

1        **Evaluation of Nucleocapsid and Spike Protein-based ELISAs for**  
2                                **detecting antibodies against SARS-CoV-2**

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4                                Running title: ELISA can be used for COVID-19 diagnosis

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20

21 **Abstract**

22 **Background:** At present, PCR-based nucleic acid detection cannot meet the demands  
23 for coronavirus infectious disease (COVID-19) diagnosis.

24 **Methods:** 214 confirmed COVID-19 patients who were hospitalized in the General  
25 Hospital of Central Theater Command of the People's Liberation Army between  
26 January 18 and February 26, 2020, were recruited. Two Enzyme-Linked  
27 Immunosorbent Assay (ELISA) kits based on recombinant SARS-CoV-2 nucleocapsid  
28 protein (rN) and spike protein (rS) were used for detecting IgM and IgG antibodies,  
29 and their diagnostic feasibility was evaluated.

30 **Results:** Among the 214 patients, 146 (68.2%) and 150 (70.1%) were successfully  
31 diagnosed with the rN-based IgM and IgG ELISAs, respectively; 165 (77.1%) and  
32 159 (74.3%) were successfully diagnosed with the rS-based IgM and IgG ELISAs,  
33 respectively. The positive rates of the rN-based and rS-based ELISAs for antibody  
34 (IgM and/or IgG) detection were 80.4% and 82.2%, respectively. The sensitivity of  
35 the rS-based ELISA for IgM detection was significantly higher than that of the  
36 rN-based ELISA. We observed an increase in the positive rate for IgM and IgG with  
37 an increasing number of days post-disease onset (d.p.o.), but the positive rate of IgM  
38 dropped after 35 d.p.o. The positive rate of rN-based and rS-based IgM and IgG  
39 ELISAs was less than 60% during the early stage of the illness 0-10 d.p.o., and that of  
40 IgM and IgG was obviously increased after 10 d.p.o.

41 **Conclusions:** ELISA has a high sensitivity, especially for the detection of serum  
42 samples from patients after 10 d.p.o, it can be an important supplementary method for

43 COVID-19 diagnosis.

44 **Key words:** COVID-19 diagnosis; ELISA; antibody; IgM; IgG; nucleocapsid protein;

45 spike protein.

## 46 Introduction

47 The ongoing outbreak of coronavirus infectious disease 2019 (COVID-19) (1),  
48 which emerged in Wuhan, China, is caused by a novel coronavirus named severe  
49 acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2-4). As of March 1, 2020,  
50 more than 80,000 laboratory-confirmed cases have been reported in China (5), and the  
51 disease has spread over 58 countries in Asia, Australia, Europe, and North America  
52 (1). On March 11, 2020, the WHO declared coronavirus a pandemic.

53 SARS-CoV-2 is the seventh member of enveloped, positive-stranded RNA viruses  
54 (4) that are able to infect humans. Genomic characterization of SARS-CoV-2  
55 identified it as a Beta coronavirus and showed it is closely related (with 96% identity)  
56 to Bat CoV RaTG13, but distinct from SARS-CoV (6). SARS-CoV-2 has a  
57 receptor-binding domain (RBD) structure similar to that of SARS-CoV. Functionally  
58 important ORFs (ORF1a and ORF1b) and major structural proteins, including the  
59 spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins, are also well  
60 annotated (7). According to previous reports, the M and E proteins are necessary for  
61 virus assembly (8, 9). The S protein is important for attachment to host cells, where  
62 the RBD of S protein mediates the interaction with angiotensin-converting enzyme 2  
63 (ACE2) (6). The S protein is located on the surface of the viral particles and has been  
64 reported to be highly immunogenic (10). The N protein is one of the major structural  
65 proteins of the virus and is involved in the transcription and replication of viral RNA,  
66 packaging of the encapsidated genome into virions (11, 12), and interference with cell  
67 cycle processes of host cells (13). Moreover, in many coronaviruses, including

68 SARS-CoV, the N protein has high immunogenic activity and is abundantly expressed  
69 during infection (14-16). Both S and N proteins may be potential antigens for  
70 serodiagnosis of COVID-19, just as many diagnostic methods have been developed  
71 for diagnosing SARS based on S and/or N proteins (10, 14-17).

72       Currently, diagnosis of COVID-19 is confirmed by RNA tests with real-time  
73 (RT)-PCR or next-generation sequencing. Studies have shown that SARS-CoV-2  
74 mainly infects the lower respiratory tract, and that viral RNA can be detected from  
75 nasal and pharyngeal swabs and bronchoalveolar lavage (BAL) (3, 6, 18). However,  
76 the collection of the lower respiratory samples (especially BAL) requires both a  
77 suction device and a skilled operator. A previous study showed that except for BAL,  
78 the sputum from confirmed patients possessed the highest positive rate, ranging from  
79 74.4% to 88.9%. The positive rate of nasal swabs ranged from 53.6% to 73.3%, and  
80 throat swabs collected  $\geq 8$  days post-disease onset (d.p.o.) had a low positive rate,  
81 especially in samples from mild cases (19). The viral colonization of the lower  
82 respiratory tract and the collection of different respiratory specimens cause a high  
83 false negative rate of real time Reverse Transcription-Polymerase Chain Reaction  
84 (RT-PCR) diagnosis. Therefore, with the current tests it is difficult to achieve a  
85 meaningful assessment of the proportion of symptomatic patients that are infected,  
86 and a rapid and accurate detection method of COVID-19 is urgently needed.

87 Serological assays are accurate and efficient methods for the screening for many  
88 pathogens, as specific IgM and IgG antibodies can be detected with ELISA, which has

89 relatively high throughput capacity and less stringent specimen requirements  
90 (uniformly serum collection) than RNA-based assays.

91 The present study was conducted to evaluate the performance of rN-based and  
92 rS-based ELISAs to detect IgM and IgG antibodies in human serum against  
93 SARS-CoV-2. A total of 214 clinical serum samples from confirmed COVID-19  
94 patients and 100 samples from healthy blood donors were tested with the rN-based  
95 and rS-based ELISAs.

96

## 97 **Materials and Methods**

### 98 **Patients and samples**

99 A total of 214 patients diagnosed with COVID-19, hospitalized in the General  
100 Hospital of the Central Theater Command of the People's Liberation Army (PLA)  
101 between January 18 and February 26, 2020, were recruited. The general information  
102 was extracted from electronic medical records. All of the patients were  
103 laboratory-confirmed positive for SARS-CoV-2 by RT-PCR using pharyngeal swab  
104 specimens, and at a median of 15 d.p.o. (range, 0–55 days). Serum samples from 100  
105 healthy blood donors were selected as controls. This study was approved by the  
106 Hospital Ethics Committee of the General Hospital of the Central Theater Command  
107 of the PLA ([2020]003-1) and the written informed consent was waived for emerging  
108 infectious diseases.

### 109 **rN-based ELISA**

110 The recombinant nucleocapsid (rN) protein-based ELISA kit (Lizhu, Zhuhai,  
111 China) was used for the detection of IgM or IgG antibody against SARS-CoV-2. For  
112 IgM detection, ELISA plates were coated with monoclonal mouse anti-human IgM ( $\mu$   
113 chain) antibody. Serum sample (100  $\mu$ L, diluted 1:100) was added to the pre-coated  
114 plates, and plates were incubated at 37 °C for 1 h. Three replicates of each sample or  
115 control were included on each plate. After washing, 100  $\mu$ L horseradish peroxidase  
116 (HRP)-conjugated rN protein of SARS-CoV-2 was added. Then, the plate was  
117 incubated at 37 °C for 30 min, followed by washing. TMB substrate solution (50  $\mu$ L)  
118 and the corresponding buffer (50  $\mu$ L) were added, and samples were incubated at  
119 37 °C for 15 min. The reaction was terminated by adding 50  $\mu$ L of 2 M sulfuric acid,  
120 and the absorbance value at 450 nm ( $A_{450}$ ) was determined. According to instruction  
121 of the kit, the cutoff value was calculated by summing 0.100 and the average  $A_{450}$  of  
122 negative control replicates. When  $A_{450}$  was below the cutoff value, the test was  
123 considered negative, and when  $A_{450}$  was greater than or equal to the cutoff value, the  
124 test was considered positive.

125 For IgG detection, ELISA plates were coated with rN protein. Serum sample (5  $\mu$ L)  
126 diluted in 100  $\mu$ L dilution buffer was added to the plates. After incubation and  
127 washing, HRP-conjugated monoclonal mouse anti-human IgG antibody was added to  
128 the plates for detection. The other operation steps were performed as described above  
129 for IgM detection. According to instruction of the kit, the cutoff value was calculated  
130 by summing 0.130 and the average  $A_{450}$  of negative control replicates. When  $A_{450}$  was  
131 below the cutoff value, the test was considered negative, and when  $A_{450}$  was greater

132 than or equal to the cutoff value, the test was considered positive.

### 133 **rS-based ELISA**

134 The rS-based ELISA kit (Hotgen, Beijing, China) was developed based on the  
135 RBD of the recombinant S polypeptide (rS). For IgM antibody testing, serum samples  
136 (diluted 1:100) and negative and positive controls were added to the wells of the  
137 rS-coated plates in a total volume of 100  $\mu$ L, and plates were incubated at 37 °C for  
138 30 min. After five wash steps with washing buffer, 100  $\mu$ L of diluted HRP-conjugated  
139 anti-human IgM antibodies was added to the wells, and samples were incubated at 37 °C  
140 for 30 min. After five wash steps with washing buffer, 50  $\mu$ L of TMB substrate  
141 solution and 50  $\mu$ L of the corresponding buffer were added, and samples were  
142 incubated at 37 °C for 10 min. The reaction was terminated by adding 50  $\mu$ L of 2 M  
143 sulfuric acid, and  $A_{450}$  was measured. The IgG antibody was determined by a standard  
144 ELISA procedure as described above, except that serum sample was diluted 1:20, and  
145 the detector was HRP-conjugated anti-human IgG. According to instruction of the kit,  
146 the cutoff values of IgM and IgG were calculated by summing 0.250 and the average  
147  $A_{450}$  of negative control replicates. When  $A_{450}$  was below the cutoff value, the test was  
148 considered negative, and when  $A_{450}$  was greater than or equal to the cutoff value, the  
149 test was considered positive.

### 150 **Statistical analysis**

151 Categorical variables were expressed as the counts and percentages and compared  
152 using the chi-square test, while the Fisher exact test was used when the data were  
153 limited. Statistical analyses were performed using SPSS version 22.0. A two-sided

154 *P*-value < 0.05 was considered statistically significant.

155

## 156 **Results**

### 157 **Performance of serological assays by rN-based and rS-based ELISA**

158 The serum samples were collected from 214 COVID-19 patients who were  
159 confirmed by qRT-PCR and hospitalized in the General Hospital of the Central  
160 Theater Command of the PLA. Of the 214 serum samples, 146 (68.2%) and 150  
161 (70.1%) were identified as positive by the rN-based IgM and IgG ELISAs (Table 1),  
162 respectively. IgM and/or IgG means that one of them or both were detected in serum  
163 samples; 172 (80.4%) serum samples were positive by rN-based ELISA (Fig. 1). The  
164 numbers of positive results with IgM, IgG, and IgM and/or IgG detected by rS-based  
165 ELISA were 165 (77.1%), 159 (74.3%), and 176 (82.2%), respectively (Table 1 and  
166 Fig. 1).

167 To study the production of antibodies in COVID-19 patients, we analyzed the  
168 positive rates of IgM and IgG in serum samples of all patients post-disease onset.  
169 Based on the number of days from disease onset to serum collection, patients were  
170 divided into seven groups: 0–5, 6–10, 11–15, 16–20, 21–30, 31–35, and >35 d.p.o.  
171 The median number of d.p.o. of serum sample collection was 15 (range, 0–55 days).  
172 The positive rates of IgM and IgG detected by the rN- and rS-based ELISAs in  
173 different groups are shown in Table 1. For rN-based ELISA, a clear increase in IgM  
174 and IgG positive rates was observed (Fig. 2a). The positive rates of IgM and IgG were  
175 low at 0–5 d.p.o. and 6–10 d.p.o., and the positive rate of IgM was higher than that of

176 IgG at 6–10 d.p.o. and much lower after 35 d.p.o. (Fig. 2a), illustrating the dynamic  
177 pattern of acute viral infection where IgG concentrations rise as IgM levels drop. The  
178 IgM and/or IgG positive rate was 88.9% at 11–15 d.p.o., and more than 90% at later  
179 stages of the disease (Table 1).

180 For rS-based ELISA, a similar trend of IgM and IgG positive rates was observed  
181 (Fig. 2b). The positive rate of IgM is a little higher than that of IgG at different  
182 disease stages, except >35 d.p.o., possibly due to the higher sensitivity of rS-based  
183 IgM detection (77.1%) than IgG detection (74.3%) (Table 1).

184 To verify the specificity of the ELISA assays, 100 samples from healthy blood  
185 donors were analyzed. No positive result was found in both rN- and rS-based IgM and  
186 IgG ELISAs.

187 **Comparison of rN- and rS-based IgM and IgG ELISAs for diagnosis of**  
188 **SARS-CoV-2**

189 The sensitivity of the rS-based IgM ELISA was significantly higher than that of  
190 the rN-based ELISA ( $P < 0.05$ ). No significant difference between rS- and rN-based  
191 ELISAs was observed for detecting IgG and total antibodies (IgM and IgG) (Fig. 1).

192 The positive rates of the rN- and rS-based ELISAs for the detection of IgM and/or  
193 IgG in serum samples collected from patients at different stages of the disease are  
194 shown in Table 1. Within 15 d.p.o., for detecting IgM, which is an important marker  
195 for early infection, rN- and rS-based ELISAs detected 57.9% (66/114) and 63.2%  
196 (72/114) of patients, indicating that the performance of the rS-based IgM  
197 immunoassay seems better than that of the rN-based assay, but there is no statistical

198 difference between the two results. The positive rate of the rS-based ELISA for IgM is  
199 significantly higher than that of the rN-based ELISA at 16–20 d.p.o.(Table 1). No  
200 significant difference between the two antigen-based ELISAs was observed for IgM  
201 detection in other groups. For IgG detection, there was no significant difference  
202 between the two kits in all groups.

203 In this study, the rN- and rS-based ELISAs for detecting IgM and IgG in 214  
204 COVID-19 patients were evaluated (Table 2). In total, the detected positive rate  
205 (174/214, 81.3%) for IgM by the two kits was significantly higher than that of the  
206 rN-based ELISA kit alone (68.2%;  $P < 0.01$ ), but was not significantly different from  
207 that of the rS-based kit alone. For IgG, 172 of 214 (80.4%) were identified positive by  
208 both kits. This sensitivity is significantly higher than that detected by only the  
209 rN-based ELISA (70.1%,  $P < 0.05$ ) and is not significantly different from that of the  
210 rS-based ELISA. For IgM and/or IgG, 186 of 214 (86.9%) were identified positive by  
211 both kits. The sensitivity is not significantly different from that of the rN-based  
212 (80.4%) or the rS-based (82.2%) ELISA alone.

213

214

## 215 Discussion

216 The recent outbreak and rapid spread of the novel coronavirus SARS-CoV-2 pose  
217 a great threat of a pandemic outbreak of COVID-19 to the world. Diagnostic methods  
218 are the frontline strategy for recognizing this disease. Currently, SARS-CoV-2 can be  
219 detected using RT-PCR, but inadequate access to reagents and equipment, the

220 requirement to upscale lab facilities with restrictive bio-safety levels and technical  
221 sophistication, and the high rate of false negative results caused mainly by an  
222 unstandardized collection of respiratory specimens have resulted in low efficiency of  
223 in-time detection of the disease. Although previous studies have shown that  
224 virus-specific IgM and IgG levels allow for serologic diagnosis of SARS (15, 20), less  
225 amount of data on the serologic diagnosis using antibodies against SARS-CoV-2 are  
226 available.

227 In this study, we evaluated immunoassays for the detection of antibodies against  
228 SARS-CoV-2. Specifically, we evaluated IgM and IgG production and their diagnostic  
229 value. rN- and rS-based ELISAs were used to detect IgM and IgG in serum samples  
230 of confirmed COVID-19 patients. The results revealed that the rS-based ELISA is  
231 more sensitive than the rN-based one in the detection of IgM antibodies (Fig. 1). We  
232 speculate that this difference is due to the relatively high sensitivity and early  
233 response to the S antigen compared to the N antigen in patients with COVID-19. We  
234 divided the patients into different groups according to the number of d.p.o. and  
235 observed an increase in the IgM and IgG positive rate with time, except the IgM  
236 positive rate decreased at >35 d.p.o. (Fig. 2). This dynamic pattern is consistent with  
237 that observed in acute viral infection, with the IgG concentration rising gradually as  
238 IgM levels drop after one month post-disease onset. The positive rates of IgM and IgG  
239 antibodies in samples tested by the rN- and rS-based ELISAs were about 30%–50% in  
240 the 0–5 d.p.o. and 6–10 d.p.o. groups (Table 1 and Fig. 2). This may be due to the low  
241 antibody titers in early stages of the disease. Our results showed that IgM and/or IgG

242 of SARS-CoV-2 might be positive (88.9% by rN-based ELISA, 90.7% by rS-based  
243 ELISA) at 11–15 d.p.o. (Table 1), which is to a certain extent in accordance with a  
244 previous publication about SARS, which reported that detection of antibodies to  
245 SARS-CoV could be positive as early as 8–10 d.p.o. and often occurs around day 14  
246 (15). We observed a decreased positive rate of IgM at > 35 d.p.o.; unfortunately, we  
247 could not collect the samples from these patients after discharge. Combined, rN- and  
248 rS-based ELISAs for IgM and IgG detection were more sensitive than the rN-based  
249 ELISA alone, but there was no significant difference when compared with the  
250 rS-based ELISA. Therefore, combined use of rN- and rS-based ELISAs is not  
251 recommended for COVID-19 diagnosis; however, if an ELISA kit coated with a  
252 cocktail of N and S polypeptides shows better results, further evaluation is needed.

253 This study demonstrated that rN- and rS-based ELISAs can be an important  
254 screening method for COVID-19 diagnosis, with high sensitivity, especially for the  
255 analysis of serum samples from patients after more than 10 d.p.o. The ELISA  
256 serodiagnosis can be a supplementary method to RT-PCR for COVID-19 diagnosis.  
257 ELISA can be used to quickly screen all febrile patients effectively, as large-scale  
258 confirmation or exclusion of patients is essential for controlling the disease.

259

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#### Conflict of interest

The authors declare that no conflict of interest exists.

#### Authors' contributions

S.Z. and W.L. conceived the study and designed experimental procedures. L.L., G.K., Y.Z., Y.D., W.N., Q.W., L.T., W.W., S.T., and Z.X. collected patients' samples. L.L. and G.K. established the ELISA and performed serological assays. W.L., S.Z., and L.L. wrote the paper. All authors contributed to data acquisition, data analysis, and/or data interpretation, and reviewed and approved the final version.

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358

359 **FIGURE LEGENDS**

360 **Figure 1.** Comparison of positive rate of antibodies detected by rN-based ELISA and  
361 rS-based ELISA. IgM means a positive result of IgM antibody detection by ELISA,  
362 IgG means a positive result of IgG antibody detection by ELISA, IgM and/or IgG  
363 means at least one of them was positive by IgM and IgG ELISA. Results were  
364 compared by chi-square tests.

365

366 **Figure 2.** Dynamic trend of the positive rate of IgM and IgG in serum of patients at  
367 the different stage of disease. Patients were divided into seven groups of 0-5,  
368 6-10,11-15, 16-20,21-30, 31-35 and >35 days post-disease onset, **a)** The dynamic  
369 trend of antibodies positive rate detected by rN-based ELISA. **b)** The dynamic trend  
370 of antibodies positive rate detected by rS-based ELISA.

Table 1. Positive rate of rN-based and rS-based ELISA for detection of IgM and IgG in serum samples of patients at different stages after disease onset.

| Days  | No. of serum samples | No. (%) positive for IgM |           |       | No. (%) positive for IgG |           |       | No. (%) positive for IgM and/or IgG |           | P     |
|-------|----------------------|--------------------------|-----------|-------|--------------------------|-----------|-------|-------------------------------------|-----------|-------|
|       |                      | rN-based                 | rS-based  | P     | rN-based                 | rS-based  | P     | rN-based                            | rS-based  |       |
| Total | 214                  | 146(68.2)                | 165(77.1) | 0.039 | 150(70.1)                | 159(74.3) | 0.332 | 172(80.4)                           | 176(82.2) | 0.620 |
| 0-5   | 22                   | 7(31.8)                  | 8(36.4)   | 0.750 | 7(31.8)                  | 9(40.9)   | 0.531 | 9(40.9)                             | 10(45.5)  | 0.761 |
| 6-10  | 38                   | 20(52.6)                 | 19(50.0)  | 0.818 | 15(39.5)                 | 19(50.0)  | 0.356 | 20(52.6)                            | 23(60.5)  | 0.488 |
| 11-15 | 54                   | 39(72.2)                 | 45(83.3)  | 0.165 | 39(72.2)                 | 41(75.9)  | 0.661 | 48(88.9)                            | 49(90.7)  | 0.750 |
| 16-20 | 55                   | 45(81.8)                 | 53(96.4)  | 0.014 | 48(87.3)                 | 51(92.7)  | 0.340 | 52(94.5)                            | 53(96.4)  | 0.684 |
| 21-30 | 32                   | 26(81.3)                 | 28(87.5)  | 0.491 | 28(87.5)                 | 27(84.4)  | 0.719 | 30(93.8)                            | 28(87.5)  | 0.386 |
| 31-35 | 6                    | 5(83.3)                  | 6(100.0)  | 0.296 | 6(100.0)                 | 5(83.3)   | 0.296 | 6(100.0)                            | 6(100.0)  | N/A   |
| >35   | 7                    | 4(57.1)                  | 6(85.7)   | 0.237 | 7(100.0)                 | 7(100.0)  | N/A   | 7(100.0)                            | 7(100.0)  | N/A   |

IgM and/or IgG: at least a positive result detected by IgM and IgG ELISA.

Days: Days post-disease onset.

N/A: Not available.

394 Table 2. Summary results for IgM and IgG detection in the 214 serum samples from  
395 patients with COVID-19.

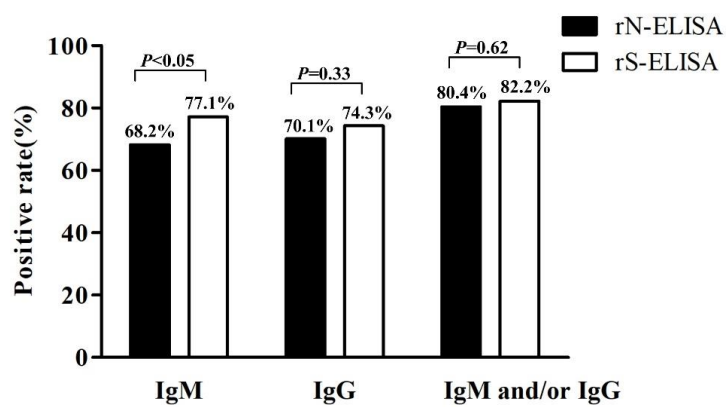
| No. (%)        | positive both rN-<br>and rS- based<br>ELISA | positive only<br>by rN-based<br>ELISA | positive only<br>by rS-based<br>ELISA | negative both<br>rN- and rS-based<br>ELISA |
|----------------|---------------------------------------------|---------------------------------------|---------------------------------------|--------------------------------------------|
| IgM            | 137(64.0)                                   | 9(4.2)                                | 28(13.1)                              | 40(18.7)                                   |
| IgG            | 137(64.0)                                   | 13(6.1)                               | 22(10.3)                              | 42(19.6)                                   |
| IgM and/or IgG | 162(75.7)                                   | 10(4.7)                               | 14(6.5)                               | 28(13.1)                                   |

396

397 IgM and/or IgG: at least a positive result detected by IgM and IgG ELISA.

398

399 **Figures**  
400

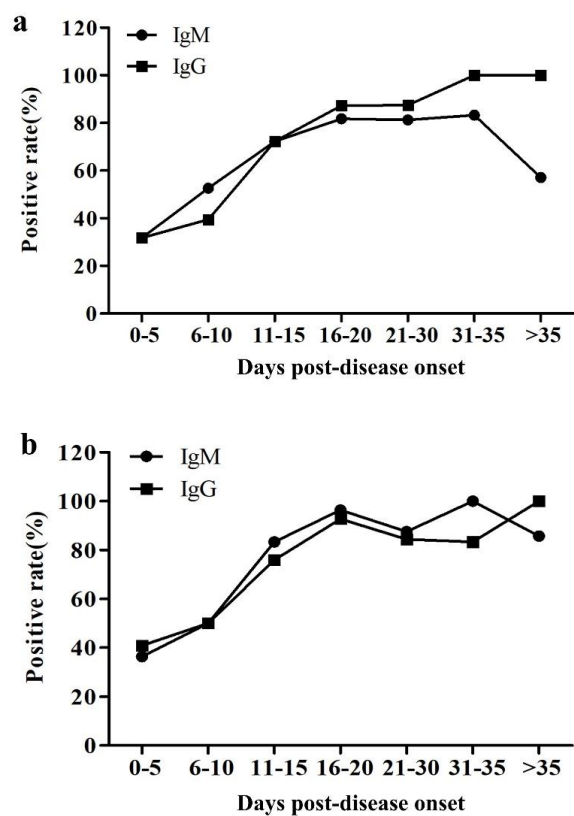


401

402 **Figure 1**

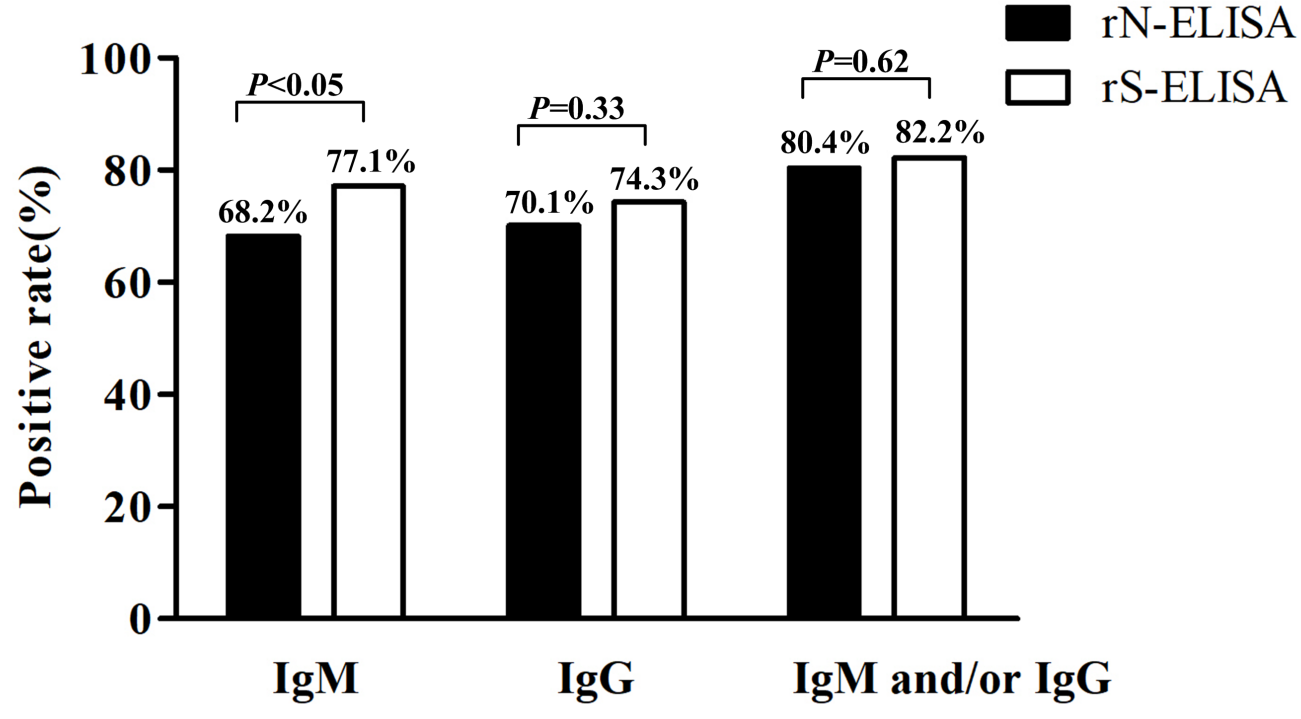
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405

406 **Figure 2**



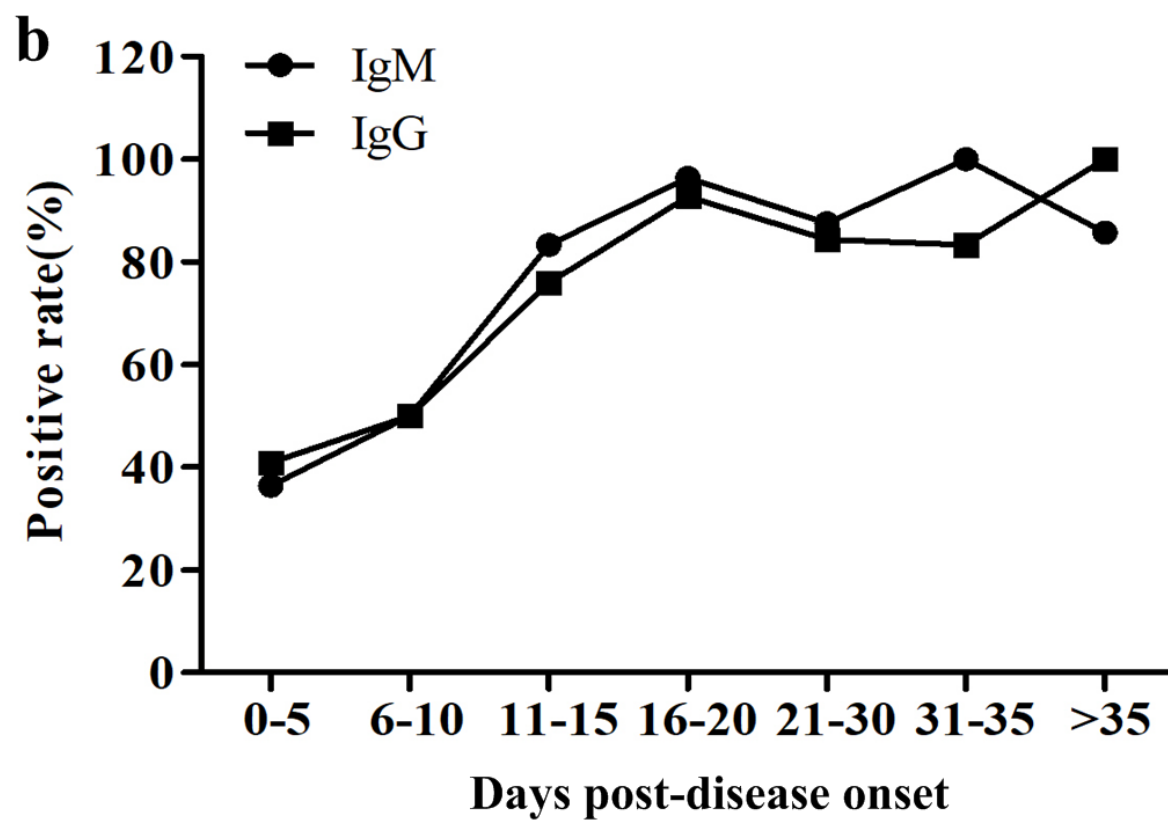
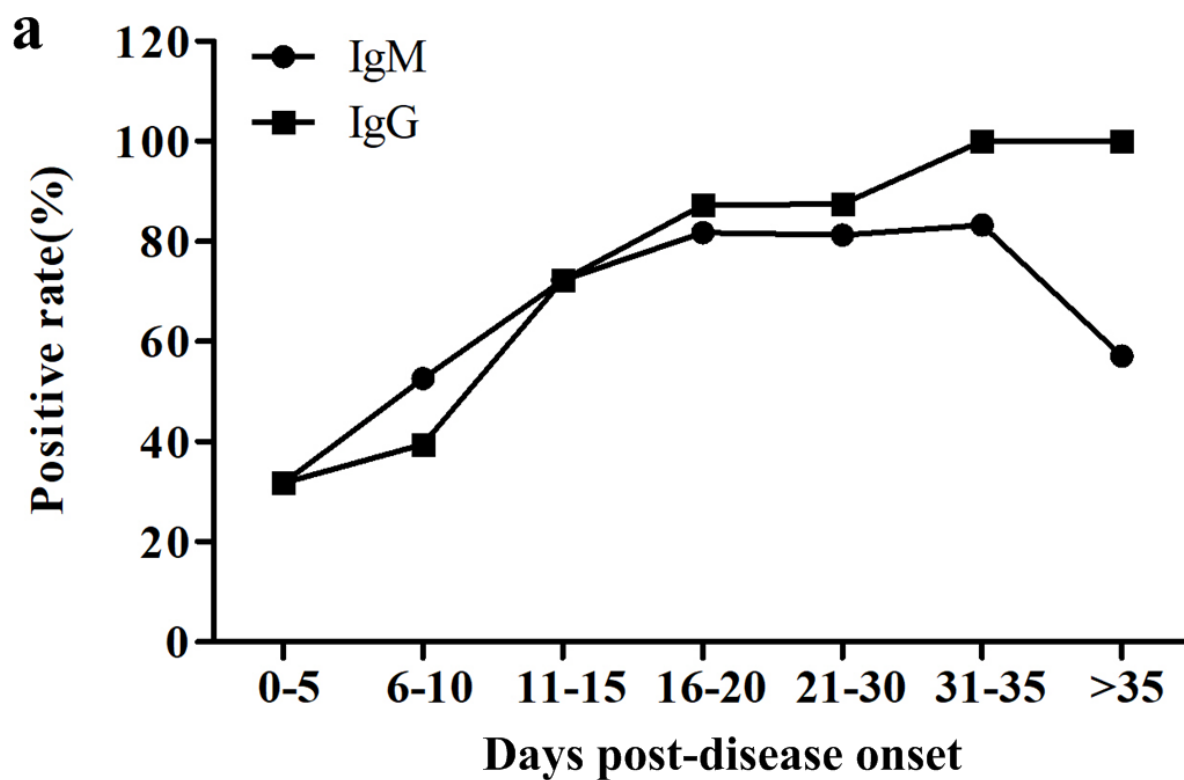


Table 1. Positive rate of rN-based and rS-based ELISA for detection of IgM and IgG in serum samples of patients at different stages after disease onset.

| Days  | No. of<br>serum<br>samples | No. (%) positive for IgM |           |       | No. (%) positive for IgG |           |       | No. (%) positive for<br>IgM and/or IgG |           |       |
|-------|----------------------------|--------------------------|-----------|-------|--------------------------|-----------|-------|----------------------------------------|-----------|-------|
|       |                            | rN-based                 | rS-based  | P     | rN-based                 | rS-based  | P     | rN-based                               | rS-based  | P     |
| Total | 214                        | 146(68.2)                | 165(77.1) | 0.039 | 150(70.1)                | 159(74.3) | 0.332 | 172(80.4)                              | 176(82.2) | 0.620 |
| 0-5   | 22                         | 7(31.8)                  | 8(36.4)   | 0.750 | 7(31.8)                  | 9(40.9)   | 0.531 | 9(40.9)                                | 10(45.5)  | 0.761 |
| 6-10  | 38                         | 20(52.6)                 | 19(50.0)  | 0.818 | 15(39.5)                 | 19(50.0)  | 0.356 | 20(52.6)                               | 23(60.5)  | 0.488 |
| 11-15 | 54                         | 39(72.2)                 | 45(83.3)  | 0.165 | 39(72.2)                 | 41(75.9)  | 0.661 | 48(88.9)                               | 49(90.7)  | 0.750 |
| 16-20 | 55                         | 45(81.8)                 | 53(96.4)  | 0.014 | 48(87.3)                 | 51(92.7)  | 0.340 | 52(94.5)                               | 53(96.4)  | 0.647 |
| 21-30 | 32                         | 26(81.3)                 | 28(87.5)  | 0.491 | 28(87.5)                 | 27(84.4)  | 0.719 | 30(93.8)                               | 28(87.5)  | 0.391 |
| 31-35 | 6                          | 5(83.3)                  | 6(100.0)  | 0.296 | 6(100.0)                 | 5(83.3)   | 0.296 | 6(100.0)                               | 6(100.0)  | N/A   |
| >35   | 7                          | 4(57.1)                  | 6(85.7)   | 0.237 | 7(100.0)                 | 7(100.0)  | N/A   | 7(100.0)                               | 7(100.0)  | N/A   |

IgM and/or IgG: at least a positive result detected by IgM and IgG ELISA.

Days: Days post-disease onset.

N/A: Not available.

Table 2. Summary results for IgM and IgG detection in the 214 serum samples from patients with COVID-19.

| No. (%)        | positive both rN-<br>and rS- based<br>ELISA | positive only<br>by rN-based<br>ELISA | positive only<br>by rS-based<br>ELISA | negative both<br>rN- and rS-based<br>ELISA |
|----------------|---------------------------------------------|---------------------------------------|---------------------------------------|--------------------------------------------|
| IgM            | 137(64.0)                                   | 9(4.2)                                | 28(13.1)                              | 40(18.7)                                   |
| IgG            | 137(64.0)                                   | 13(6.1)                               | 22(10.3)                              | 42(19.6)                                   |
| IgM and/or IgG | 162(75.7)                                   | 10(4.7)                               | 14(6.5)                               | 28(13.1)                                   |

IgM and/or IgG: at least a positive result detected by IgM and IgG ELISA.