Diagnostic indexes of a rapid immunoglobulin G/immunoglobulin M combined antibody test for severe acute respiratory syndrome coronavirus 2

Ying Liu[1], Yue-Ping Liu[2], Bo Diao[2], Jin-Ya Ding[1], Meng-Xue Yuan[2], Fei-Fei Ren[1], Yue Wang[1], Qian-Chuan Huang[1]

[1]Department of Laboratory Medicine, General Hospital of the Central Theater Command, Wuhan, Hubei 430015, China;
[2]Department of Medical Laboratory Center, General Hospital of the Central Theater Command, Wuhan, Hubei 430015, China.

Detection of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a supplemental option for reverse transcription polymerase chain reaction (RT-PCR) detection, regardless of whether it is immunoglobulin M (IgM), immunoglobulin G (IgG) or combined detection. However, there are inadequate data on the sensitivity and specificity of IgM/IgG detection, which is of paramount importance for the decision of whether, when, and how to use IgM/IgG antibody detection. The aim of this study was to evaluate the sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of IgM/IgG antibody detection, especially the anti-interference ability and kappa coefficient compared with the SARS-CoV-2 RT-PCR, which was defined as a standard diagnosis in this study.

A total of 179 participants who visited or were admitted to General Hospital of the Central Theatre Command between January 1, 2020 and March 12, 2020 were enrolled in this retrospective observational study. Approximately 5 mL fasting blood samples were drawn from all the enrolled participants for SARS-CoV-2 IgG/IgM antibodies detection (Innovita Biological Technology Co., Ltd, Tangshan, Hebei, China). The nasal or pharyngeal swab specimens for all the 179 participants were also collected for SARS-CoV-2 RT-PCR (DAAN Gene Co., Ltd, Guangzhou, Guangdong, China). A confirmed COVID-19 case was defined as a patient who has clinical background, including the acute respiratory infection syndromes and/or abnormalities in chest computed tomography images, and detectable SARS-CoV-2 RNA in respiratory sample since illness onset for at least one time.

This study was approved by the Ethics Commission of General Hospital of the Central Theatre Command (No. [2020]017-1) and was exempted from the need for informed consent from patients. The selected 179 cases were divided into two groups, namely the confirmed COVID-19 group and non-confirmed COVID-19 group, which consisted of 90 cases and 89 cases, respectively. The median age of patients in the confirmed and non-confirmed group were 76 years and 56 years, respectively. The confirmed group consisted of 46 mild/common cases and 44 severe/critical cases according to the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 5),[1] while the non-confirmed group comprised five clinically confirmed cases (suspected cases who had typical pneumonia imaging features but did not have the positive RT-PCR results), 20 suspected cases (these 20 patients were not finally diagnosed as COVID-19 until their discharge) and 64 cases with other diseases, including ten cases of Sjogren syndrome, eight cases of diabetes, six cases of systemic lupus erythematosus, five cases of rheumatoid arthritis, two cases of dermatomyositis, two cases of connective tissue disease, one case of scleroderma, and 30 cases of common injuries with no underlying diseases.

Of the 90 samples of confirmed cases, 77 were tested positive according to the SARS-CoV-2 IgG/IgM test kit, yielding a sensitivity of 85.6% (77/90). In addition, of the 89 samples of non-confirmed cases, eight samples were tested positive, resulting in a specificity of 91.0% (81/89). The PPV, NPV, and accuracy of this test kit were 90.6% (77/85), 86.2% (81/94), and 88.3% (158/179), respectively. Our study also calculated the kappa coefficient between the SARS-CoV-2 IgG/IgM antibody test kit and the standard diagnosis, which yielded a result of 0.75

Correspondence to: Dr. Qian-Chuan Huang, Department of Laboratory Medicine, No. 627 Wuluo Road, Wuhan, Hubei 430015, China
E-Mail: hqcwuhan@126.com

Table 1: Accuracy of IgG/IgM detection in different subgroups of confirmed and non-confirmed COVID-19 patients.

| | Confirmed cases (n = 90) | | Non-confirmed cases (n = 89) | | | |
| | | | | | Other diseases | |
Items	Mild/common cases	Severe/critical cases	Clinically confirmed cases	Suspected cases	Autoimmune diseases	Common injuries
n	46	44	5	20	34	30
IgG and IgM-positive, n	11	21	1	4	0	0
IgG-positive, n	22	21	1	1	0	0
IgM-positive, n	1	1	0	1	0	0
Accuracy	73.9%	97.7%	60.0%	70.0%	100.0%	100.0%

IgG: Immunoglobulin G; IgM: Immunoglobulin M; COVID-19: Coronavirus disease 2019.

($P < 0.001$), which further indicated the moderate agreement between the two methods.

The confirmed group was further divided into two subgroups, the mild/common and severe/critical subgroups, while the non-confirmed group was further divided into three subgroups, the clinically confirmed, suspected case, and other disease subgroups. The accuracies of IgG/IgM detection in different subgroups ranged from 60% to 100%, which were detailed in Table 1.

IgM antibody could be detected in patient blood 3 to 6 days after SARS-CoV infection, while IgG antibody could be detected 8 days after infection.[2] SARS-CoV-2 belongs to the same large family of viruses as that of the virus which caused SARS. Hence, it is very important to calculate the diagnostic indexes of this test kit in different groups stratified by the time from illness onset to sample collection. A total of 115 inpatients that included 90 confirmed cases, five clinically confirmed cases, and 20 suspected cases were divided into three groups according to the time from illness onset to sample collection: the "0 to 7 days" group (25 cases), "8 to 15 days" group (eight cases) and "≥16 days" group (82 cases). The test kit showed the worst performance in the "0 to 7 days" group, yielding a sensitivity of 18.8% (3/16), a specificity of 77.8% (7/9) and an accuracy of 40.0% (10/25). This test kit performed the best in the "≥16 days" group, yielding a sensitivity of 100.0% (68/68) and an accuracy of 93.9% (77/82), though with a relatively low specificity (64.3%, 9/14). In the "8 to 15 days" group, the diagnostic indexes of this test kit were 100.0% (6/6) for sensitivity, 50.0% (1/2) for specificity, and 87.5% (7/8) for accuracy.

Because the symptoms of COVID-19 are not unique but similar to those of other diseases, testing is the only way to determine whether someone is infected with SARS-CoV-2.[3] Mass testing is therefore of paramount importance to curb the SARS-CoV-2 epidemic. Viral nucleic acid RT-PCR is not suitable for large-scale screening owing to its inherent properties. In contrast, the qualitative detection of SARS-CoV-2 IgG/IgM antibodies in human serum with a test that is configured like a home pregnancy test has the ability to be used for mass testing. Positive IgM test means that it is likely that the individual became infected with SARS-CoV-

2 recently or is in the early stage of infection. If only IgG is positive, then it is probable that the person had an infection sometime in the past or is in the late stage of virus infection. Hence, to be applicable for different stages of COVID-19, combined detection of IgG and IgM antibodies is recommended.

The diagnostic indexes, including sensitivity, specificity, PPV, NPV, and accuracy, were evaluated to determine the diagnostic usefulness. Our results revealed that the abovementioned five indexes were 85.6%, 91.0%, 90.6%, 86.2%, and 88.3%, respectively, which was in agreement with a recently published study.[4] Based on the definition of sensitivity, we can suggest that the manufacturer try to improve the detection sensitivity of the IgG/IgM test kit. The lower the sensitivity is, the more false-negative results occur. False-positive cases can be further confirmed by other detection methods; however, false-negative cases would be likely to result in the infection of people with whom the individual makes contact.

Any person who obtains a positive IgG/IgM test result may want to know what the chance is that he or she actually has the disease. Our study showed that the PPV of the IgG/IgM test kit was 90.6%, which indicated that the proportion of people with a positive test result who actually had the disease was 90.6%. On the other hand, the NPV of this test kit was just 86.2%%, which demonstrated that the proportion of those with a negative result who do not have the disease was 86.2%. This result strongly suggests that negative IgG/IgM test results cannot exclude virus infection, and repeated examination after approximately 1 week is strongly suggested. When interpreting PPV and NPV results, one thing should keep in mind that PPV and NPV differed according to different target population.

The accuracy of the test kit in five clinically confirmed cases showed that two out of the five cases were tested positive, while the RT-PCR results were negative. SARS-CoV-2 infection starts in the lung and not in the upper respiratory tract; hence, the sampling process has a large effect on the final RT-PCR results,[5] which might partially explain the high false-negative rate. However, this should not have any effects on the SARS-CoV-2 IgG/IgM test kit because only venous blood is needed for this test. The IgG/IgM test kit

can likely remedy the issue with false negatives that is inherent in the use of respiratory swab samples and can serve as a complementary option for RT-PCR.

Our study also recorded the time from illness onset to sample collection for each patient. From the results, we can see that only three out of the 16 cases with positive PCR results in the "0 to 7 days" group were tested positive according to the SARS-CoV-2 IgG/IgM test kit, generating a sensitivity of just 18.8% (3/16). The time from illness onset to sample collection for the 16 patients in the "0 to 7 days" group was between 1 and 2 days. Patients in this group may be at the initial stage or "window period" of SARS-CoV-2 infection, and the concentration of antibodies was too low to be detected. In the "8 to 15 days" subgroup and "≥16 days" subgroup, the sensitivity of the IgG/IgM test kit increased to 100%, whereas the specificity in these two groups was relatively low, 50.0% and 64.3%, respectively. This was very difficult to explain, because we were unsure whether the low specificity was caused by the false negative results of RT-PCR, considering the high false-negative rate of RT-PCR, or by the false positive results of the IgG/IgM test kit itself or the small sample size. The enrollment of 34 autoimmune disease patients was necessary to determine the anti-interference ability of the IgG/IgM test kit. Our results showed that the IgG/IgM test kit performed well in these patients.

However, some limitations should also be noted. First, due to the limited time, diagnostic indexes were only evaluated in serum but not in other types of blood samples, such as fingertip blood and plasma. Second, the small sample size should also be taken into consideration. Third, further demonstration of the grouping time points in larger populations will be more sufficient and can provide more insights into the seroconversion.[6]

Above all, the sensitivity and specificity of this ease-of-use IgG/IgM combined test kit were good, and the kit has a short turnaround time and no specific requirements for additional equipment or skilled technicians, all of which can collectively contribute to its usefulness for mass testing. At the current stage, it cannot take the place of SARS-CoV-2 nucleic acid RT-PCR but can serve as a complementary option for RT-PCR.

Conflicts of interest

None.

References

1. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 5). Beijing: National Health Commission & National Administration of Traditional Chinese Medicine; 2020. Available from: http://www.gov.cn/zhengce/zhengceku/2020-02/05/content_5474791.htm. [Accessed February 5, 2020].
2. Lee HK, Lee BH, Seok SH, Baek MW, Lee HY, Kim DJ, et al. Production of specific antibodies against SARS-coronavirus nucleocapsid protein without cross reactivity with human coronaviruses 229E and OC43. J Vet Sci 2010;11:165–167. doi: 10.4142/jvs.2010.11.2.165.
3. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061–1069. doi: 10.1001/jama.2020.1585.
4. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol 2020;92:1518–1524. doi: 10.1002/jmv.25727.
5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506. doi: 10.1016/S0140-6736(20)30183-5.
6. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26:845–848. doi: 10.1038/s41591-020-0897-1.