# Detection of serum IgM and IgG for COVID-19 diagnosis

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Dear Editor,

Infection with the novel coronavirus (SARS-CoV-2, which is the virus responsible for the coronavirus disease 2019 (COVID-19)) was first reported in Wuhan, China on December 31, 2019. The outbreak of COVID-19 remains ongoing and was linked to more than 80,000 infected patients and more than 3,000 deaths in China as of March 7, 2020 (Holshue et al., 2020).

Currently, the real-time RT-PCR assay is the gold-standard methodto diagnose SARS-CoV-2. However, false-negative cases have been reported due to problems with sample collection and transportation, RNA extraction, enzyme inhibitors, and the RT-PCR method (Yang et al., 2020; Lu et al., 2020). By contrast, conventional serological assays, such as the enzyme-linked immunoassay (ELISA) for specific IgM and IgG antibodies, have a high-throughput advantage, and they avoid false -negative cases that occur with the RT-PCR method (Xiao et al., 2020).

To assay the concentration of antibodies (IgM and IgG)

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responsible for the coronavirus spike (S) and nucleocapsid (N) proteins in the serum, we developed ELISA and chemiluminescence methods for serum samples to detect IgM and IgG antibodies responsible forS and N proteins, respectively. The N gene of the SARS-CoV-2 was cloned into a pET28a vector, and the S gene was cloned into a pMFcIg vector. The proteins were expressed in *E. coli* and FreeStyle 293-F cells and purified by affinity chromatography. Both immunological methods were compared to the nucleic acid detection assay. Sample size calculation was based on the desired sensitivity and specificity (Negida et al., 2019) and the operator received curve (ROC) was constructed with SPSS version 25.0.

The N proteins appeared as an obvious band at approximately 50 kD, which is consistent with their theoretical molecular weight of 49.1 kD (Figure S1A and S1C). The the RBD-mFc band was consistent with its estimated molecular weight of approximately 60 kD (Figure S1B). Because there were several glycosylation sites, with most located in the S1 subunit of the S protein of the coronavirus, the RBD-mFc band was larger than the theoretical molecular weight of the RBD of the S protein (51 kD). This change in the molecular weight of S protein of SARS-Co was also shown in a previous study (Yuan et al., 2017). The $A_{450}$ boosted with the increase in the concentration of RBD-mFc expressed by our method described above in the interaction assay of the RBD-mFc with human ACE2 (Figure S1D). In our study, the median age was 48 years (ranging from 18 years to 82 years), and 34.0% of the patients were male. To investigate the efficacy of the ELISA method, samples from 47 COVID-19 participants who were nucleic acid-positive (Table S1) and 300 healthy controls were analyzed. The 47 positive serum samples produced a median $A_{450}$ of 1.078 for rN-based IgG, which was much higher than it was in all healthy controls (median $A_{450}$ of 0.036) ($t$=42.20, $P$<0.0001) (Figure 1A). The mean $A_{450}$ value (rS-based IgG) for the serum samples obtained from the COVID-19 patients was 0.405 (range 0.032 to 1.029), and the normal controls had a median $A_{450}$ value of 0.079 ($t$=17.83, $P$<0.0001) (Figure 1B). These results suggest that the ELISA was able to detect the COVID-19 IgG antibody in both rN-based IgG and rS-based IgG because all the nucleic acid-positive results detection had higher values than that in the normal controls. However, the range of $A_{450}$ value (from 0.032 to 0.402) in rS-based IgG testing results involve not only positive cases but also control subjects, which indicated that further testing and observation should be conducted to ensure the correct conclusion. For rN-based IgM, patients had a median value of 1.453 (range 0.381 to 2.217). The negative controls had lower $A_{450}$ values, with a median of 0.059 ($t$=49.36, $P$<0.0001) (Figure 1C). For rS-based IgM, the median of positive patients was 1.398 (ranging from 0.111 to 2.818), which was higher than that of the control group ($t$=31.58, $P$<0.0001) (Figure 1D). The

results showed that the AUC of rN-based IgG reached 0.999 ($P$<0.001, Figure 1G). The optimal cutoff value was 0.443, with sensitivity and specificity values of 97.9% and 99.7%, respectively. According to the cutoff value, there was only one patient who had a positive nucleotide result and a negative ELSIA result. The results showed that the AUC of rS-based IgG was 0.949 ($P$<0.001, Figure 1H). Its optimal cutoff value was 0.176, with sensitivity and specificity values of 95.7% and 85.7%, respectively. The sensitivity and specificity values of rN-based IgM were 97.9% and 99.7%, respectively, with an AUC of 0.994 ($P$<0.001, Figure 1I). For rS-based IgM, the optimal cutoff value was 0.167, with sensitivity and specificity values of 89.1% and 97.0%, respectively ($P$<0.001, Figure 1J).

In chemiluminescence, the positive patients showed much higher level in IgG, compared with normal controls ($t$=7.20, $P$<0.0001) (Figure 1E). In IgM, the statistical difference existed between the positive and negative ($t$=11.40, $P$<0.0001) (Figure 1F). An analysis of the ROC curve for IgG in chemiluminescence demonstrated an optimal cut off value of 0.199 ($P$<0.001, sensitivity of 95.6% and specificity of 96.6%). The AUC was 0.996 (Figure 1K). For IgM, the results showed that the AUC reached 0.993 ($P$<0.001, Figure 1L). The optimal cutoff value was 0.230, with sensitivity and specificity values of 97.7% and 95.2%, respectively. For patients who were confirmed positive by the nucleic acid assay but had a negative chemiluminescence result, we analyzed the corresponding $A_{450}$ by the ELISA method. Two confirmed patients had a negative rN-based IgG analysis result. Their average $A_{450}$ values were 0.651 and 1.122. One confirmed patient was negative in the rS-based IgM analysis, with an $A_{450}$ value of 1.647. The patients' $A_{450}$ values exceeded the corresponding median value of the normal controls. Therefore, the two methods could be simultaneously performed to monitor the titer of antibodies and provide more comprehensive information.

Although detection of the RNA by either PCR or sequencing is the gold standard for COVID-19 diagnosis, false negative results of the nucleotide assay have been reported due to problems related to sample collection and/or detection methods. In this study, both the ELISA and the chemiluminescence methods exhibited good consistency with nucleic acid detection. For the ELISA, rN-based IgG was seemingly more sensitive than rS-based IgG for discriminating between the patients and the controls. The differential sensitivity of the rS-based and rN-based IgG ELISA that was demonstrated in the present report may have originated from different restricted IgG-dominated antibody responses (Leung et al., 2004). In contrast, both rN-based IgM and rS-based IgM were able to discriminate the positive patients from the controls, so it may be a good tool to use with the nucleic acid test. If a patient has a high $A_{450}$ value for IgM but has anegative nucleic acid test result, they should be followed and
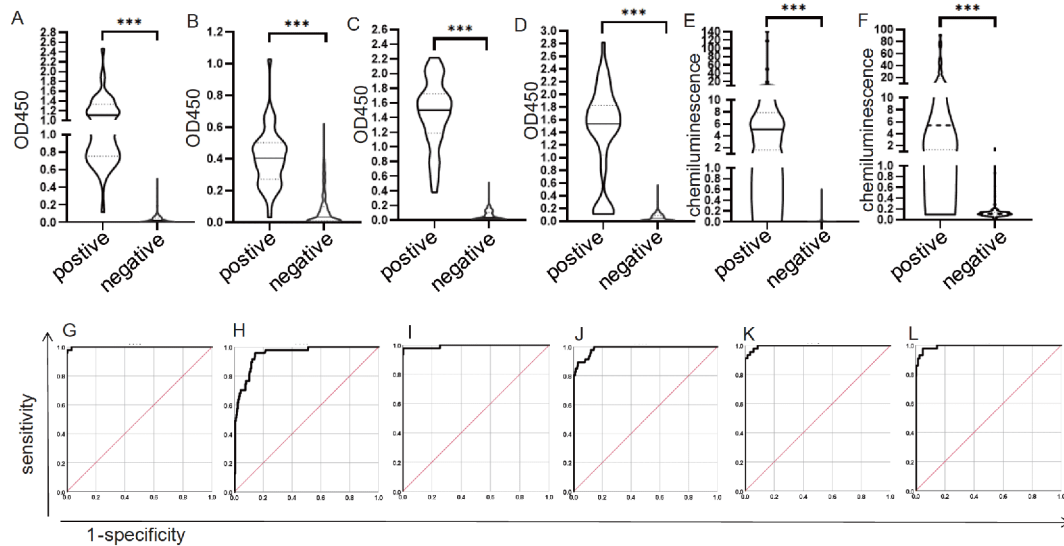
**Figure 1** (Color online) Results and ROC curve of antibodies for SARS-CoV-2 detected by ELISA and chemiluminescence. A, $A_{450}$ values of the two groups (positive and negative controls) tested by rN-based IgG. B, $A_{450}$ values analyzed by rS-based IgG in different groups. C, $A_{450}$ values in two groups from rN-based IgM testing. D, For rS-based IgM analysis, different groups showed differing $A_{450}$ values. E, For IgG analysis in chemiluminescence, different groups showed differing values. F, For IgM analysis in chemiluminescence, different groups showed differing values. G, ROC curve for rN-based IgG. H, ROC curve analyzed by rS-based IgG. I, ROC curve from rN-based IgM testing. J, ROC curve for rS-based IgM analysis. K, The ROC curve for IgG testing in chemiluminescence. L, The ROC curve analyzed by IgM testing in chemiluminescence. ***, $P < 0.0001$.

re-tested for the virus by PCR. False-negative results from PCR may be misleading. The rS-based IgM ELISA was reported to be more sensitive than the rN-based IgM ELISA because the S1 protein is a transmembrane protein, which may easily stimulate the body to produce the IgM antibody, especially during early infection (Woo et al., 2005). The limitation of serum IgM and IgG for COVID-19 diagnosis may be that the time span after disease onset could affect the $A$ value, which may reduce the sensitivities of the assays. If blood samples were collected druing the early stage of the infection, they may produce false negative results. Other molecules including interferon, rheumatoid factor, non-specific IgM and so on, might cause false positive. Moreover, since the identity of the N protein of SARS-CoV-2 and SARS-Co reached up to 91.2%, there is probably to a cross-reaction between the N protein of SARS-CoV-2 and antibodies against other human coronaviruses. It is better to verify the detection results with the combination of assay against both the N protein and S proteins.

In conclusion, our results showed that ELISA and chemiluminescence methods to detect IgG and IgM antibodies by the recombinant N and S proteins of SARS-CoV-2 were more consistent with the nucleic acid detection assay. They not only eliminated a possible cross reaction with other coronaviruses but also avoided potential risks in the process of inactivation. The two methods are simple and cheap and require only a small amount of serum, suggesting that they could be a good prospect for wide application in seroepidemiologic monitoring and may play a valuable role in practical applications for the diagnosis and epidemic control

of COVID-19 in the future.

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## SUPPORTING INFORMATION

**Figure S1**    SDS-PAGE analysis and Western blot of the N protein, the RBD of the S protein, and the binding curve of the RBD-mFc to human ACE2.

**Table S1**    Demography and clinical classification of COVID-19 patients

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