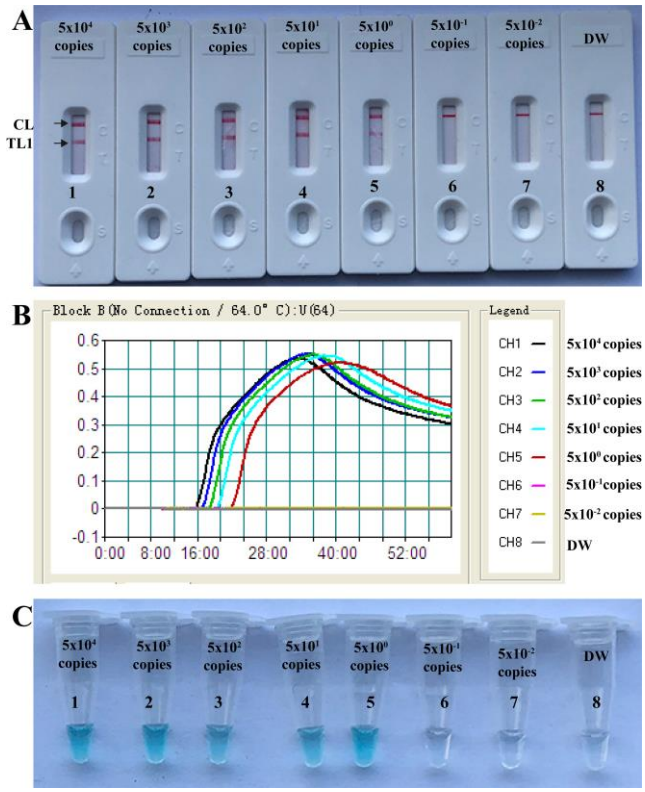

Figure S5. Analytical sensitivity of N-RT-MCDA-BS assay using serially diluted N-plasmid templates

A, BS applied for reporting the results; **B**, Real-time turbidity applied for reporting the results; **C**, VDR applied for reporting the results. BS (A)/Signals (B)/Tubes (C) 1-8 represented the N-plasmid levels of 5×10^4, 5×10^3, 5×10^2, 5×10^1, 5×10^0, 5×10^{-1}, 5×10^{-2} copies per reaction and blank control (DW). The plasmid levels of 5×10^4 to 5×10^0 copies per reaction produced the positive reactions.

Figure S6. The optimal duration of time required for COVID-19 RT-MCDA-BS assay

Four reaction times (**A**, 15 min; **B**, 25 min; **C**, 35 min; and **D**, 45 min) were tested and compared at 64 °C. COVID-19 RT-MCDA reactions were conducted using the LoD level of templates ($5x10^0$ copies of each of F1ab-plasmid and N-plasmid), and the templates at the LoD level could be detected when the RT-MCDA reaction only lasted for 25 min (**Biosensor 2**).