

Evaluations of serological test in the diagnosis of 2019 novel coronavirus (SARS-CoV-2) infections during the COVID-19 outbreak

Dachuan Lin^{1†}, Lei Liu^{2‡}, Mingxia Zhang², Yunlong Hu¹, Qianting Yang², Jiubiao Guo¹, Youchao Dai¹, Yuzhong Xu³, Yi Cai¹, Xinchun Chen¹, Kaisong Huang^{1*}, Zheng Zhang^{2*}

¹Guangdong Provincial Key Laboratory of Regional Immunity and Disease, Department of Pathology Biology, School of Medicine, Shenzhen University, Shenzhen, China

²National clinical research center for infectious diseases, Guangdong Key Lab for Diagnosis & Treatment of Emerging Infectious Diseases, Shenzhen Third People's Hospital, Southern University of Science and Technology, Shenzhen, China

³Department of clinical laboratory, Shenzhen Baoan people's hospital, The Second Affiliated Hospital of Shenzhen University, Shenzhen, China

* Correspondence to kaisong@szu.edu.cn and zhangzheng1975@aliyun.com

† These authors contributed equally to this work

Abstract

The ongoing SARS-CoV-2 outbreak has killed over twenty-one thousand and sickened over four hundred thousand people worldwide, posing a great challenge to global public health. A sensitive and accurate diagnosis method will substantially help to control disease expansion. Here, we developed a chemiluminescence-immunoassay method based on the recombinant nucleocapsid antigen and the magnetic beads for diagnosis of SARS-CoV-2 infections and surveillance of antibody changing pattern.

25 Serums from 29 healthy individuals, 51 tuberculosis patients, and 79 SARS-CoV-2 confirmed patients
26 were employed to evaluate the performance of this approach. Compared to the IgM testing, the IgG
27 testing was more reliable in which it identified 65 SARS-CoV-2 infections from the 79 confirmed
28 patients and only two false-positive cases from the 80 control group with a sensitivity and specificity
29 reaching 82.28% and 97.5%, respectively. However, only a slight difference (not statistically
30 significant) in the detected cases of SARS-CoV-2 infections was observed between the IgM and IgG
31 testing manner in patients at a different time of onset of disease. A performance comparison
32 between an ELISA kit using the same nucleocapsid antigen and our chemiluminescence method was
33 undertaken. The same false-positive cases were seen in both methods from the paired control group,
34 while ELISA kit can only detect half of the SARS-CoV-2 infections from paired SARS-CoV-2 confirmed
35 patients group than that of the chemiluminescence method, indicating a higher performance for the
36 chemiluminescence-immunoassay approach. Together, our studies provide a useful and valuable
37 serological testing tool for the diagnosis of SARS-CoV-2 infections in the community.

38 **Keywords:** SARS-CoV-2, serological testing, chemiluminescence immunoassay, IgM and IgG

39 **Introduction**

40 *Coronavirus*, belonging to the family of *Coronaviridae* and order of *Nidovirales*, is a group of
41 enveloped, non-segmented positive-sense RNA virus that has been reported to be able to infect
42 humans and a wide range of animals including cattle, swine, chicken, cat, horse, camels, rodent, bats
43 and snakes and so forth (1-3). Based on the genetic properties, coronavirus was further divided into

44 four genera, namely *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*
45 (4). Prior to December 2019, a total of six coronaviruses have been documented to be capable of
46 causing disease in humans. These include two strains from *Alphacoronavirus* (HCoV-229E and HKU-
47 NL63) and four from *Betacoronavirus* subfamily (HCoV-OC43, HCoV-HKU1, SARS-CoV and MERS-CoV)
48 (5-10). Among them, the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East
49 Respiratory Syndrome coronavirus (MERS-CoV) are the most well-described as they directly led to two
50 deadly large-scale outbreaks globally, with 8,096 cases infections and roughly 10 percent mortality
51 and 2,494 cases and 34.4 percent mortality, respectively(9, 10).

52 Recently, the outbreak of a severe pneumonia COVID-19 was confirmed to be caused by the 2019
53 novel coronavirus infections (SARS-CoV-2) that was originated from a seafood wholesale market in
54 Wuhan, China(11). So far, this novel coronavirus has spread throughout the whole of China and over
55 198 countries globally, causing over 468,905 laboratory-confirmed cases of infections with 21,200
56 people dead posing a great threat to the global public health (<http://2019ncov.chinacdc.cn/2019-nCov/>).
57 Besides, there are still numerous suspected cases and a myriad of medical monitoring people who
58 were quarantined in specialized hospitals or at homes because of their previous epidemiological link
59 to confirmed SARS-CoV-2 patients. All of these put an extreme burden on the emergency, hospital
60 and public health system particularly the epidemic zone worldwide. Therefore, a timely, sensitive and
61 accurate diagnosis approach is urgently needed and of pivotal importance for surveillances of disease
62 dissemination and the prevention of further expansions. Conventional diagnosis methods such as
63 virus culture and microscopic analysis are generally time-consuming and labor-intensive with limited
64 sensitivity (12, 13). In contrast, the last decade emerged molecular biologic and serologic approaches,

65 such as TaqMan Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR), Enzyme-linked
66 immunosorbent assay, colloidal gold immunochromatography and direct chemiluminescence
67 immunoassay (CLIA), can be developed into a rapid and effective tool for detections of respiratory
68 pathogens infections, even though in certain circumstances molecular biologic method like RT-PCR
69 had a low sensitivity for specimens from upper respiratory tract (14-17).

70 In this study, we developed a chemiluminescence immunoassay method to specifically detect the
71 induced antibody IgM and IgG by SARS-CoV-2 using the recombinant nucleocapsid (YP_009724397.2)
72 and evaluate its sensitivity and specificity in detections of SARS-CoV-2 infected patients. High
73 sensitivity and specificity results indicate this chemiluminescence immunoassay method in
74 combination with RT-PCR method can serve as highly sensitive and accurate tools for the diagnosis
75 and screen of SARS-CoV-2 infections in the community.

76 **Material and methods**

77 **Participants and specimens**

78 In Shenzhen city, China, patients infected by SARS-CoV-2 were all eventually admitted into a
79 specialized hospital (the third people's hospital of Shenzhen) for quarantines and treatments. In this
80 study, a total of 29 healthy individuals, 51 tuberculosis patients and 79 SARS-CoV-2 patients were
81 enrolled for serological testing. Twenty-nine healthy people were recruited from the Shenzhen
82 University aging from 16 to 72 years old. Fifty-one tuberculosis patients were enrolled from the
83 Shenzhen Baoan hospital, and their mycobacterium tuberculosis infections were previously confirmed
84 by sputum smear acid-fast-bacilli analysis, chest radiograph and the QuantiFERON®-TB Gold test.

85 COVID-19 patients were randomly enrolled from the third people's hospital of Shenzhen, and their
86 SARS-CoV-2 infections were confirmed by combinations of epidemiological risk, clinical features and
87 positive detections of SARS-CoV-2 RNA in respiratory specimens using the National Medical
88 Production Administration authorized GeneoDX kit according to the official instruction for diagnosis
89 and treatment of 2019 novel coronavirus infections issued by the National Health Commission of the
90 People's Republic of China. All healthy cohorts and tuberculosis patients had no epidemiological risks,
91 and they were persistently negative for SARS-CoV-2 RNA detections in at least three respiratory
92 specimens' tests using above mentioned GeneoDX kit. Peripheral blood samples were collected into
93 EDTA and sodium heparin containing tubes, and then the serum was separated by centrifugations
94 (800g ×10 minutes) for immediate testing or stored at -80°C until used. Verbal Informed consent was
95 obtained from all individual participants.

96 **SARS-CoV-2 Nucleic acid**

97 Total nucleic acid for collected respiratory specimens was extracted in BSL-3 laboratory using the
98 nucleic acid extraction and purification kit from Huayin Bio-Tech (Shenzhen, China) according to the
99 manufacturer protocol. Detections of SARS-CoV-2 RNA were performed using the National Medical
100 Production Administration authorized GeneoDX kit (Taqman RT-PCR method, targeting the ORF1ab
101 and N genes) according to the manufacturer instructions.

102 **Development of chemiluminescence immunoassay and test procedures**

103 After the transcription of extracted SARS-CoV-2 genome RNA into the cDNA, the coding regions
104 (YP_009724397.2) were then amplified and cloned into the pET30a vector. The recombinant full-

105 length nucleocapsid antigen was expressed in engineering *E. coli* BL21 (DE3) strains and purified using
106 the Ni-NTA resin (Darui Biotech, China). Magnetic beads Magnosphere™ MS300 used in this study are
107 commercially available in the JSR Corporation, Tokyo, Japan. Recombinant nucleocapsid antigens
108 were coupled to these tosyl magnetic beads using the catalytic reagent solution (3M Ammonium
109 sulfate / 0.1M Borate buffer, pH9.5) according to the manufacture's instruction, and the resultant
110 beads were further blocked by 0.05% BSA for six hours at 37 °C. The following testing and detection
111 procedure was automated on a chemical immuno-luminescence analyzer ACCRE6 (Tianshen Tech,
112 Shenzhen, China). It was comprised of those following steps. 50 microliter pure serum was firstly
113 incubated with the magnetic beads that were coupled with antigens for 5 minutes at 37 °C.
114 Subsequently, the unbound substance was gently removed and then washed by Tris buffer for three
115 times. Alkaline phosphatase labeled anti-human immunoglobulin (50µg/ml) was added and further
116 incubated for 5 minutes at 37 °C in the Mes Buffer. After three times washing to remove unbound
117 materials, the lumigen APS-5 substrate (50ug/ml) was eventually added. Ultimately, the light signal
118 was measured by the photomultiplier in ACCRE6 (Tianshen Tech, Shenzhen, China) as relative light
119 units, and the whole testing can be completed in 23 minutes. Confirmed SARS-CoV-2 positive-serum
120 and negative-serum were used as controls in each set test.

121 **Detections of IgG and IgM by a commercial ELISA kit**

122 In parallel testing, the commercial enzyme-linked immunosorbent assay kit (Darui Biotech, CHINA) for
123 detections of the anti-SARS-CoV-2 IgG and IgM antibody was used to measure the SARS-CoV-2
124 antibody level in above mentioned COVID-19 patients and control individuals. For the principle of this
125 ELISA kit, the specific SARS-CoV-2 nucleocapsid protein and anti-human IgM monoclonal antibody

126 were firstly coated on the plates, respectively. Subsequently, the 100 μ l of 100- fold diluted serum
127 was added and then incubated for 60 minutes at 37 °C. After five times washing by PBST buffer, the
128 horseradish peroxidase (HRP) labeled mouse anti-human IgG antibody or HRP-labeled SARS-CoV-2
129 nucleocapsid antigen was added for 30 minutes incubation at 37 °C. Fifty microliter TMB substrate
130 was then added for 15 minutes incubation after the second time washing by PBST buffer. The
131 stopping solution was eventually added to suspend the reaction, and OD 450/630 values were
132 immediately measured using the Varioskan LUX™ Multimode Microplate Reader. The cutoff values for
133 positive were set based on the manufacturer’s recommendations.

134 **Ethical statement**

135 The internal use of collected samples for diagnoses of etiological agents and serological research was
136 approved by the Ethical Committee in the third people’s hospital of Shenzhen (SZTHEC2016001).

137 **Statistical analysis**

138 All statistical analysis was performed in GraphPad Prism 7 software. The One-way ANOVA test was
139 used to analyze the average RLU values difference between different participant groups. The chip-
140 square and Fisher’s exact test was used for comparing the difference between the analyzed groups.
141 The difference was considered significant when a p-value is < 0.05.

142

143 **Results**

144 **Detections of IgG and IgM antibodies induced by SARS-CoV-2 in serum and overall specificity and**
145 **sensitivity assessments**

146 To assess the specificity and sensitivity of the chemiluminescence immunoassay method developed
147 based on the recombinant nucleocapsid antigen, serum from 29 healthy individuals, 51 tuberculosis
148 patients and 79 confirmed SARS-CoV-2 patients were employed and tested. More than six-fold and
149 eight folds higher average RLU (relative light units) values were observed in the SARS-CoV-2 patients
150 group in the IgM testing compared to that of the healthy cohort and tuberculosis patients (Figure 1A).
151 This average RLU difference is more dramatic when it comes to the IgG testing reaching 60 and 70
152 fold increase in SARS-CoV-2 patients in comparison with the healthy and tuberculosis group,
153 respectively (Figure 1B). A Receiver Operating Characteristic curve was then obtained based on these
154 RLU values for the SARS-CoV-2 patients group and control group that consists of healthy cohort and
155 tuberculosis patients. According to the ROC curve and analysis results, we recommend a cutoff setting
156 for IgM (RLU 162296) and IgG (336697), in which the calculated sensitivity and specificity for IgM were
157 82.28% and 81.25%, and 82.28% and 97.5% for IgG, respectively.

158 Based on this cutoff and using the IgM testing, we identified 15 cases and 65 cases as SARS-CoV-2
159 positive from the control group (80 cases) and the SARS-CoV-2 confirmed group (79 cases),
160 respectively (Table 1). In contrast, using the IgG testing, we only detected two false-positive cases
161 from the control group, which is in line with the higher specificity for IgG (97.5%) compared to that of
162 the IgM testing (81.25%) as above described.

163

164 **The links between disease onset time, ages and IgM and IgG productions and detection efficiency**

165 To explore whether the onset time was significantly linked with the detection sensitivity by this
166 serological chemiluminescence method, comparison and statistical analysis of the sensitivity rates
167 between different onset time patient categories was undertaken. No statistically significant
168 difference was observed between the IgM and IgG testing results in the patients with the same onset
169 time, although two more cases from 12 cases were detected by IgM testing compared to that of IgG
170 testing in patients less than the one-week onset of disease (Table 2, p-value >0.05). In stark contrast,
171 two more cases SARS-CoV-2 patients were identified by IgG testing than that of IgM testing in patients
172 with more than two weeks onset of disease (p-value >0.05). In addition, we also compared the
173 detection rates between the different age groups people, and we found that a significantly lower
174 detection rate in both IgM and IgG testing manner for individual group younger than 18 years old was
175 observed compared to that of people aging from 18 to 65 (p-value < 0.01) or over 65 years old (p-
176 value >0.05) (Supplementary Table S1). No statistically significant differences were observed for male
177 and female groups as well.

178

179 **Comparisons with other ELISA kit**

180 To further characterize the patient's immune response to the SARS-CoV-2 antigens and to prove the
181 feasibility of the practical application of this serological testing kit in clinical diagnosis, 64 paired
182 serum from the above-mentioned control cohorts and 65 COVID-19 patients were also examined
183 using a recently developed commercial available ELISA Kit. A total of 14 false-positive cases (21%)
184 were identified by IgM testing in both methods. A very lower false-positive rate was observed in IgG
185 testing in both methods. Compared to the ELISA kit, a significantly higher detection rate for SARS-CoV-

186 2 in both IgM and IgG testing manners was seen in our chemiluminescence method, suggesting a
187 higher sensitivity of our approach compared to the tested ELISA kit (Table 3, p-value < 0.001).

188 **Discussion**

189 Compared with the conventional virological methods, the molecular biologic TaqMan RT-PCR method
190 has been widely used for clinical diagnosis of respiratory pathogens infections particularly in the initial
191 phase of disease because of its high specificity property (18, 19). Nevertheless, a relatively low
192 sensitivity (30%- 50%) in single upper respiratory specimen testing has been commonly reported
193 including the well-appreciated methods for SARS-CoV detections (20). Furthermore, since the SARS-
194 CoV-2 expansion from 2020 January, several cases reported that consecutive negative detections of
195 SARS-CoV-2 RNA were observed for upper respiratory specimens testing like throat swabs in patients
196 with apparent clinical symptoms, and the positive results can only be achieved by collecting the
197 bronchoalveolar lavage fluid for re-testing. Hence, a sensitive serological diagnosis method can serve
198 as a very useful compensation tool for current clinical diagnosis situations. Our results demonstrated
199 that a single IgG testing is feasible in the clinical diagnosis for SARS-CoV-2, as a higher specificity and
200 sensitivity were observed in our chemiluminescence method. In the humoral immune response, the
201 antibody IgM was generally produced earlier than the IgG isotype as the IgM can be expressed
202 without the isotype switching. Unexpectedly, we only observed a slight detection rate difference (not
203 statistically significant) between these two antibody isotype testing manner in patients in the first
204 week or more than two weeks after onset of disease. However, compared to the IgG approach, our
205 IgM method showed a lower specificity (higher false-positive cases) in our testing. As the IgM and IgG

206 using the same pure recombinant antigen and coupling condition (supplementary figure S1), the
207 detection specificity difference is more likely linked to intrinsic antibody traits and concentration
208 differences in the patients' blood.

209 We noted that four patients with clinical symptoms less than four days were simultaneously detected
210 by both IgM and IgG testing. A close examination of their medical record reveals that all of them had
211 previous contact with confirmed SARS-CoV-2 individuals in at least 16 days ago, pointing to the
212 possibility that they were probably asymptotically infected by SARS-CoV-2 for certain time already.
213 Fourteen cases (17.7%) from 79 SARS-CoV-2 confirmed patients were not identified by our serological
214 testing method (both the IgM and IgG manner). Interestingly, of them, seven people were younger
215 than 8-year-old or over 70-year-old. These people generally have low immunity in which a clinical
216 symptom may occur rapidly upon exposure to the SARS-CoV-2, and we speculate that the antibodies
217 in these people may not develop well yet when testing. More investigations are warranted to
218 uncover the real situations. When comparing the detection rates in different age groups by our
219 method, we noted that a significantly lower detection rate in both IgM and IgG testing manner for the
220 individual group younger than 18 years old was observed compared to that of people aging from 18 to
221 65. An in-depth look at the days after onset for these 12 individuals younger than 18-years-old, the
222 symptom onset time for all the 12 people are less than 14 days with six people even less seven days
223 (Supplementary Table S2). The lower detection rate for these 12 people younger than 18-years-old
224 was likely associated with no or less production of antibodies in them yet when we collected the
225 serums.

226 In our parallel performance testing, the same antigen of nucleocapsid protein was used in both the
227 commercially available ELISA kit and our chemiluminescence immunoassay. Unexpectedly, a
228 significantly higher sensitivity was observed in our method compared to the ELISA kit. This sensitivity
229 difference may be partially attributed to the difference in the serum amount for the first incubation
230 step. On the other side, the intrinsic method difference including the aspect of binding surface
231 interaction and mode of separations of the unbound material can also contribute to the sensitivity
232 difference in the chemiluminescence immunoassay and ELISA method.

233 In conclusion, in this study, we developed and evaluated a serological chemiluminescence
234 immunoassay testing technique for clinical diagnosis of SARS-CoV-2 infections using the recombinant
235 nucleocapsid antigen. This high sensitivity and specificity chemiluminescence immunoassay method
236 combined with the RT-PCR method can doubtless significantly improve the clinical diagnosis for SARS-
237 CoV-2 and contribute to the control of COVID-19 expansion globally.

238

239 **Acknowledgment**

240 We are very grateful to Prof. Yingsong Wu at Southern Medical University for his kind gifts of several
241 proteins for our trail test and helpful suggestions for the whole project. This work is supported by
242 Guangdong Provincial Science and Technology Program (No. 2019b030301009), the National Natural
243 Science Funds of China (81802060), the start-up funding of Shenzhen University and the National
244 Science and Technology Major Project (2017ZX10201301).

245

246 Figure 1. Detections and measurements of the SARS-CoV-2 IgM and IgG antibody in healthy people,
247 tuberculosis patients and SARS-CoV-2 confirmed patients using the chemiluminescence immunoassay method
248 (A and B). The average results were expressed as mean \pm SEM of all individuals. Receiver Operating
249 Characteristic curves for IgM (C) and IgG(D) were obtained based on the RLU for the SARS-CoV-2 patient group
250 and the control group consisting of healthy cohorts and tuberculosis patients.

251 Supplementary figure S1. SDS-PAGE of the purified recombinant nucleocapsid antigen, M band indicates the
252 marker, 1-3 band are the purified recombinant nucleocapsid protein.

253

- 254 1. Wang LF, Shi Z, Zhang S, Field H, Daszak P, Eaton BT. 2006. Review of bats and SARS. *Emerg Infect Dis*
255 12:1834-40.
- 256 2. Lim XF, Lee CB, Pascoe SM, How CB, Chan S, Tan JH, Yang X, Zhou P, Shi Z, Sessions OM, Wang LF, Ng LC,
257 Anderson DE, Yap G. 2019. Detection and characterization of a novel bat-borne coronavirus in
258 Singapore using multiple molecular approaches. *J Gen Virol* 100:1363-1374.
- 259 3. Fehr AR, Perlman S. 2015. Coronaviruses: an overview of their replication and pathogenesis. *Methods*
260 *Mol Biol* 1282:1-23.
- 261 4. Chan JF, To KK, Tse H, Jin DY, Yuen KY. 2013. Interspecies transmission and emergence of novel viruses:
262 lessons from bats and birds. *Trends Microbiol* 21:544-55.
- 263 5. St-Jean JR, Jacomy H, Desforges M, Vabret A, Freymuth F, Talbot PJ. 2004. Human respiratory
264 coronavirus OC43: genetic stability and neuroinvasion. *J Virol* 78:8824-34.
- 265 6. Chiu SS, Chan KH, Chu KW, Kwan SW, Guan Y, Poon LL, Peiris JS. 2005. Human coronavirus NL63
266 infection and other coronavirus infections in children hospitalized with acute respiratory disease in
267 Hong Kong, China. *Clin Infect Dis* 40:1721-9.
- 268 7. Woo PC, Lau SK, Yip CC, Huang Y, Yuen KY. 2009. More and More Coronaviruses: Human Coronavirus
269 HKU1. *Viruses* 1:57-71.
- 270 8. Vassilara F, Spyridaki A, Pothitos G, Deliveliotou A, Papadopoulos A. 2018. A Rare Case of Human
271 Coronavirus 229E Associated with Acute Respiratory Distress Syndrome in a Healthy Adult. *Case Rep*
272 *Infect Dis* 2018:6796839.
- 273 9. Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. 2015. Middle East respiratory syndrome
274 coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clin Microbiol Rev* 28:465-
275 522.
- 276 10. Cheng VC, Lau SK, Woo PC, Yuen KY. 2007. Severe acute respiratory syndrome coronavirus as an agent
277 of emerging and reemerging infection. *Clin Microbiol Rev* 20:660-94.
- 278 11. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang
279 D, Xu W, Wu G, Gao GF, Tan W, China Novel Coronavirus I, Research T. 2020. A Novel Coronavirus from
280 Patients with Pneumonia in China, 2019. *N Engl J Med* doi:10.1056/NEJMoa2001017.

- 281 12. Sarasini A, Percivalle E, Rovida F, Campanini G, Genini E, Torsellini M, Paolucci S, Baldanti F, Marchi A,
282 Grazia Revello M, Gerna G. 2006. Detection and pathogenicity of human metapneumovirus respiratory
283 infection in pediatric Italian patients during a winter--spring season. *J Clin Virol* 35:59-68.
- 284 13. Rose RM, Pinkston P, O'Donnell C, Jensen WA. 1987. Viral infection of the lower respiratory tract. *Clin*
285 *Chest Med* 8:405-18.
- 286 14. Vemula SV, Zhao J, Liu J, Wang X, Biswas S, Hewlett I. 2016. Current Approaches for Diagnosis of
287 Influenza Virus Infections in Humans. *Viruses* 8:96.
- 288 15. Goy G, Croxatto A, Posfay-Barbe KM, Gervaix A, Greub G. 2009. Development of a real-time PCR for the
289 specific detection of *Waddlia chondrophila* in clinical samples. *Eur J Clin Microbiol Infect Dis* 28:1483-6.
- 290 16. Mahony JB, Petrich A, Smieja M. 2011. Molecular diagnosis of respiratory virus infections. *Crit Rev Clin*
291 *Lab Sci* 48:217-49.
- 292 17. Poon LL, Chan KH, Wong OK, Yam WC, Yuen KY, Guan Y, Lo YM, Peiris JS. 2003. Early diagnosis of SARS
293 coronavirus infection by real time RT-PCR. *J Clin Virol* 28:233-8.
- 294 18. Li J, Li W, Mao L, Hao F, Yang L, Zhang W, Jiang J. 2016. Rapid detection of novel caprine parainfluenza
295 virus type 3 (CPIV3) using a TaqMan-based RT-qPCR. *J Virol Methods* 236:126-131.
- 296 19. Weinberg GA, Schnabel KC, Erdman DD, Prill MM, Iwane MK, Shelley LM, Whitaker BL, Szilagyi PG, Hall
297 CB. 2013. Field evaluation of TaqMan Array Card (TAC) for the simultaneous detection of multiple
298 respiratory viruses in children with acute respiratory infection. *J Clin Virol* 57:254-60.
- 299 20. Wang B, Potter SJ, Lin Y, Cunningham AL, Dwyer DE, Su Y, Ma X, Hou Y, Saksena NK. 2005. Rapid and
300 sensitive detection of severe acute respiratory syndrome coronavirus by rolling circle amplification. *J*
301 *Clin Microbiol* 43:2339-44.

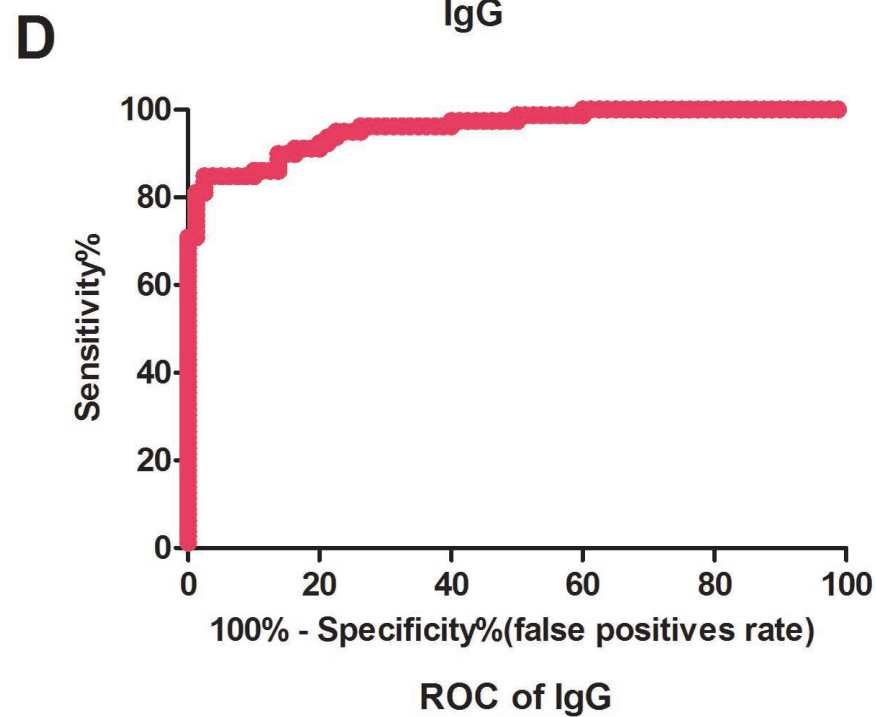
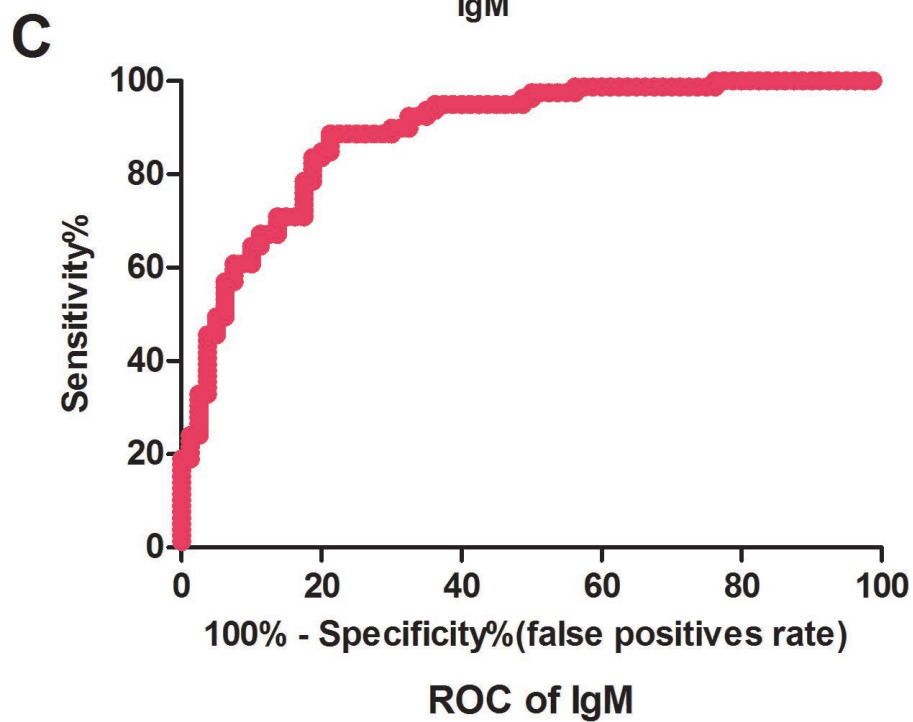
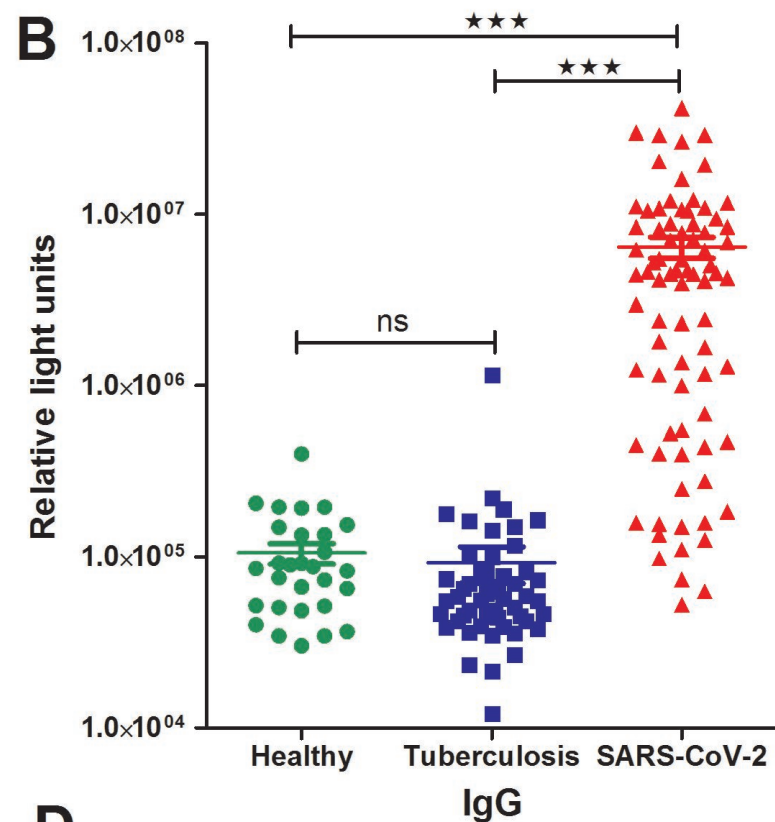
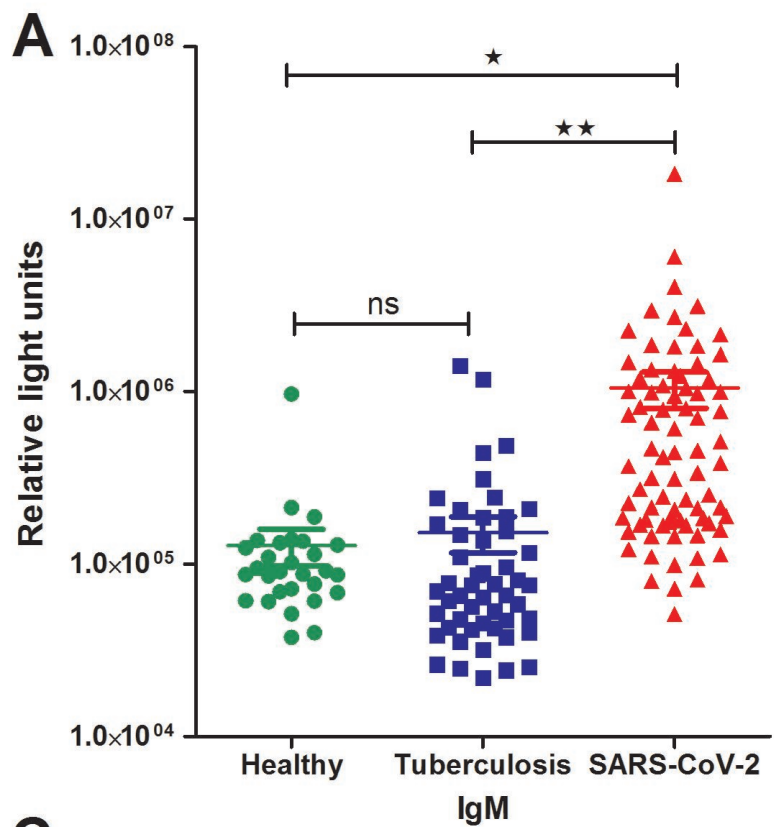


Table 1 Evaluations of a chemiluminescence immunoassay method for diagnosis of SARS-CoV-2 by detections of specific IgM and IgG in the patient's serum.

Participants category /total cases	IgM positive cases / (%)	IgG positive/ Cases / (%)	IgM or IgG positive cases/ (%)	IgM and IgG positive cases (%)
Healthy cohorts and tuberculosis patients/80 cases	15 /18.75%	2 /2.50%	16 /20.00%	1 /1.25%
SARS-CoV-2 confirmed patients /79 cases	65 /82.28%	65 /82.28%	72 /91.14%	58/73.42%

Table 2 Comparison of SARS-CoV-2 detections results in patients with different onset time between the IgM and IgG approach

Days after onset	Total cases	IgM positive cases / (%)	IgG positive/ cases / (%)	IgM or IgG positive cases/ (%)	IgM and IgG positive cases (%)
0-3	4	4/100%	4/100%	4/100%	4/100%
4-7	8	6/75%	4/50%	6/75%	4/50%
8-14	33	24/72.73%	24/72.73%	29/87.88%	19/57.58%
>14	34	31/91.18%	33/97.06%	33/97.06%	31/91.18%

Table 3 Detection differences between the chemiluminescence and ELISA method

Methods	Control group (total 64 cases)		SARS-CoV-2 confirmed patients (total 65 cases)	
	IgM/false-positive /%	IgG/ false-positive /%	IgM positive /%	IgG positive /%
ELISA	14 /21.8%	0 / 0%	30 / 46.1%	15 / 23%
chemiluminescence	14 /21.8%	2 / 3.1%	55 / 84.6%	53 / 81.5%
Identified in both	3 / 4.6%	0 / 0%	28 / 43%	15 / 23%