

1 **Development of a quantum-dot lateral flow immunoassay strip based portable**
2 **fluorescence smart-phone system for ultrasensitive detection of IgM/IgG to**
3 **SARS-CoV-2**

4 Bochao Liu^{1†}, Jinfeng Li^{1,2†}, Xi Tang^{3,1†}, Ze Wu¹, Jinhui Lu¹, Chaolan Liang¹,
5 Shuiping Hou¹, Ling Zhang¹, Tingting Li¹, Wei Zhao⁴, Yongshui Fu⁵, Yuebin Ke^{2*},
6 Chengyao Li^{1*}

7 ¹ Department of Transfusion Medicine, School of Laboratory Medicine and
8 Biotechnology, Southern Medical University, Guangzhou, China;

9 ² Shenzhen Key Laboratory of Molecular Epidemiology, Shenzhen Center for Disease
10 Control and Prevention, Shenzhen, China

11 ³ Infection Department, The First People's Hospital of Foshan, Foshan, China

12 ⁴Laboratory of Biosafety, School of Public Health, Southern Medical
13 University, Guangzhou, China

14 ⁵Guangzhou Blood Center, Guangzhou, China

15 [†]These authors contributed equally to this work.

16 ***Corresponding author:**

17 Chengyao Li or Yuebin Ke

18 School of Laboratory Medicine and Biotechnology, Southern Medical University.

19 No. 1838 North Guangzhou Avenue, Guangzhou 510515, China.

20 Tel: 0086-20-61649360, Email: chengyaoli@hotmail.com (Li C) or

21 keyke@szu.edu.cn (Ke Y)

22 ABSTRACT

23 **Background:** Since December 2019, the outbreak of coronavirus disease (COVID-
24 19) has been occurred by novel coronavirus (SARS-CoV-2). The rapid and sensitive
25 immunoassays are urgently demanded for detecting specific antibodies as assistant
26 diagnosis for primary screening of asymptomatic individuals, close contacts,
27 suspected or recovered patients of COVID-19 during the pandemic period.

28 **Methods:** The recombinant receptor binding domain of SARS-CoV-2 spike protein
29 (S-RBD) was used as the antigen to detect specific IgM and the mixture of
30 recombinant nucleocapsid phosphoprotein (NP) and S-RBD were used to detect
31 specific IgG by the newly designed quantum-dot lateral flow immunoassay strip (QD-
32 LFIA), respectively.

33 **Results:** A rapid and sensitive QD-LFIA based portable fluorescence smart-phone
34 system was developed for detecting specific IgM/IgG to SARS-CoV-2 from 100
35 serum samples of COVID-19 patients and 450 plasma samples from healthy blood
36 donors. Among 100 COVID-19 patients diagnosed with NAT previously, 3 were
37 severe, 35 mild and 62 recovered cases. By using QD-LFIA, 78 (78%) and 99 (99%)
38 samples from 100 COVID-19 patients' serum were detected positive for anti-SARS-
39 CoV-2 IgM or IgG, respectively, but only one sample (0.22%) was cross-reactive
40 with S-RBD from 450 healthy blood donor plasmas that were collected from different
41 areas of China.

42 **Conclusion:** An ultrasensitive and specific QD-LFIA based portable fluorescence
43 smart-phone system was developed for detection of specific IgM and IgG to SARS-
44 CoV-2 infection, which could be used for investigating the prevalence or assistant
45 diagnosis of COVID-19 in humans.

46

47 **Running Title:** A quantum-dot smart-phone system for SARS-CoV-2 antibodies

48

49 **Key words:** SARS-CoV-2, IgM/IgG detection, Quantum dot, 3D printing, Smart-
50 phone system

51

52 INTRODUCTION

53 In December 2019, a coronavirus pneumonia (COVID-19) was firstly discovered
54 in Wuhan, China and then largely reported within a few months in the rest of world,
55 which was caused by the novel coronavirus designated as SARS-CoV-2 [1,2]. The
56 small part of patients rapidly developed acute complications such as acute respiratory
57 distress syndrome (ARDS) and acute respiratory failure [3]. The main routes of
58 SARS-CoV-2 transmission in humans were respiratory droplets and virus exposure
59 between individuals. By the middle of July 2020, more than 13 million people were
60 diagnosed as COVID-19 by viral nucleic acid test (NAT) and over 500 thousand
61 deaths were announced globally, among them more than 80 thousands cases and 4000
62 deaths were reported in China. SARS-CoV-2 has been found more infectious than
63 SARS coronavirus, especially for the middle-aged and elderly people [4]. Due to the
64 existence of close contacts, suspected cases and asymptomatic patients or resolvers of
65 COVID-19 can be discovered by detecting the antibodies specific to SARS-CoV-2.

66 The SARS-CoV-2 is a single strand sense RNA virus and belongs to β genus of
67 coronavirus family [5-7], and has nearly 30,000 bases of nucleotides in length [8].
68 Based on the known genome sequence (GenBank MN908947), testing of SARS-CoV-
69 2 RNA was commonly used for detection of virus. Due to the stability of reagents and
70 primers, some nucleic acid tests were not sensitive enough to detect SARS-CoV-2
71 from nasal or pharynx swabs [9-12]. Many patients had to be tested repeatedly before
72 they were finally diagnosed [13,14]. However, testing of specific antibodies to SARS-
73 CoV-2 can be a helpfully assistant method.

74 Serum IgM or IgG specific to SARS-CoV-2 might co-exist with viral RNA,
75 which could be used for preliminary screening and auxiliary diagnosis of COVID-19
76 [15]. A previous study reported that less than 40% of antibodies were presence within
77 1-week among patients since onset, and rapidly increased to 100% (either IgM or
78 IgG), 94.3% (IgM) and 79.8% (IgG) since day-15 after onset, while IgM declined to
79 the cutoff level in 39 days but IgG persisted in the high level [16,17]. Several
80 antibody assays have been developed for detection of SARS-CoV-2 infection [18-21].
81 However, the more specific, sensitive, rapid and simple tests are still required strongly.
82 In this study, we designed a quantum-dot lateral flow immunoassay strip (QD-LFIA)
83 based portable fluorescence smart-phone system for detection of specific IgM/IgG to
84 SARS-CoV-2 in human serum samples. This assay has two innovative techniques: (1)

85 a new format assay includes a portable fluorescence detection platform which can be
86 operated in biological safety hoods or cabinets for avoiding possible aerosol
87 contamination, and in wireless connecting smart-phone for processing data efficiently;
88 (2) the testing can be carried out within 15 min. This novel assay is ultrasensitive,
89 cost-effective, rapid and simple, which can be used for on-site detection of serum
90 IgM/IgG specific to SARS-CoV-2 infection in humans.

91

92 **MATERIALS AND METHODS**

93 *Blood specimen*

94 The numbers of 100 serum samples of COVID-19 patients were provided by the
95 Shenzhen Center for Disease Control and Prevention (CDC), which were confirmed
96 positive for SARS-CoV-2 RNA by real-time RT-PCR (RT-qPCR). A total of 450
97 plasma samples of healthy blood donors were obtained from blood centers of
98 Shenzhen and Guangzhou (south), Harbin (northeast), Chengdu (southwest) and Xi'an
99 (northwest), which were collected during the period of August to October of 2019
100 before the COVID-19 outbreak in Wuhan (middle), China. These blood donor
101 samples were used as negative control. This study was approved by the Medical
102 Ethics Committees of Shenzhen CDC and Southern Medical University, and followed
103 the ethical guidelines of the 1975 Declaration of Helsinki.

104 *Reagents and equipment*

105 Bovine serum albumin (BSA) and goat anti-mouse polyclonal immunoglobulin
106 G (G-mIgG) were purchased commercially (Sigma-Aldrich, St. Louis, USA). Mouse
107 anti-human IgG (M-hIgG) and IgM (M-hIgM) were purchased from Beijing Bersee
108 Science and Technology Co. Ltd (Beijing, China). Recombinant spike protein
109 receptor binding domain fused with mouse IgG-Fc fragment (S-RBD) and
110 nucleocapsid phosphoprotein (NP) of SARS-CoV-2 were purchased from Bioeast
111 Biotech Co. Ltd (Hangzhou, China) and S-RBD was produced from HEK293 cells
112 and NP was produced in *E. coli*, respectively. Polystyrene-coated quantum dot
113 nanoparticles (QDs) with 160 nm diameter were purchased from Huge Biotech Ltd
114 (Shanghai, China). Nitrocellulose (NC) membranes, glass fibers and absorbent pads
115 were purchased from Millipore Corporation (Bedford, MA, USA). A 3D-printed
116 portable fluorescence strip reader was designed by this study. The colloid gold lateral

117 flow immunoassay strip (CG-LFIA; Lvshiyuan Biotechnology Co. Ltd., Shenzhen,
118 China) was used as comparative assay for detection of antibodies to SARS-CoV-2.

119 *Preparation of QD-LFIA strips*

120 QD-LFIA strip was composed of a 25 mm sample pad, 20 mm NC membrane and
121 15 mm absorbent pad, and all of those were pasted on an adhesive backing card. The
122 conjugate pad was saturated with treatment buffer (10mM PBS, pH 7.4 containing 4%
123 sucrose, 0.5% Tween-20, 0.5% PVP and 1% BSA) for 1 h and dried at 37 °C for 24h.

124 An aliquot of QDs (25μL) were conjugated with 10 μg M-hIgG or S-RBD by
125 covalent attachment, respectively. The M-hIgG@QD and S-RBD@QD conjugates
126 were mixed, and then dispensed onto the conjugate pad at 2μL/cm by a Biolet XYZ-
127 3060 Quantidispenser, followed by drying at 37°C before storing in a dry cabinet at
128 room temperature for ready use. The G-mIgG (0.5μg/cm), mixture of M-hIgG and NP
129 (0.5μg each protein/cm) or M-hIgM (1μg/cm) were spotted on the NC membrane
130 (from absorbent pad to sample pad direction) to generate the C, T2 and T1 lines,
131 respectively (Fig. 1A). The coated NC was dried at 65 °C for 30min. The
132 hemofiltration sample pad was pre-treated with the blocking buffer (10mM PBS, pH
133 7.4 containing 2% sucrose, 0.4% Tween-20, 0.5% S9 and 10% casein) and dried at
134 37°C for overnight.

135 QD-LFIA testing was performed by mixing 1μL of serum sample with 120μL
136 sample buffer (10mM PBS, pH 7.4 containing 0.05% Tween-20 and 1% BSA). Then
137 the reactive sample solution was taken onto sample pad of the testing strip for
138 immunochromatography. Fluorescence signals on T1/T2 and C lines of strip were
139 measured in 10 min by a portable fluorescence strip reader and were uploaded to the
140 smart-phone via wifi for processing the data.

141 *Statistical analysis*

142 All experiments were performed at least three times independently. The data
143 were analyzed using the statistical package SPSS v. 16.0. The results were presented
144 as the mean±SD. Difference between groups was analyzed by using Student's t-test,
145 and *P*-value < 0.05 was considered statistically significant.

146

147

148 **RESULTS**

149 *Invention of QD-LFIA based portable fluorescence smart-phone system*

150 In order to report the testing results effectively, a portable fluorescence smart-
151 phone system was invented for QD-LFIA (Fig.1). This system included a QD-LFIA
152 strip with C and T1/T2 lines, a portable fluorescence strip reader with laser, real-time
153 camera and wifi, and a mobile phone with signal receiver and data processor. When a
154 reactive strip was inserted into the fluorescence reader, the fluorescent images on
155 T1/T2/C lines were taken and transited to a mobile smart-phone via wifi. The
156 fluorescence lines were selected and the fluorescent intensity of T1, T2 or C lines was
157 converted to the peak area. The value of IgG/control (T2/C) or IgM/control (T1/C)
158 was calculated, and then the reaction of sample was reported for negativity or
159 positivity after comparing with the cut-off value by artificial intelligence (AI)
160 processor.

161 *Detection of COVID-19 IgM/IgG by QD-LFIA*

162 Fifty healthy blood donor plasma samples (provided by Guangzhou blood center)
163 were tested negative for IgM/IgG to SARS-CoV-2 by ELISA. By using these negative
164 control samples, the appropriate cut-off values of IgM/IgG in QD-LFIA system were
165 defined as 0.0964 or 0.0952 (mean plus 3 SD), respectively. In comparison with CG-
166 LFIA, 100 serum samples of COVID-19 patients and 450 plasma samples of healthy
167 blood donors were detected by QD-LFIA for specific IgM/IgG to S-RBD or NP
168 mixture, respectively. Among 100 COVID-19 cases diagnosed previously, 3 were
169 severe, 35 mild and 62 recovered patients. Each of three severe patients had 3 follow-
170 up samples (S1-S3), containing the high level of both IgM and IgG responses to either
171 S-RBD or NP in QD-LFIA (Fig.2). Among 35 mild patients, 32 positive IgM to S-
172 RBD and 22 positive IgM to NP were detected by QD-LFIA, in which S-RBD
173 presented higher IgM response than NP in QD-LFIA excepting for three cases (Fig.
174 3A and 3B, $P < 0.05$). For detection of specific IgG among 35 mild patients, 30 cases
175 were tested positive by S-RBD or NP based QD-LFIA, respectively (Fig. 3C and 3D),
176 while each antigen based QD-LFIA had 5 positive samples uniquely, but the
177 combined S-RBD and NP antigens were all positive for 35 samples. Among 62
178 recovered patients, specific IgM was detected in 43 cases by S-RBD-based QD-LFIA
179 and 9 cases by NP-based QD-LFIA, overall were 43 cases by two antigens but no

complementary (Fig. 4A and 4B). For IgG detection, 61/62 recovered patients were detected positive by the combined S-RBD and NP antigens in QD-LFIA, while a sample was negative by QD-LFIA (Fig.4C and 4D). The reactivity of IgG to S-RBD or NP was similar among recovered patients, in which 10 samples were higher reactive to S-RBD but 8 to NP, respectively.

In addition to 50 Guangzhou blood donors, 400 plasma samples of healthy blood donors from South, Southwest, Northeast and Northwest of China were tested for reactive IgM/IgG to S-RBD and NP antigens by QD-LFIA, respectively. All samples were negative to S-RBD and NP based QD-LFIA excepting for that a sample from Chengdu (Southwest of China) had IgG reactive to S-RBD in QD-LFIA.

Sensitivity and specificity of QD-LFIA

According to above results obtained by two kinds of S-RBD and NP antigens, S-RBD presented better sensitivity than NP to IgM in QD-LFIA, while S-RBD and NP had mutual complementary reactivity for detection of specific IgG in QD-LFIA. Compared with conventional CG-LFIA, one serum sample with serial tenfold dilutions were measured for reacting with IgM or IgG by QD-LFIA, which showed that QD-LFIA detected IgM/IgG in 1:1000 diluted sample (Fig.5), which was 10-100 times sensitive than CG-LFIA. Among 100 COVID-19 cases diagnosed previously by NAT at hospitals, the detection rate for reactive IgM was 78% (78/100) by S-RBD based QD-LFIA, and for reactive IgG was 99% (99/100) by the combined S-RBD and NP based QD-LFIA (Table 1). From comparison with control assay, CG-LFIA only had 32% of IgM and 71% of IgG positivity (Table 1).

For identifying the specificity of QD-LFIA, none (0/450) of cross-reactivity was found IgM positive and only one sample (1/450) was detected IgG positive from 450 healthy blood donors, which suggested that the specificity of QD-LFIA was 100% for IgM and 99.8% for IgG detection, which were identical with conventional CG-LFIA (Table 1).

DISCUSSION

A brand new coronavirus from a patient's pharyngeal swab sample was discovered by the Chinese Center for Disease Control and Prevention (CDC) on January 7, 2020, which was temporarily named 2019-nCoV, and subsequently designated as SARS-CoV-2 by the World Health Organization (WHO) [22]. SARS-

213 CoV-2 has caused a global pandemic with novel coronavirus pneumonia disease
214 (COVID-19). More than 5 millions of people are diagnosed with COVID-19 and over
215 300 thousands of deaths are reported so far. This pandemic has made many
216 communities or cities closed, which caused huge economic losses
217 worldwide. Currently, the re-opening or re-starting of social activities are initiating in
218 Asian and European countries where the SARS-CoV-2 infection has been contained.
219 Along with nucleic acid testing of SARS-CoV-2, the antibody tests are largely needed
220 as well. The development of rapid and accurate antibody detection methods is highly
221 welcomed. Detection of specific IgM and IgG to SARS-CoV-2 can reflect the status
222 of virus infection among different populations, and also can provide serological
223 evidence for clinical diagnosis of COVID-19 patients.

224 The colloid gold lateral flow immunoassay strip (CG-LFIA) is a commonly used
225 rapid immunochromatography assay for antibody detection. It normally takes 10-15
226 minutes to report result without requiring of special equipment. However, CG-LFIA
227 has the lower sensitivity and the testing is judged by eyes, which may give false result
228 by human errors. In this study, we developed a quantum-dot based LFIA (QD-LFIA)
229 and combined with a portable fluorescence real-time camera reader, which increased
230 its sensitivity in 10-100 times than conventional CG-LFIA. For detection rates of
231 specific IgM/IgG from 100 COVID-19 patients diagnosed by NAT previously, QD-
232 LFIA identified 78% of IgM and 99% of IgG reactive samples, while CG-LFIA had
233 only 32% of IgM and 71% of IgG reactive to SARS-CoV-2 antigens in patients' sera,
234 respectively ($P < 0.05$). In view of biosafety, the testing and reading of QD-LFIA strip
235 could be performed in the biosafety hood, while the data could be transmitted via wifi
236 and received outside the closed experiment cabinet by a mobile smart-phone within
237 the laboratory. It's only takes 15min to obtain the results safely.

238 The S-RBD or NP of SAR-CoV-2 in single and combined forms were examined
239 as antigens to detect specific IgM or IgG of blood samples from COVID-19 patients
240 in this study (Fig. 1-4). According to the reactive rate, S-RBD was finally determined
241 to detect IgM and S-RBD and NP mixture was used to detect IgG in QD-LFIA. No
242 cross-reaction of these S-RBD and NP antigens was found with IgM but 1 (0.22%)
243 case with IgG from 450 healthy blood donors, which suggesting that the specificity of
244 QD-LFIA was 100% for IgM and 99.78 % for IgG for detection of antibodies from
245 COVID-19 patients. Also, this cross-reactive IgG sample from a 45 years old woman

(Chengdu, Southwest) was tested positive by the control assay CG-LFIA, which was considered as non-specific to S-RBD of SARS-CoV-2, indicating the donor might be infected previously by a general flu coronavirus such as human coronavirus 229E (HuCoV-229E), NL63 (*Alphacoronavirus*), HKU1 or OC43 (*Betacoronavirus*) (23), but not identified yet.

In conclusion, a QD-LFIA based portable fluorescence smart-phone system was invented for detecting specific IgM and IgG to SARS-CoV-2 infection, which could be applied for investigating the epidemiology or assistant diagnosis of COVID-19 in epidemic areas of world.

Disclosure statement

The authors declare no conflicts of interest.

Acknowledgments

The author thanks for Guangzhou, Shenzhen, Chengdu, Haerbing and Xian blood centers for their providing the healthy blood donor samples.

This study has partly received the financial and technical supports from Guangzhou Bai Rui Kang or Breakthrough (BRK) Bioscience and Technology Limited Company, Guangzhou, China.

Author contribution

C.L., Y.K. and B.L. designed study; B.L., J.L., X.T., Z.W. and S.H. performed experiments; J.L., B.L., C.L. and T.L. analyzed data; Y. F. provided materials and B.L., L.Z., C.L. and Y.K. wrote the paper.

271

272 **References**

- 273 [1] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia
274 outbreak associated with a new coronavirus of probable bat origin. NATURE
275 2020;579:270-3.
- 276 [2] Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of
277 global health concern. LANCET 2020;395:470-3.
- 278 [3] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and
279 clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan,
280 China: a descriptive study. LANCET 2020;395:507-13.
- 281 [4] Phan T. Novel coronavirus: From discovery to clinical diagnostics. INFECT
282 GENET EVOL 2020;79:104211.
- 283 [5] Phelan AL, Katz R, Gostin LO. The Novel Coronavirus Originating in Wuhan,
284 China: Challenges for Global Health Governance. JAMA 2020.
- 285 [6] Chu D, Pan Y, Cheng S, Hui K, Krishnan P, Liu Y, et al. Molecular Diagnosis
286 of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. CLIN
287 CHEM 2020;66:549-55.
- 288 [7] Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, Yuen KY. Genomic
289 characterization of the 2019 novel human-pathogenic coronavirus isolated from a
290 patient with atypical pneumonia after visiting Wuhan. EMERG MICROBES INFECT
291 2020;9:221-36.
- 292 [8] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus
293 associated with human respiratory disease in China. NATURE 2020;579:265-9.
- 294 [9] Patra S, Tellapragada C, Vandana KE, Mukhopadhyay C. Diagnostic utility of
295 in-house loop-mediated isothermal amplification and real-time PCR targeting virB
296 gene for direct detection of Brucella melitensis from clinical specimens. J APPL
297 MICROBIOL 2019;127:230-6.
- 298 [10] Kim JH, Kang M, Park E, Chung DR, Kim J, Hwang ES. A Simple and
299 Multiplex Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid
300 Detection of SARS-CoV. BIOCHIP J 2019;13:341-51.
- 301 [11] Tanner NA, Zhang Y, Evans TJ. Visual detection of isothermal nucleic acid
302 amplification using pH-sensitive dyes. BIOTECHNIQUES 2015;58:59-68.

303 [12] Lim DR, Kim HR, Park MJ, Chae HG, Ku BK, Nah JJ, et al. An improved
304 reverse transcription loop-mediated isothermal amplification assay for sensitive and
305 specific detection of serotype O foot-and-mouth disease virus. J VIROL METHODS
306 2018;260:6-13.

307 [13] Zhang N, Wang L, Deng X, Liang R, Su M, He C, et al. Recent advances in
308 the detection of respiratory virus infection in humans. J MED VIROL 2020;92:408-17.

309 [14] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al.
310 Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. EURO
311 SURVEILL 2020;25.

312 [15] Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical
313 application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection
314 diagnosis. J MED VIROL 2020.

315 [16] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to
316 SARS-CoV-2 in patients of novel coronavirus disease 2019. CLIN INFECT DIS
317 2020.

318 [17] Xiao D, Gao DC, Zhang DS. Profile of Specific Antibodies to SARS-CoV-2:
319 The First Report. J Infect 2020.

320 [18] Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical
321 performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and
322 antibody kinetics. CLIN CHEM LAB MED 2020.

323 [19] Pan Y, Li X, Yang G, Fan J, Tang Y, Zhao J, et al. Serological
324 immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-
325 19 patients. J INFECT 2020.

326 [20] Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, et al. Diagnostic value and
327 dynamic variance of serum antibody in coronavirus disease 2019. INT J INFECT DIS
328 2020.

329 [21] Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, et al. Evaluation of
330 Nucleocapsid and Spike Protein-based ELISAs for detecting antibodies against
331 SARS-CoV-2. J CLIN MICROBIOL 2020.

332 [22] Cheng ZJ, Shan J. 2019 Novel coronavirus: where we are and what we know.
333 INFECTION 2020;48:155-63.

334 [23] Warnes SL, Little ZR, Keevil CW. Human Coronavirus 229E Remains
335 Infectious on Common Touch Surface Materials. mBio. 2015 Nov 10;6(6):e01697-15.

336

337

338 **Table 1.** Sensitivity and specificity of QD-LFIA for testing of anti-SARS-CoV-2 IgM
339 and IgG

Population	Sample (Nb*)	QD-LFIA		CG-LFIA	
		(Nb/%)		(Nb/%)	
COVID-19		IgM	IgG	IgM	IgG
Severe	3	3 (100%)	3 (100%)	3 (100%)	3 (100%)
Mild	35	32 (94.4)	35 (100%)	23(65.7%)	26(74.3%)
Recovered	62	43(69.4%)	61(98.4%)	6(9.7%)	42(67.7%)
Overall	100	78 (78%)	99 (99%)	32 (32%)	71(71%)
Blood donor	450	0 (0)	1 (0.22%)	0 (0)	1 (0.22%)

340 * Number.

341

342

343

344

345

346

347

348

349

350

351

352

353

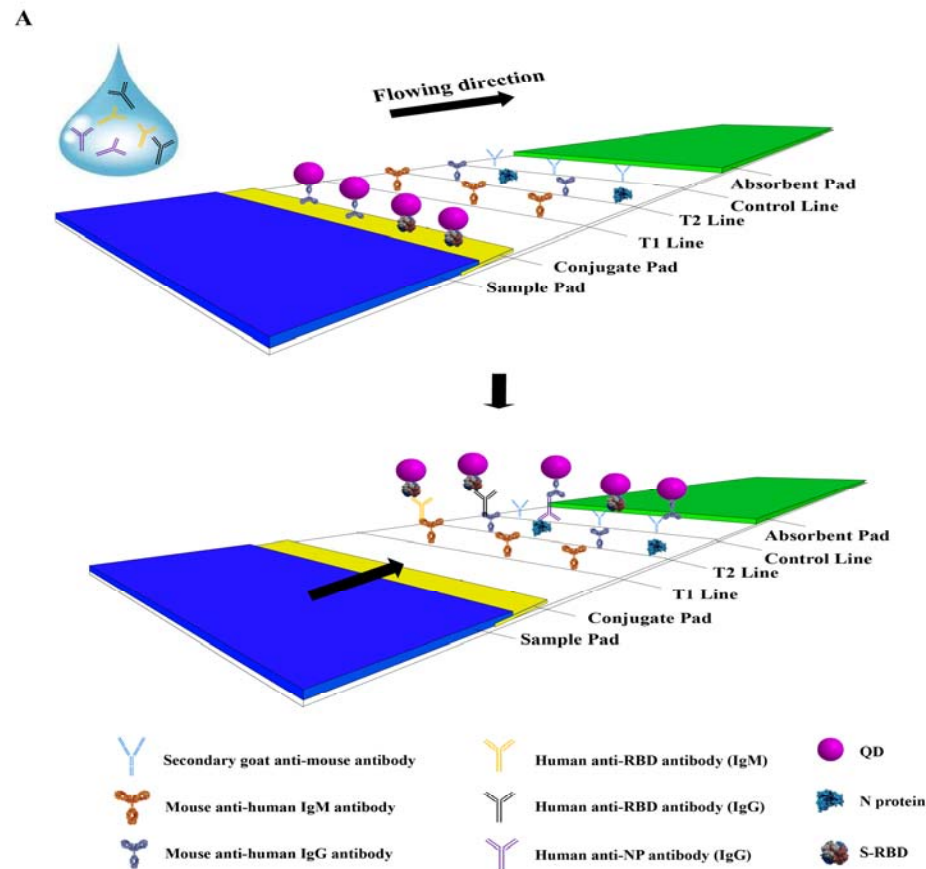
354

355

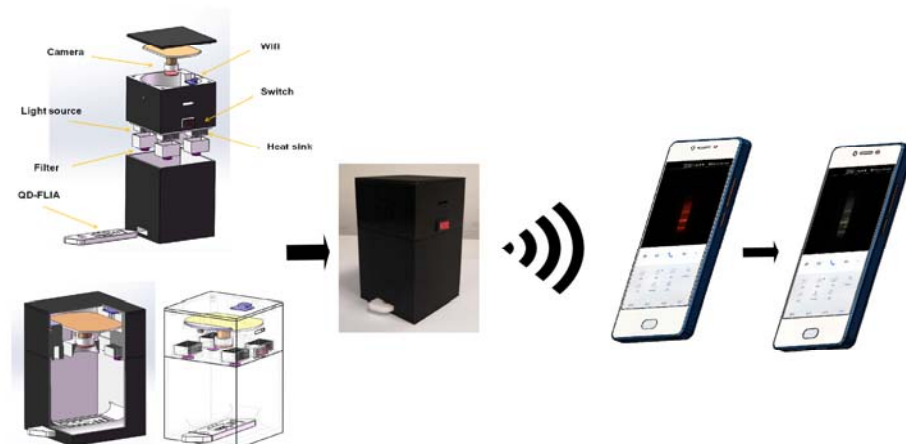
356

357

358 **Figure and figure legends**

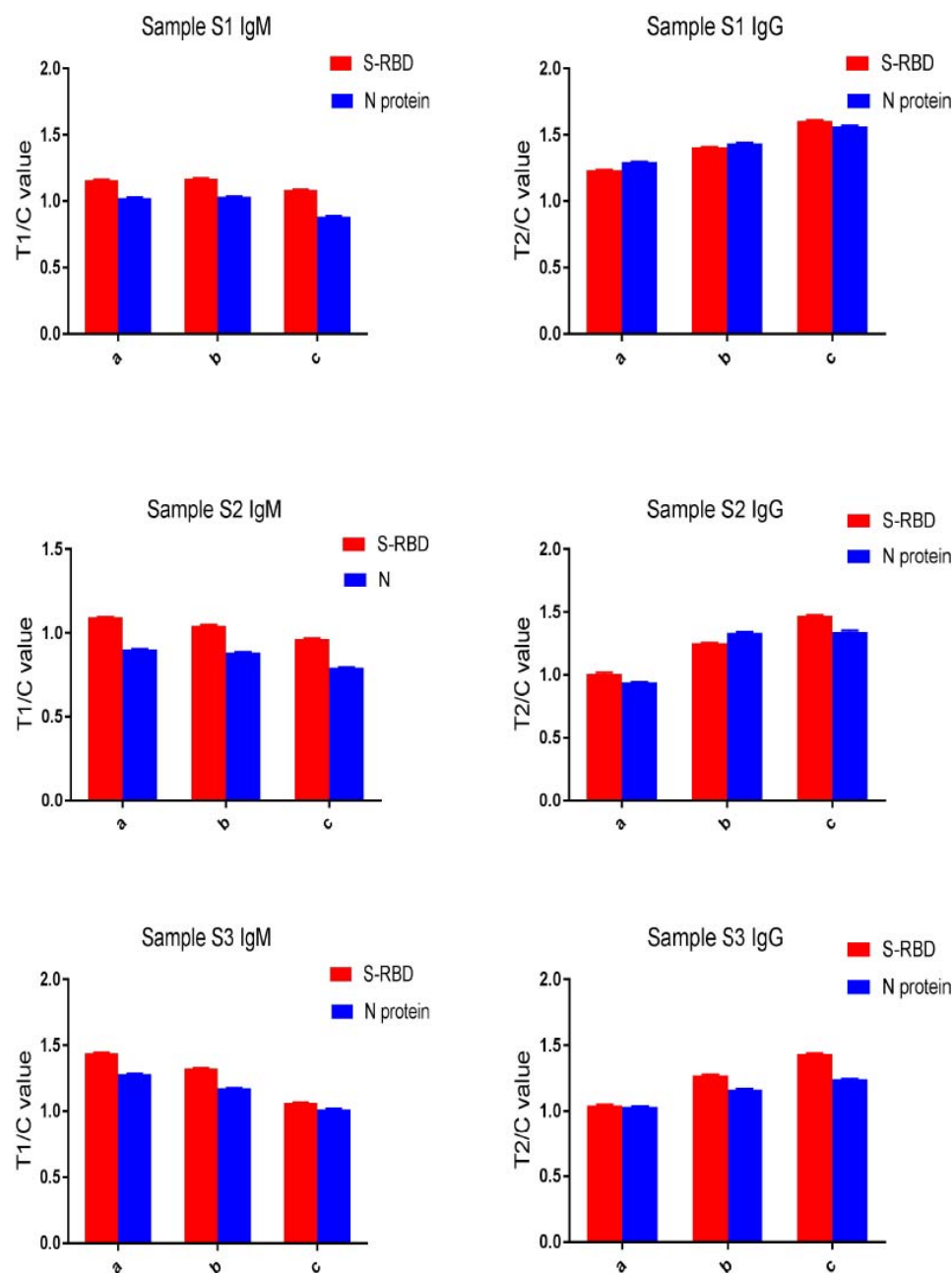


B



359

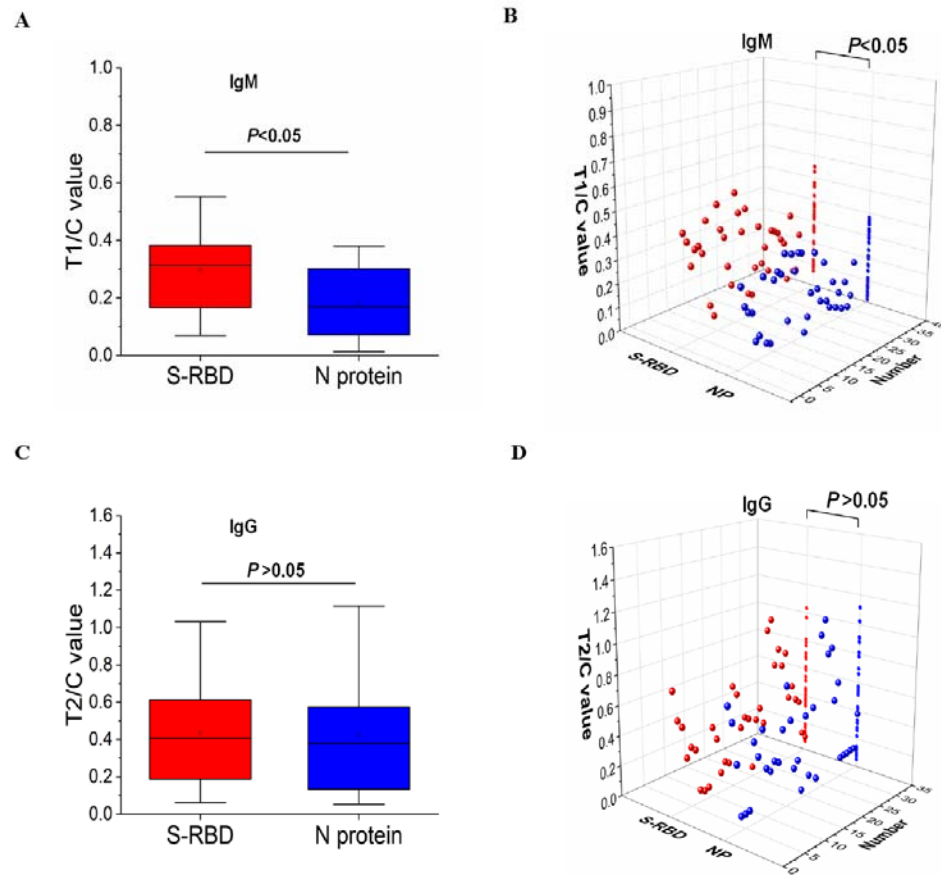
360 **Fig. 1.** The schematic diagram of QD-LFIA (A) based portable fluorescence smart-
361 phone system (B) for detection of specific IgM/IgG to SARS-CoV-2 infection in
362 humans.



363
364 **Fig. 2.** T/C values of QD-LFIA for detection of serum IgM or IgG reactive to S-RBD
365 and NP of SARS-CoV-2 from three severe COVID-19 patients (samples S1-S3). S-
366 RBD and NP were used as detection antigens in the assay.

367

368



369

370 **Fig. 3.** T/C values of QD-LFIA for detection of serum IgM (A, B) or IgG (C, D)
 371 specific to SARS-CoV-2 from 35 mild COVID-19 patients, respectively. S-RBD and
 372 NP were used as detection antigens in QD-LFIA. * $P < 0.05$.

373

374

375

376

377

378

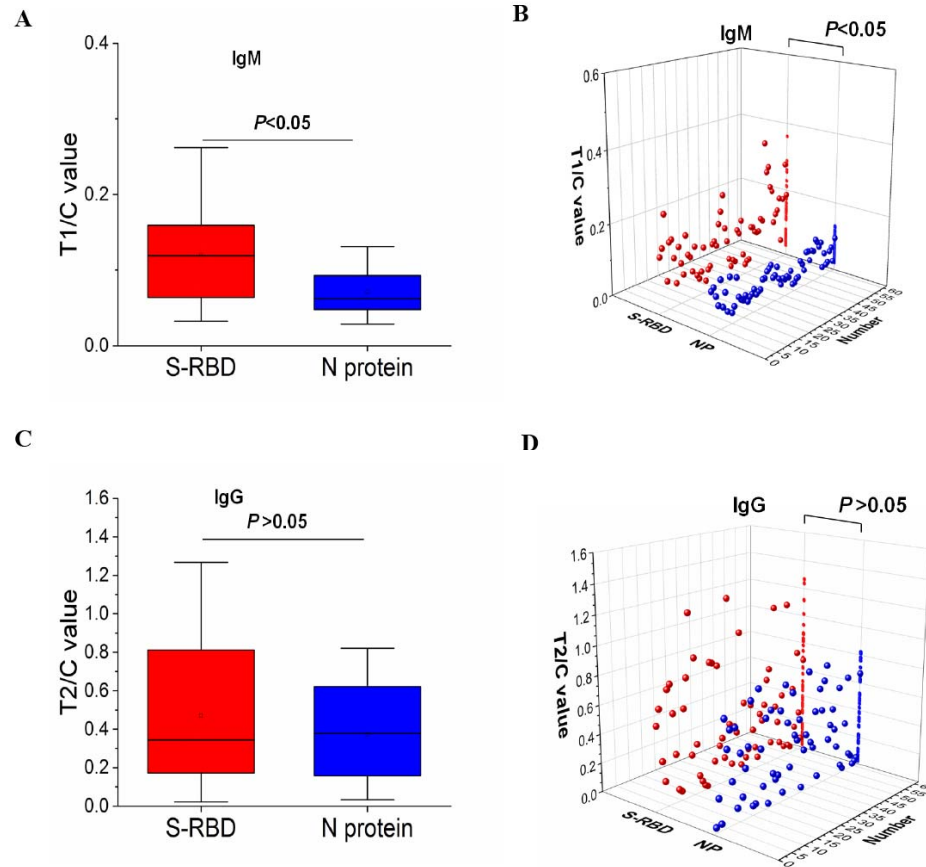
379

380

381

382

383



384

385 **Fig. 4.** T/C values of QD-LFIA for detection of serum IgM (A, B) or IgG (C, D)

386 reactive to SARS-CoV-2 from 62 recovered COVID-19 patients using S-RBD or NP

387 as detection antigens, respectively. * $P < 0.05$.

388

389

390

391

392

393

394

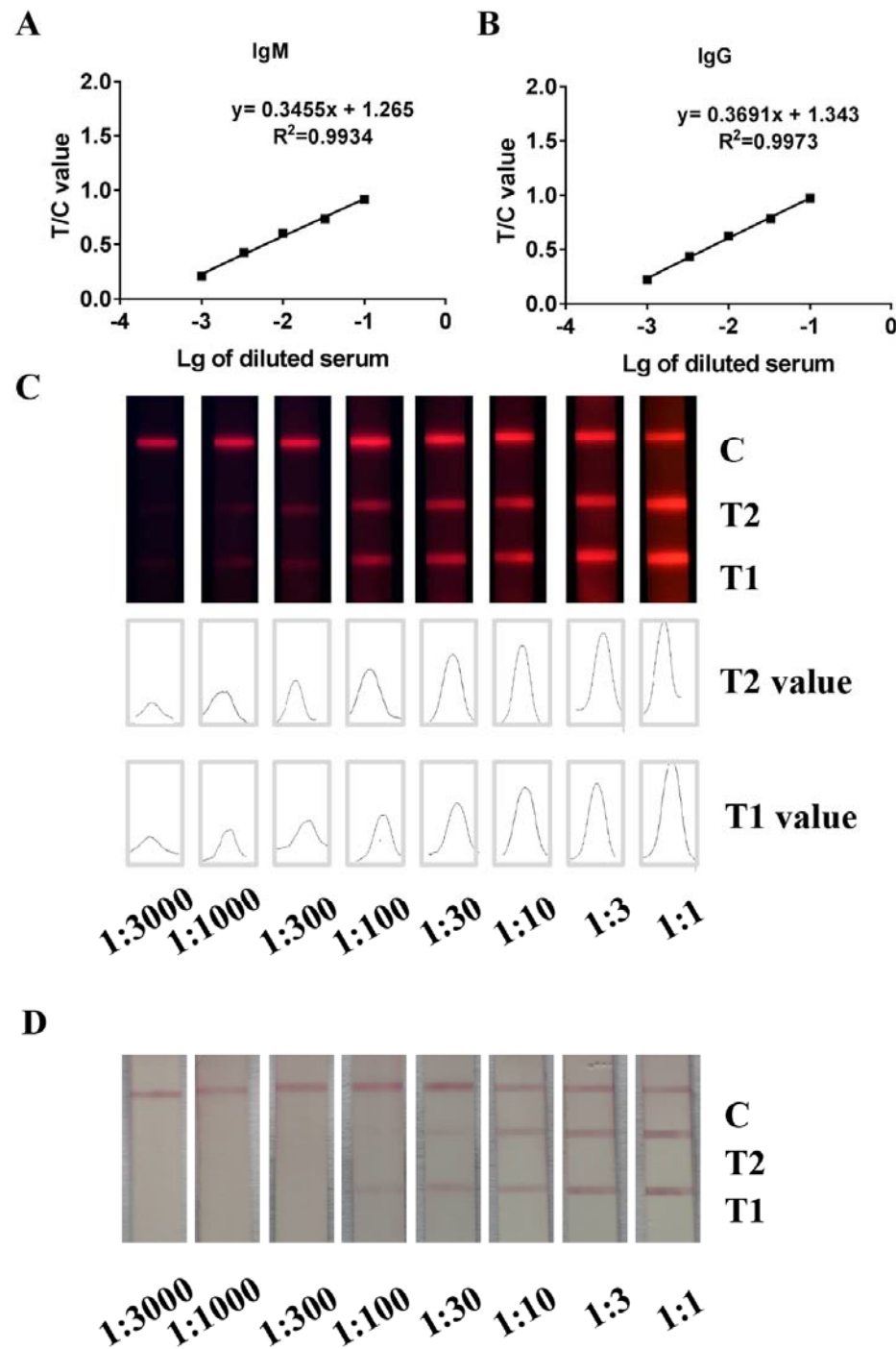
395

396

397

398

399



400

401 **Fig. 5.** QD-LFIA linear regression of quantitative testing of IgM/IgG to SARS-CoV-2

402 in blood samples from COVID-19 patients. (A, B) The linear regression curves for

403 IgM and IgG in serum sample. (C) The fluorescent images of reactive C, T2 and T1

404 lines and the converted areas of T1/T2 fluorescence density in QD-LFIA. (D) The
405 images of reactive C, T2 and T1 lines in CG-LFIA.