Supplementary Information for

Multiple Aptamers Recognition based Quantum dots Lateral Flow Platform: An Ultrasensitive Point-of-Care Testing of Respiratory Infectious Diseases

Hengxuan Li,[a,b] Xiaoyi Fu,*[b,c] Qimin You,[d] Dawei Shi,[e] Lingxuan Su,[f] Minghui Song,[b] Ruizi Peng,[b] Ting Fu,[b] Peng Wang *[b] and Weihong Tan *[b,g]

a. Medical School, Faculty of Medicine, Tianjin University, Tianjin 300072, P. R. China.
b. Zhejiang Cancer Hospital, The Key Laboratory of Zhejiang Province for Nucleic Acids, Hangzhou Institute of Medicine (HIM), Chinese Academy of Sciences, Hangzhou, 310022, P. R. China. E-mail: chemwangp@hnu.edu.cn, tan@hnu.edu.cn
c. Hangzhou Aptech Biotechnology Company Limited, Hangzhou 310022, P. R. China. E-mail: fxy_study@163.com
d. Ustar Biotechnologies (Hangzhou) Company Limited, Hangzhou 310051, P. R. China.
e. National Institutes for Food and Drug Control, Beijing 100050, P. R. China.
f. Key Laboratory of Public Health Detection and Etiological Research of Zhejiang Province, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou 310050, P. R. China.
g. Institute of Molecular Medicine (IMM), Renji Hospital, Shanghai Jiao Tong University School of Medicine, and College of Chemistry and Chemica Engineering, Shanghai Jiao Tong University, Shanghai, 200240, P. R. China.

Supplementary Methods

Materials and Instruments

All DNA sequences were synthesized by Sangon Biotech (Shanghai, China). All sequences are listed in Table S1. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC), N-Hydroxysuccinimide (NHS), 2-Morpholinoethanesulphonic acid (MES) and Streptavidin (SA) was acquired from Solarbio (Beijing, China). (3-Mercaptopropyl) trimethoxysilane (MPTS), n-hexane octyl trimethoxysilane (OTMS), ammonia 3-aminopropyltriethoxysilane (APTES), and Succinic anhydride were purchased from Sigma-Aldrich (Missouri, USA). Oil-Soluble Quantum Dots were acquired from Wuhanjiayuan Quantumdots (Wuhan, China). Influenza B (B/Victoria/705/2018) virus nucleoprotein, SARS-CoV-2 (2019-nCoV) N protein and other coronavirus N protein were purchased from Sino Biological (Beijing, China). Anti-influenza B virus nucleoprotein antibody and anti-SARS-CoV-2 N protein antibody were purchased from Fapon Biotech (Dongguan, China). Recombinant anti-SA antibody was purchased from Bioeast (Hangzhou, China). All other reagents were purchased from the National Pharmaceutical Group Chemical Reagent (Shanghai, China). The polyvinylchloride (PVC) backing card (YN-Jn8100), glass fiber membrane sample pad (YN-Jn8301), polyester fiber membrane conjugate pad (YN-Jn8309), nitrocellulose (NC) membrane (YN120), absorbent pad (YN-Jn8201) and plastic card case (YNC-9) were purchased from YiNeng Biotech Co., Ltd. (Guangzhou, China). The dispensing platform was obtained from Kinbio Tech Co., Ltd. (Shanghai, China).

RT PCR was performed on the Light Cycler 96 Real-Time PCR System (Roche). Surface plasmon resonance (SPR) experiments were used to test the affinity of aptamers toward different kinds of proteins by BIAcore T200 biosensor system. Transmission electron microscopy (TEM) analyses and elemental mapping scanning were performed on the JEM-F200 field emission TEM. Scanning electron microscopy (SEM) analyses were performed on the JSM-IT800 field emission SEM. Dynamic light scattering and zeta (ζ) potentials were measured with the Zetasizer Nano ZS ZEN3600 particle and molecular charge analyzer.

The portable fluorescence reader was co-developed with Ustar Biotechnologies (Hangzhou) Company Limited, which consists of photodiode, focusing mirror, 625 nm pass filter, dichroic mirror, focusing/collimator group, step motor, LED light source and narrow band filter. All components of the portable fluorescence reader are derived from commercially available components and parts. The device control and data reading are performed using serial port/network data debugging software (SSCOM V 5.13.1).

Synthesis of Dendritic mesoporous silica nanoparticles (DMSN)

DMSNs applied to enrich QDs were synthesized according to reported method.[1] In a typical synthesis process, 0.3 mg/mL 80 mL TEA aqueous solution was stirred at 80°C for 30 min, followed by the addition of 130 mg CTAB and 56 mg NaSal and stirred for 1 h at 80°C. The solution was injected with 1.3 mL TEOS and allowed to be gently stirred for 2 h at 80 °C. The dimension and pore size of nanospheres could be tuned by adjusting reaction parameters as previously reported[2]. Subsequently, the precipitate was collected after centrifugation at 10000 rpm for 5 min. The mixture was finally dispersed in 50 mL HCl/methanol 1:1 mixture and stirred at 60 °C for 6 h, and then repeat once to extract the residue organic templates. The extraction was repeated once and the dendritic silica spheres were finally dispersed in 35 mL of ethanol. The above dendritic silica ethanol solution was added with 850 µL of ammonia and 350 µL of MPTS, followed by vigorous stirring at room temperature for 12 h. The finally product DMSNs was harvested by centrifugation, dispersed in 16.7 mL ethanol.

Synthesis of DMSN/QDs/SiO_2 (MQS)

For the assembling of QDs by DMSNs, the thiolated DMSNs were used for OTMS-capped QDs incorporation. In brief, a wet precipitate of 6mg DMSN was added with 1mL 3mg/mL QDs chloroform solution and sonicated for 5 min to obtain a clear solution. The DMSN/QDs composites were recovered by centrifugation at 10000 rpm for 5 min and dissolved by 50uL OTMS, 7.5mL methanol and 95 µL ammonia. The aqueous phase transfer of composites was realized by sonicating the above mixture for 30 min and then centrifugation. To grow the silica shell by Stöber method[3], the precipitate incubate with 10mL ethanol, 2.5 mL pure water, 315 µL ammonia and 6.25 µL TEOS for 2 h. After centrifugation, we obtained the DMSNs/QDs/SiO2 spheres (MQS). The MQS added with 250 µL ammonia and 10 µL APTES and reacted for 12 h to obtain amino-group. The amino-terminated MQS dispersed in 5mL DMF containing 5 mg/mL succinic anhydride and reacted for 4 h to form the carboxyl-terminated MQS. The production was washed with ethanol and water several times, and dispersed in 0.1M MES buffer (pH 6).

Pretreatment of the sample pad and absorbent pad

The pretreatment steps for sample pads and conjugate pads in LFIA are critical for optimizing the performance of the assay. Soaked the sample pad in the buffer solution (Tris-HCl, Tween-20, PVP k30, sucrose in the appropriate proportions) for 30 minutes, then dried the sample pad overnight at 37°C

until completely dry. Treated the conjugate pad the same way as the sample pad, and the buffer solution for treating the conjugate pad included Tris-HCl, PVP k30, isopropanol, NaCl and trehalose.

Supplementary Figures

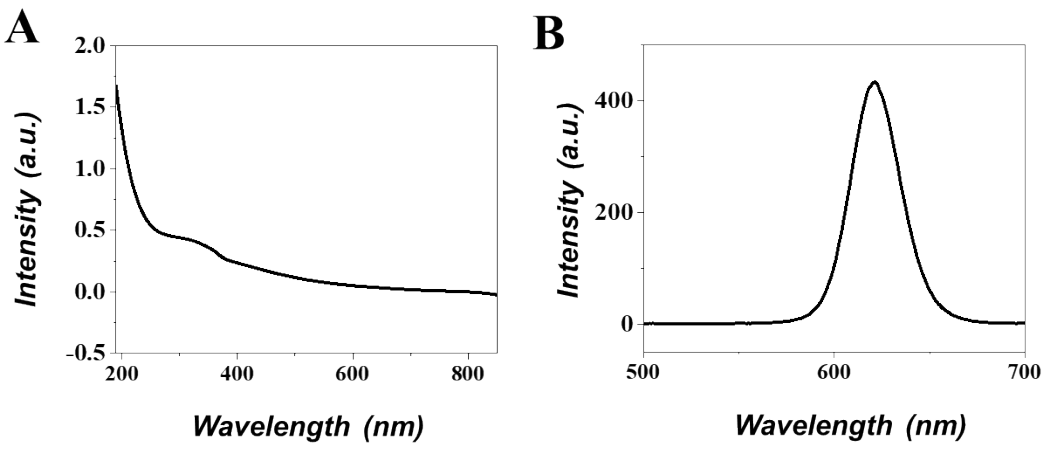

Fig. S1 (A) The UV-Vis absorption spectrum of ApMQS. (B) The fluorescence emission spectrum of ApMQS. (Excitation: 365 nm; Emission: 625 nm)

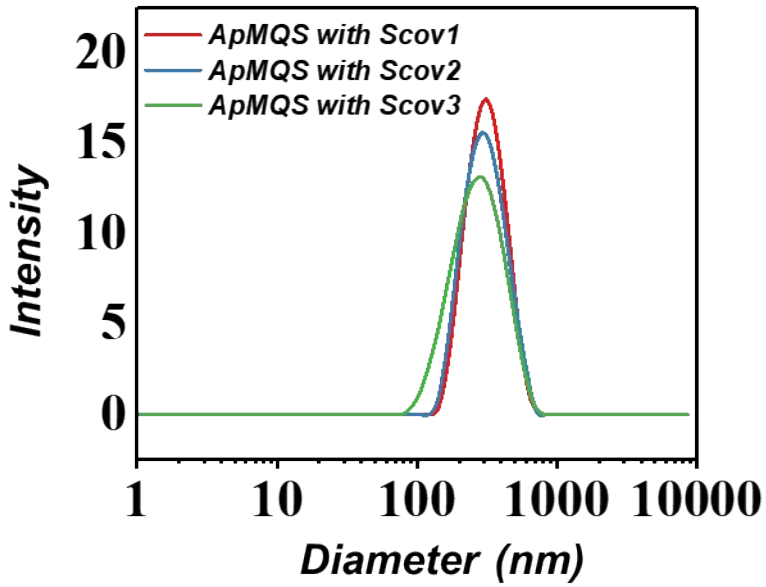

Fig. S2 The hydrodynamic diameter distributions of ApMQS with different aptamers (Scov1, Scov2, Scov3).

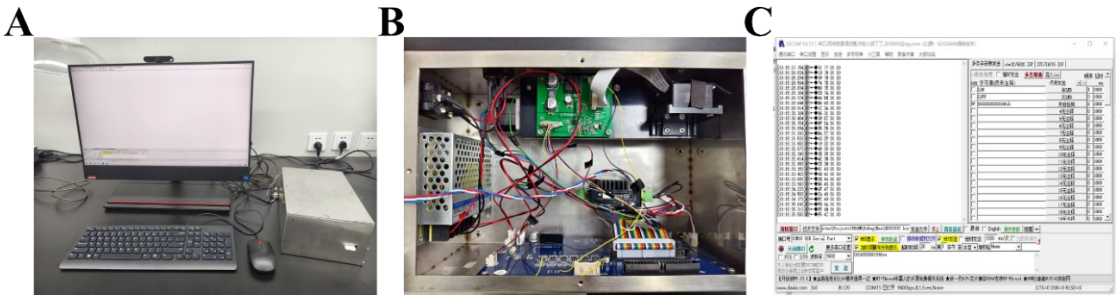

Fig. S3 (A) The appearance photo of the portable fluorescence reader with the matching computer. (B) The internal structure photo of the portable fluorescence reader. (C) The main interface of the fluorescence signal processing software.

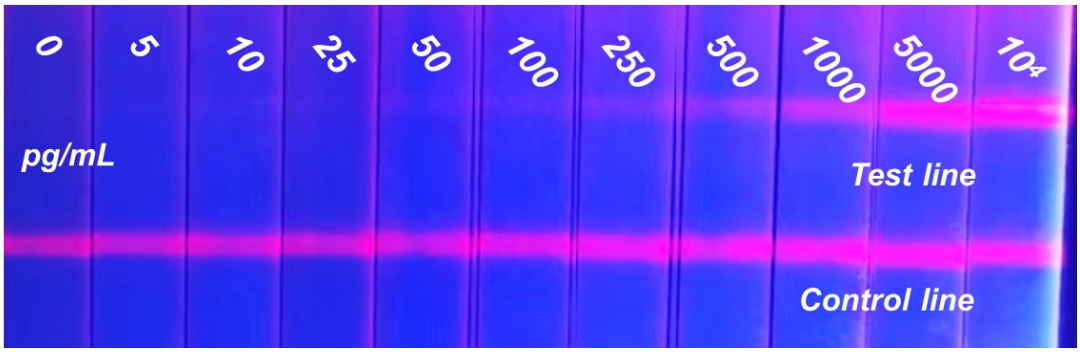

Fig. S4 The photograph of ApMQS-LFA detection results of N protein concentration (10000, 5000, 1000, 500, 250, 100, 50, 25, 10, 5, 0 pg/mL) under UV light (Light source from right).

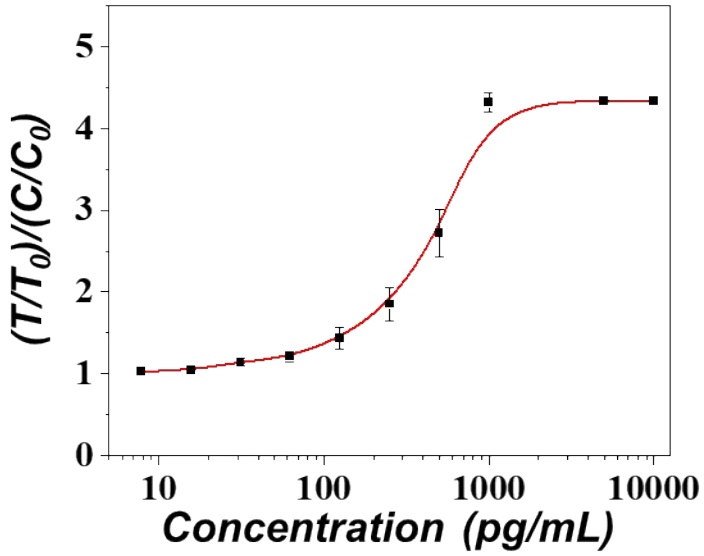

Fig. S5 Curve of SARS-CoV-2 N protein concentration (10000, 5000, 1000, 500, 250, 100, 50, 25, 10, 5, 0 pg/mL) and T/C value (T and C represent the detection values of T and C lines under the corresponding N protein concentration, T_0 and C_0 represent the detection values of T and C lines under the blank control). Error bars represent the standard deviation of three parallel experiments.

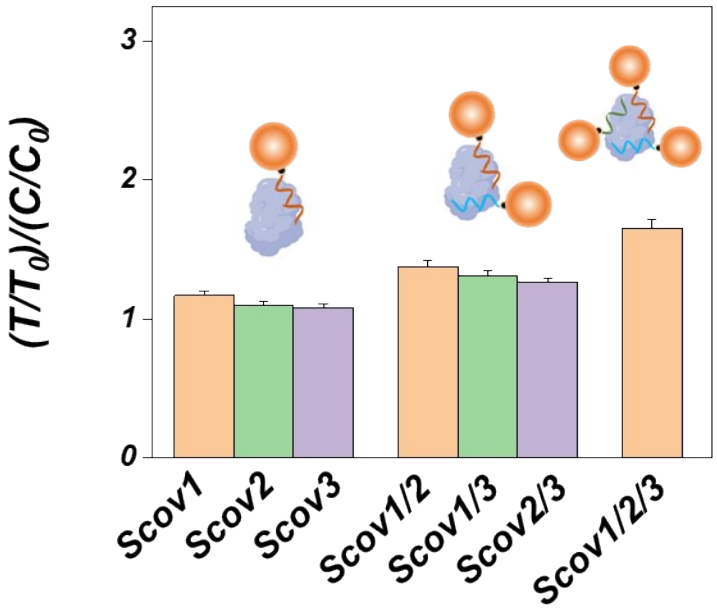

Fig. S6 Detection results of different combinations of Scov1, Scov2 and Scov3 under 100 pg/mL N protein concentration.

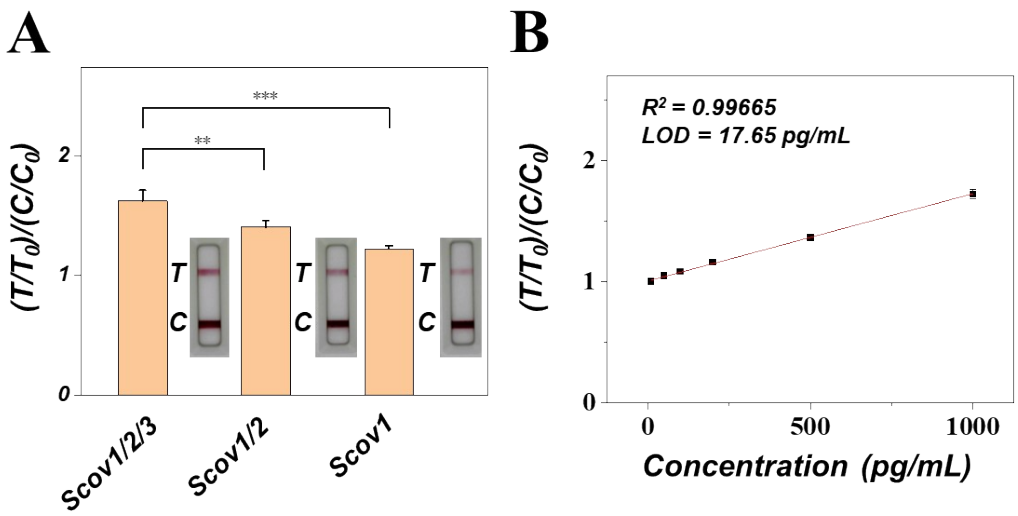

Fig. S7 (A) Detection results of different combinations of Scov1, Scov2 and Scov3 under 1 ng/mL SARS-CoV-2 N protein concentration by AuNPs-LFIA. (B) The standard curve of SARS-CoV-2 N protein detection on AuNPs-LFIA. * p<=0.05, ** p<=0.01, *** p<=0.001.

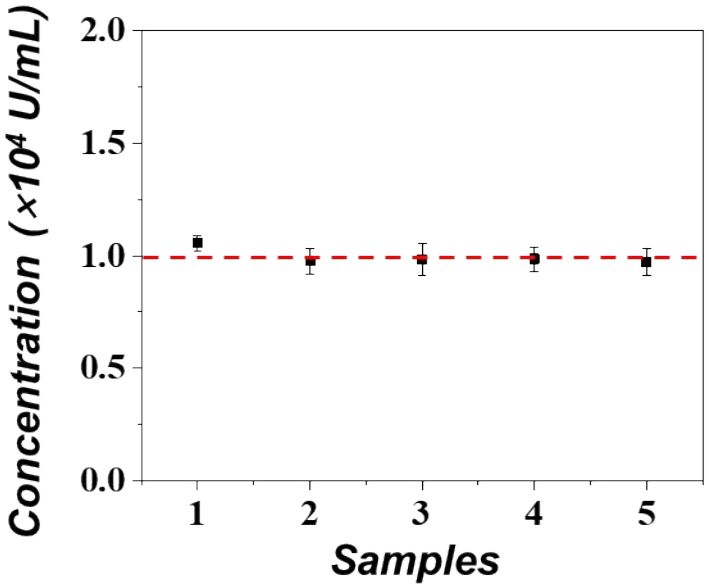

Fig. S8. Detection results of inactivated SARS-CoV-2 recovery (the red line represents the concentration: 10^5 U/ml). Error bars represent the standard deviation of three parallel experiments.

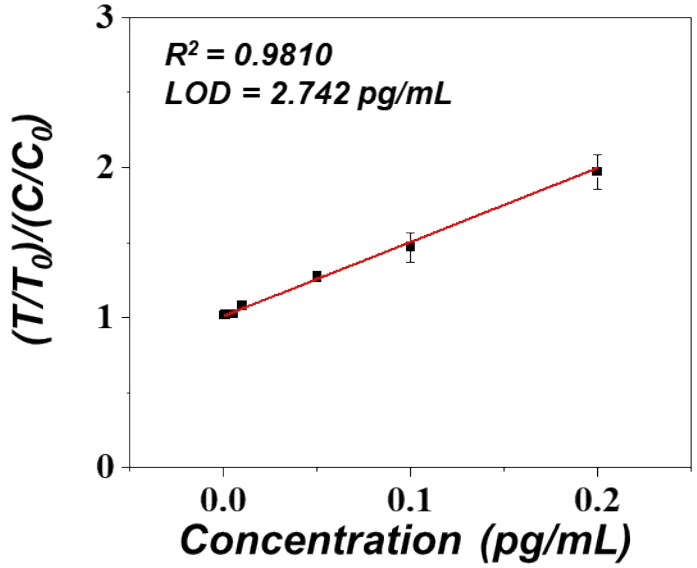

Fig. S9. The standard curve of the influenza B virus nucleoprotein concentration and $(T/T_0)/(C/C_0)$ value.

Supplementary Tables

Tab. S1 The sequences of SARS-CoV-2 N protein aptamers and the initial library and the primers used in SELEX.

DNA species	Sequence (5' to 3')
DNA library1	*GTTCGTGGTGTGCTGGATGT(N)$_{36}$TGACACATCCAGCAGCACGA*
Primer1-S	*GTTCGTGGTGTGCTGGATGT*
Primer1-S-FAM	*FAM-GTTCGTGGTGTGCTGGATGT*
Primer1-A	*TCGTGCTGCTGGATGTGTCA*
Scov1	*CGCCTCCTTCCTCTCGGGGTGTGTAGGGTCAGGGAGTGTGAGAGGA GGAGACGAGATCGGCG*
Scov2	*CACGTCGGGGGGGTCACACATGAACCGTGCGGATACGGAGACGAG*
Scov3	*CGCCTCCTTCCACGGGATCGGATTCCCCACTCGGCTCTATCGGATTGG AGACGAGATCGGCG*

$(N)_{36}$ represents 36 nucleotides with equimolar incorporation of A, G, C, and T at each position.

Tab. S2 The sequences of Influenza B virus nucleoprotein aptamers and the initial library and the primers used in SELEX.

Aptamer	Sequence(5' to 3')
DNA library2	*TTCAGCACTCCACGCATAGC(N)$_{36}$CCTATGCGTGCTACCGTGAA*
Primer2-S1	*TTCAGCACTCCACGCATAGC*
Primer2-S2-FAM	*FAM-TTCAGCACTCCACGCATAGC*
Primer2-A	*TTCACGGTAGCACGCATAGG*
FluB1	*TTTACGCATAGCGGAGGGACGCCCAACAGTTCCGGAGGTTTATCGGG GCCTATGCGT*
FluB2	*TTTACGCATAGCAAGTCCGCGTCGTTCCCGGTCATTGCCTTGTGGTGC CTATGCGT*
FluB3	*TTTACGCATAGCCGATCCGGTTGATGCACCGGTTAAGGCCTTGTGGT GCCTATGCGT*

Tab. S3 The ka, kd, and KD values of aptamers targeting the SARS-CoV-2 N protein.

Injection variables Analyte Solution	1:1 binding ka (1/Ms)	kd(1/s)	KD(M)
Scov1	8.20e+4	1.97e-4	**2.41e-9**
Scov2	1.68e+5	5.43e-4	**3.24e-9**
Scov3	5.90e+5	2.54e-3	**4.31e-9**

Tab. S4 The ka, kd, and KD values of aptamers targeting the influenza B virus nucleoprotein.

Injection variables Analyte Solution	1:1 binding ka (1/Ms)	kd(1/s)	KD(M)
FluB1	1.28e+5	1.01e-3	**7.88e-9**
FluB2	1.20e+5	7.52e-4	**6.29e-9**
FluB3	1.29e+5	1.10e-3	**8.53e-9**

Tab. S5 Two-sample equal variance hypothesis t-test analysis between group of Scov1/2/3 and Scov1 at the concentration of 100 pg/mL of N protein.

Group	Scov1/2/3	Scov1
Mean	1.641646	1.200359
Variance	0.005158	0.000777
Observation	5	5
Pooled Variance	0.002967	
Hypothetical Mean Difference	0	
Degrees of Freedom	8	
t Statistic	12.80907	
P(T <=t) One-Tailed	6.51E-07	
t Critical One-Tailed	1.859548	
P(T <= t) Two-Tailed	**1.3E-06**	
t Critical Two-Tailed	2.306004	

Tab. S6 Two-sample equal variance hypothesis t-test analysis between group of Scov1/2/3 and Scov1/2 at the concentration of 100 pg/mL of N protein.

Group	Scov1/2/3	Scov1/2
Mean	1.641646	1.454455867
Variance	0.005158	0.001923017
Observation	0.00354	5
Pooled Variance	0.00354	
Hypothetical Mean Difference	0	
Degrees of Freedom	8	
t Statistic	4.974292	
P(T <=t) One-Tailed	0.000544	
t Critical One-Tailed	1.859548	
P(T <= t) Two-Tailed	**0.001087**	
t Critical Two-Tailed	2.306004	

Tab. S7 Two-sample equal variance hypothesis t-test analysis between group of Scov1/2/3 and Scov1 at the concentration of 5 pg/mL of N protein.

Group	Scov1/2/3	Scov1
Mean	1.166706	1.016008
Variance	0.002107	0.000814
Observation	5	5
Pooled Variance	0.00146	
Hypothetical Mean Difference	0	
Degrees of Freedom	8	
t Statistic	-6.23497	
P(T <=t) One-Tailed	0.000125	
t Critical One-Tailed	1.859548	
P(T <= t) Two-Tailed	**0.00025**	
t Critical Two-Tailed	2.306004	

Tab. S8 Two-sample equal variance hypothesis t-test analysis between group of Scov1/2/3 and Scov1/2 at the concentration of 5 pg/mL of N protein.

Group	Scov1/2/3	Scov1/2
Mean	1.166706	1.099941
Variance	0.002107	0.001188
Observation	5	5
Pooled Variance	0.001648	
Hypothetical Mean Difference	0	
Degrees of Freedom	8	
t Statistic	-2.60068	
P(T <=t) One-Tailed	0.015792	
t Critical One-Tailed	1.859548	
P(T <= t) Two-Tailed	**0.031585**	
t Critical Two-Tailed	2.306004	

Tab. S9 Detection results of inactivated SARS-CoV-2 in diluted nasopharyngeal swab samples from healthy individuals

	Column 1	Column 2	Column 3
Sample	Added (10^5 U/mL)	Founded (10^5 U/mL)	Recovery (%)
1	1	1.05637	105.64%
2	1	0.9756	97.56%
3	1	0.981366	98.14%
4	1	0.98556	98.56%
5	1	0.969558	96.96%

Tab. S10 Welch t-test analysis of detection results inactivated SARS-CoV-2 in diluted nasopharyngeal swab samples from healthy individuals.

SUMMARY				
Group	Number of observations	Sum	Averge	Variance
Column 1	5	4.968454	0.993691	0.001264
Column 2	5	5	1	0

Variance analysis						
Source of variance	SS	df	MS	F	**P-value**	F crit
interblock	9.95E-05	1	9.95E-05	0.157452	**0.701888**	5.317655
interclass	0.005056	8	0.000632			
Total	0.005156	9				

Tab. S11 Subgroup results of MARQ-LFIA with clinical diagnosis of COVID-19 (N = 50)

	Clinical Results		Total
	Positives	Negatives	
Positives	26	0	26
Negatives	4	20	24
Total	30	20	50

Tab. S12 Subgroup results of MARQ-LFA with clinical diagnosis of Influenza B (N = 77)

	Clinical Results		Total
	Positives	Negatives	
Positives	44	0	44
Negatives	13	20	33
Total	57	20	77

Tab. S13 Compositions and specifications of the National Reference Panel for COVID-19（SARS-CoV-2）Antigen Detection Kit

Reference type	Reference number	Pathogen category
Positive Reference	P1	*SARS-CoV-2 (Wild type)*
Negative Reference	N1	*Staphylococcus aureus*
	N2	*Streptococcus pneumoniae*
	N3	*Measles virus*
	N4	*Mumps virus*
	N5	*Adenovirus 3*
	N6	*Mycoplasma pneumoniae*
	N7	*Parainfluenza virus 2*
	N8	*Metapneumovirus*
	N9	*Coronavirus OC43*
	N10	*Coronavirus 229E*
	N11	*Bordetella pertussis*
	N12	*Influenza B virus Victoria*
	N13	*Influenza B virus Yamagata*
	N14	*Influenza A virus H1N1 2009*
	N15	*Influenza A virus H3N2*
	N16	*Avian influenza virus H7N9*
	N17	*Avian influenza virus H5N6*
	N18	*Epstein-barr virus*
	N19	*Enterovirus CA16*
	N20	*Rhinovirus*
Detection Limit Reference	S	*SARS-CoV-2 (Wild type)*

Tab. S14 Compositions and specifications of the 2nd National Reference Panel for Influenza B Viral Antigens Detection Kit

Reference type	Reference number	Pathogen category
Positive Reference	P1	*Influenza B virus Yamagata*
Negative Reference	N1	*Staphylococcus aureus*
	N2	*Streptococcus pneumoniae*
	N3	*Measles virus*
	N4	*Mumps virus*
	N5	*Adenovirus 3*
	N6	*Adenovirus 7*
	N7	*Mycoplasma pneumoniae*
	N8	*Parainfluenza virus 2*
	N9	*Metapneumovirus*
	N10	*Coronavirus OC43*
	N11	*Coronavirus 229E*
	N12	*Bordetella pertussis*
	N13	*Epstein-barr virus*
	N14	*Enterovirus CA16*
	N15	*Respiratory syncytial virus*
	N16	*Rhinovirus A*
	N17	*Neisseria meningitidis*
	N18	*SARS-CoV-2 Omicron*
	N19	*Negative swab*
	N20	*Interference sample (Dexamethasone sodium phosphate, 0.4mg/mL)*
Detection Limit Reference	S	*Influenza B virus (Victoria)*

Tab. S15 The clinical COVID-19 positive samples detection original data of MARQ-LFIA

No.	C Value	T Value
2023-31032	10398940	1112835
2023-31033	11459213	6888464
2023-31034	10124549	4041524
2023-31037	11449218	7176999
2023-31038	10755859	4794779
2023-31039	11360164	1989929
2023-31040	11072139	5844426
2023-31041	11685217	7922533
2023-31042	11985293	6013233
2023-31043	10853856	148950
2023-31044	11327775	860939
2023-31045	10276262	6304522
2023-31046	11621179	5379917
2023-31047	11881102	7206131
2023-31048	11916161	5829075
2023-31049	10306946	4181180
2023-31050	11608469	107903
2023-31051	10946499	3690656
2023-31052	11804628	3811305
2023-31053	11396280	7073993
2023-31054	11710889	3149389
2023-31055	11138778	88907
2023-31056	10833777	2339463
2023-31057	10202404	2771588
2023-31058	10789175	117062
2023-31059	11384512	437806
2023-31060	10349539	652418
2023-31061	10655764	3910601
2023-31063	11936650	3420451
2023-31065	11734746	2902792
2023-31080	11069288	297787

2023-31081	10770695	151562
2023-31082	10414407	235188
2023-31083	11546419	87239
2023-31084	10274561	105806
2023-31085	10520323	95786
2023-31086	11569857	295807
2023-31087	10640152	75098
2023-31088	10136816	169367
2023-31089	11335932	185730
2023-31090	11048309	99612
2023-31091	11747354	261444
2023-31092	10570315	153930
2023-31093	11932833	136241
2023-31094	11728615	214184
2023-31095	10672833	135771
2023-31096	10364673	107729
2023-31097	11973452	226471
2023-31098	11611284	102483
2023-31099	11645529	231865

Tab. S16 The clinical Flu B positive samples detection original data of MARQ-LFIA.

No.	C Value	T Value
1002234709	10382813	1047989
0102234701	11977320	7982106
0108234703	11951375	7659704
0102234805	11462965	5251923
0108234803	10131095	1934427
0802234709	11264851	193752
0602234812	11108388	158424
203234537	11801633	5283190
0503235210	11674210	4189879
F232119	11171868	273282
F232120	11715321	1909577
F20231162	11447069	255430
F23-1180	11362068	1933714
231110	10330038	113710
F231961	11580466	743126
1002234827	11249632	1557679
23-1-1310	11989494	6529002
20231298	10635027	4809275
F20231265	10179738	504240
F20231271	11003505	6751042
F20231359	10912824	158401
F20231388	10694563	452094
F20231389	11379323	1919620
2023-2311	10850711	7152954
1002234923	11449540	202339
1002234924	10487109	7779544
1002234934	10446232	4847200
1002235218	10712880	6528535
1002235229	11968340	173013
1002235234	10490225	2057546
0902234611	11031140	517118

0902234829	10440885	2921914
1082234701	11087527	2137520
1082234830	11957943	458067
0102234707	10119101	3963132
0108234905	11647757	871547
0802234713	10145206	296437
0802235005	11540209	3743988
0782234904	11894167	70362
0602234611	11499825	5774919
0602234602	10492431	4530180
203234549	11670274	133491
203234558	11846281	6108641
0784234603	10882771	113472
0784234811	10136278	800285
1102234710	10732619	3740706
1102234911	11376194	6891307
0303234608	10460449	5284494
0303234802	11232206	3687856
0503234704	10748856	1301908
0502234811	11684526	5997662
Flu20231064	10737246	7895771
JH20231093	10168129	3726927
F20231061	10986557	5241390
F20231147	10745128	2115567
231148	11290526	141629
15342	10299274	4524969
23-2-1176	11148209	274209
23-2-1181	10645193	239096
20240106	10405477	100661
20240107	11235008	169941
20240108	11785249	277551
20240109	10092146	108881
20240113	11088184	153143

20240116	10983410	156585
20240120	11119238	231300
20240121	10172772	222898
20240122	10665659	176864
20240125	10269603	201974
20240129	11427844	134585
20240130	10816946	248407
20240131	11443474	266324
20240132	11199202	74668
20240133	11345167	163582
20240134	11924283	235036
20240135	11234731	160600
20240136	11789281	87043

References

1. L. Huang, T. Liao, J. Wang, L. Ao, W. Su and J. Hu, *Adv. Funct. Mater.*, 2018, **28**, 1705380.
2. T.-H. Le, S. Kim, S. Chae, Y. Choi, C. S. Park, E. Heo, U. Lee, H. Kim, O. S. Kwon and W. B. Im, *J. Colloid Interface Sci.*, 2020, **564**, 88-98.
3. A. d. S. da Silva and J. H. Z. Dos Santos, *Adv. Colloid Interface Sci.*, 2023, **314**, 102888.