

## Serological test is an efficient supplement for detecting RNA to confirm SARS-CoV-2 infection

Ningshao Xia<sup>1</sup>, Guiqiang Wang<sup>2</sup>, Wenfeng Gong<sup>3</sup>

<sup>1</sup>School of Public Health, Xiamen University; <sup>2</sup>Peking University First Hospital; <sup>3</sup>Bill & Melinda Gates Foundation

Corresponding authors: Ningshao Xia, email: [nsxia@xmu.edu.cn](mailto:nsxia@xmu.edu.cn); Guiqiang Wang, email: [john131212@sina.com](mailto:john131212@sina.com); Wenfeng Gong, email: [Wenfeng.Gong@gatesfoundation.org](mailto:Wenfeng.Gong@gatesfoundation.org)

### Abstract

To date, viral RNA detection is almost the only way to confirm SARS-CoV-2 infection in practice. However, various reasons can cause low sensitivity for RNA detection, and this poses a serious challenge to disease control. We tested the performance of detecting total antibody (Ab) and IgM levels in serum by the methods of chemiluminescence, enzyme-linked immunosorbent assay (ELISA), and colloidal gold detection. The data showed that the sensitivity and specificity for detecting total Ab and IgM levels were high by all three methods, and the sensitivity was higher for detecting total Ab than for detecting IgM. Evidence from studies has shown that viral RNA testing combined with serological testing could increase the diagnostic sensitivity while maintaining a high specificity. Specific serology tests for SARS-CoV-2 have great value for clinical practice and public health.

**Keywords:** SARS-CoV-2; diagnosis; antibody; serology; screening

On Apr 4, 2020, the World Health Organization (WHO) declared that more than 200 countries, territories and areas reported more than a million cases of COVID-19, and more than 56,000 COVID-19-related deaths.<sup>1</sup> Given the rapid spread of COVID-19, WHO has raised the risk level of COVID-19 from "high" to "very high" on February 28, 2020, and called on all countries to give the highest priority to the prevention and control of COVID-19 epidemics.<sup>2</sup> Currently, viral RNA detection by quantitative real-time polymerase chain reaction (qRT-PCR) and/or sequencing is almost the only way to confirm the diagnosis of SARS-CoV-2 infection in practice. However, the reported positive rate of qRT-PCR results in COVID-19 patients was not high, and patients often need to be sampled several times and at multiple sites before a final diagnosis. Some highly suspected patients who were strongly epidemiologically linked to SARS-CoV-2 exposure with typical lung radiological findings retained negative qRT-PCR results for viral RNA from their upper respiratory tract samples. In addition, RNA detection requires high-quality swab specimens. Consequently, these issues pose a serious challenge to disease control and preventive quarantine strategies.

Patients with asymptomatic infection and those who are within the latent period of the

disease cannot be observed clinically and will become a major threat for the prevention and control of COVID-19. Guan et al. analyzed the clinical characteristics of 1,099 patients with laboratory-confirmed SARS-CoV-2 results from 552 hospitals in 30 provinces, autonomous regions, and municipalities in China and found that radiographic or CT abnormalities were not present in 157 of 877 patients (17.9%) with nonsevere disease, and only 87.7% and 67.8% of patients experienced typical symptoms of fever and cough, respectively, during hospitalization.<sup>3</sup> Another study suggested that among 72,314 patients, 889 patients (1%) diagnosed by positive viral nucleic acid test results were asymptomatic.<sup>4</sup> Data from Wölfel et al implies that for COVID-19 patients, the several days before the onset of clinical symptoms might be the most highly infectious.<sup>5</sup> Therefore, a convenient, high-throughput and rapid test is urgently needed to widely screen a large number of patients with mild symptoms and asymptomatic carriers for the purposes of prompt quarantine and timely treatment.

To date, several antibody testing reagents have been approved by the National Medical Products Administration (NMPA) of China to assist the diagnosis of SARS-CoV-2 infection, and these include kits for testing total Ab, IgM or IgG by the methods of enzyme-linked immunosorbent assay (ELISA), chemiluminescence or colloidal gold detection. In the diagnosis and treatment scheme for coronavirus disease 2019 (the seventh edition) from China, which was released on March 3, 2020, the detection of specific antibodies was included as one element of etiological evidence to make the diagnosis.<sup>5</sup>

In our systematic evaluation of confirmed COVID-19 patients, the chemiluminescence method, ELISA, and the colloidal gold method were used for the detection of total Ab and IgM against SARS-CoV-2. The double-antigen sandwich method was applied for total Ab detection, and the  $\mu$ -capture method was applied for IgM detection (Table 1). The sensitivity and specificity for detecting total Ab and IgM were high by all three methods, and the sensitivity was higher for detecting total Ab than for detecting IgM.

Table 1. The sensitivity and specificity of total antibody and IgM detected by different methods

Method	Antibodies	Sensitivity ( $n_1/N_1$ ) <sup>1</sup>	Specificity ( $n_2/N_2$ ) <sup>2</sup>
Fully automatic tubular chemiluminescence method	Total Ab	86.9% (192/221)	99.2% (358/361)
	IgM	74.3% (165/222)	99.2% (358/361)
ELISA	Total Ab	94.8% (289/305)	100% (333/333)
	IgM	86.9% (265/305)	100% (333/333)
Colloidal gold method	Total Ab	96.2% (127/132)	95.2% (199/209)
	IgM	87.9% (116/132)	98.1% (205/209)

Note. 1.  $n_1$ : the number of patients with a positive result;  $N_1$ : the number of tested patients; 2.  $n_2$ : the number of healthy persons with a negative result;  $N_2$ : the number of tested healthy persons.

In a study on the SARS-CoV-2 antibody response in COVID-19 patients by Shenzhen Third People's Hospital, Xiamen University<sup>6</sup>, 173 confirmed patients were tested for total antibodies (Ab), IgM and IgG against SARS-CoV-2. The results showed that the combined use of RNA and total Ab tests increased the diagnostic sensitivity to 99.4% compared with 67.1% for the RNA-only test. Even at the early stage of the disease course (within 7 days after seeking medical advice), during which the patients are highly infectious, the diagnostic sensitivity of the combination of RNA and total Ab was 78.7%, which was 12% higher than the RNA-only test (78.7% vs 66.7%). Compared with total Ab, the sensitivity was lower for a single indicator of IgM or IgG or by combining the 2 indicators of IgM and IgG. In addition, the specificity of the assays for total Ab, IgM and IgG was determined to be 99.1%, 98.6% and 99.0%, respectively, by testing samples collected from healthy individuals before the outbreak of SARS-CoV-2. Thus, the combined use of RNA and total Ab tests can improve diagnostic sensitivity while maintaining high specificity, and this is thought to have great value for clinical practice and public health.

According to a report in Science magazine on February 27, 2020, investigators in Singapore first applied Ab testing in tracking COVID-19 contact.<sup>7</sup> After Abs were detected in an asymptomatic contact with a negative nucleic acid test result, the source of infection was successfully traced, and a connection between two clustered cases was established. This breakthrough discovery also confirmed that serological testing is of great significance for tracking the source with inapparent infections.

The diagnosis and treatment scheme for coronavirus disease 2019 (the seventh edition) from China indicates that the seropositivity of IgM and IgG, seroconversion of IgG, or 4-fold or greater increase in the serum IgG titer in the recovery period compared with the acute period are pathogenic evidence for confirming cases<sup>5</sup>. In general, the IgM antibody is primarily induced and promptly reaches a peak after infection, but the persistence time is relatively short. While the IgG antibody is mainly produced in the middle and late stages of the infection, it can persist for a long time at a high level, even after recovery. Since SARS-CoV-2 is an emerging pathogen and started infecting humans in December 2019, which was less than half year ago, the prevalence of anti-SARS-CoV-2 antibodies in the general population is close to zero. In general, the double-antigen sandwich method used for total antibody detection has methodological advantages over  $\mu$ -capture (commonly used for IgM antibody detection) and indirect ELISA (commonly used for IgG antibody detection): 1) simultaneous detection of IgM, IgG, IgA and other types of antibodies; 2) detection requires that the two Fab fragments of the antibody molecule bind to the coating and labeled antigens, and this characteristic significantly reduces the nonspecific binding caused by the heterogeneous proteins in the coating and labeled antigens, thus ensuring a higher specificity; and 3) due to the very limited interference of heterologous proteins in the antigen, increasing the concentration of the coating and labeled antigens can further improve the sensitivity of the detection.

Zhao et al. performed antibody testing on a series of 535 plasma samples from 173

confirmed patients at different time points after the onset of COVID-19.<sup>8</sup> Based on the methodological advantages, the average seroconversion time of the total Ab was 1 and 3 days earlier than that of IgM and IgG, respectively, and the signal-to-noise ratio of the positive response of the total Ab was also higher. The above suggests that the total Ab could increase the diagnostic sensitivity while maintaining the specificity and may be a better serological diagnostic indicator for early diagnosis. The study from Li et al. also reported that the sensitivity of combining IgM with IgG was higher than that of utilizing IgM or IgG alone, while the specificity remained similar.<sup>9</sup>

Overall, Ab testing by ELISA and chemiluminescence methods have the characteristics of convenient sampling, high throughput, and high efficiency and can be quantitative or semiquantitative, and the method of colloidal gold is good for real-time and point-of-care testing. The above Ab testing methods should be selected as an effective supplement for detecting RNA under different circumstances to improve the diagnostic sensitivity and specificity. The applications of Ab testing can be summarized as follows:

#### **Screening and Management of Clinical Patients and Close Contacts:** 1 ) For

suspected patients and clinically diagnosed patients who have negative RNA tests, COVID-19 diagnosis can be made with positive Ab test results; 2) with a positive Ab result, an asymptomatic close contact should be deemed as being a current carrier or previously infected. For a close contact, if RNA testing results of the upper respiratory tract specimens remain negative during the 14 days of quarantine, a positive Ab test result implies more days of quarantined observation, and further collection and nucleic acid testing for lower respiratory tract and stool specimens should be considered; and 3) Ab testing could be used to quantitatively measure antibody levels in the plasma of recovery patients, considering that plasma with a high-titer antibody may be helpful for the treatment of severe COVID-19 patients.

**Screening of Key Populations:** A combination of RNA and Ab testing could be applied to screen travelers from countries or regions with a high incidence of COVID-19, participants of important conferences or events, and populations returning to work or school from high incidence areas. For people with a negative RNA result and a positive Ab result, quarantine and health monitoring may also be conducted to timely identify potential cases and decrease the risk of transmission.

**Epidemiology Research:** 1) By combining the serological test with the nucleic acid test and clinical symptoms to analyze the full spectrum of SARS-CoV-2 infection, the distribution of inapparent, atypical, mild symptom, and severe symptom infections and the contribution of each kind of infection to the transmission of the virus and disease can be revealed; 2) by conducting a population-based serological survey, the actual prevalence and pathogenicity of SARS-CoV-2 infection in different regions and populations, including populations of different ages, occupations or with different underlying diseases, can be understood, thus suggesting appropriate disease control

interventions; 3) by collecting a series of samples from suspected and confirmed cases and testing for serological, nucleic acid and other laboratory indicators, their dynamics can be analyzed, thus improving COVID-19 diagnosis and treatment strategies; 4) by analyzing the spectra of antibody epitopes in convalescent COVID-19 patients, scientific guidance can be provided for the design and evaluation of vaccines and therapeutic antibodies in the future; and 5) by supporting the search for potential animal hosts of SARS-CoV-2, the total Ab detected by the double-antigen sandwich method is not restricted to specific species.

**Competing Interest Statement**

The authors have declared no competing interests.

**Funding**

This study is supported by a grant from the Bill & Melinda Gates Foundation.

## References

1. Coronavirus disease 2019 (COVID-19) situation report-75. In: World Health Organization; 2020: [https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200404-sitrep-75-covid-19.pdf?sfvrsn=99251b2b\\_2](https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200404-sitrep-75-covid-19.pdf?sfvrsn=99251b2b_2). Accessed 2020/04/05.
2. Coronavirus disease 2019 (COVID-19) situation report-39. In: World Health Organization; 2020: [https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200228-sitrep-39-covid-19.pdf?sfvrsn=5bbf3e7d\\_2](https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200228-sitrep-39-covid-19.pdf?sfvrsn=5bbf3e7d_2). Accessed 2020/04/05.
3. Guan W-j, Ni Z-y, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine*. 2020.
4. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA*. 2020.
5. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020.
6. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019 [J]. *Clin Infect Dis*, 2020 (in press). Doi: 10.1093/cid/ciaa344/5812996.
7. Normile D. Singapore claims first use of antibody test to track coronavirus infections. *Scinece*. 2020/02/27, 2020.
8. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. 2020.
9. Hui L, Yongyin L, Zhigao Z, et al. Establishment and clinical performance evaluation of 2019 novel coronavirus antibody colloidal gold detection method. *Chinese journal of infectious diseases*. 2020;00:E017-E017.