



Antibody Testing for SARS-CoV-2: Role in Management of the Disease

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Summary

1. Detection of viral RNA by PCR has become a mainstay of disease detection. However, this technique has several limitations including the requirement for technical expertise, the occurrence of false negative results and an inability to detect individuals who may be immune.
2. Antibody testing has a role to play in supplementing PCR in diagnosis, screening of contacts and possibly in the determination of population immunity. Sensitivity varies with the stage of infection; it is low in the first week and then rises. Antibodies are highly specific.
3. Combined PCR and antibody testing may be the optimal strategy for initial diagnosis given the dynamics of the infection and host response and the limitations of PCR testing.
4. Significant questions remain with regard to the performance of individual test methods and the degree of immunity associated with the antibody response.

Purpose

The purpose of this report is to outline the role of testing for antibodies against SARS-CoV-2 in the management of COVID-19.

Rationale and background

In addition to clinical judgment, supplemented if possible by chest CT, two testing strategies have emerged to aid in diagnosis of the disease. A third approach is to combine PCR and antibody testing.

a) PCR testing

PCR detects viral RNA and has become a mainstay of diagnosis. However, this method has several limitations. Viral RNA is usually obtained from a nasopharyngeal swab, but the virus is predominantly a lower respiratory pathogen. It may not be present in sufficient quantity in the upper respiratory tract leading to false negative results. The virus may be present in low titer in the incubation period. Technically the test requires expertise and sophisticated laboratory equipment which may be in short supply especially in the developing world. False negative tests may occur (Comparative accuracy of oropharyngeal and nasopharyngeal swabs for diagnosis of COVID-19. Centre for Evidence-based medicine.

<https://www.cebm.net/covid-19/comparative-accuracy-of-oropharyngeal-and-nasopharyngeal-swabs-for-diagnosis-of-covid-19/>. March 26, 2020). Furthermore, the PCR is not useful in identifying patients who are post infection and may be immune.

b) Antibody testing

A humoral response with production of antibodies to pathogens is part of the normal host response. SARS-CoV-2 exhibits a number of antigenic sites. Most attention has focused on the spike and nucleocapsid proteins. IgM and IgG antibodies to these sites can be detected by a number of methods including ELISA and immunochromatographic testing.

Several groups have published their results of the time profile of antibody detection. In the initial report from Wuhan the serology of 34 patients was reported (Ai Tang Xiao , Chun Gao, Sheng Zhang, Profile of Specific Antibodies to SARS-CoV-2: The First Report, Journal of Infection (2020). Accepted March 11 2020. doi: <https://doi.org/10.1016/j.jinf.2020.03.012>). IgM and IgG were analyzed by chemiluminescent immunoassay. With the exception of two patients tested in the first week after infection, all included patients had IgM and IgG tests two weeks after symptom onset. IgM and IgG both rose towards week 2 with IgM. A subsequent report (Wanbing Liu, Lei Liu, Guomei Kou, Yaqiong Zheng, Yinjuan Ding et al. JCM Accepted Manuscript Posted Online 30 March 2020

J. Clin. Microbiol. doi:10.1128/JCM.00461-20) of 214 patients, confirmed with the disease by positive PCR from pharyngeal swabs, outlined the results of ELISA testing for IgM and IgG antibodies to recombinant nucleocapsid (rN) and spike (rS) proteins. The positivity rates for rN based IgM and/or IgG was 80.4% and for rS 82.2%. The positivity rate for IgM dropped after 35 days and for both assays was less than 60% in the first 10 days after infection. It appears, therefore, that infection with the virus is associated with a detectable immune response. IgM levels decrease after about a month indicating that it may be a useful marker of more recent infection. IgG may be a reliable marker of past infection as levels persist for longer.

c) Combined PCR and antibody testing.

In another report from Shenzhen, China (Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Juanjuan Zhao, Quan Yuan, Haiyan Wang et al. Clin Infect Dis. 2020 Mar 28. pii: ciaa344. doi: 10.1093/cid/ciaa344.) the results of total antibodies, IgM and IgG were reported in 173 patients. The presence of antibodies was <40% in the first week after onset of symptoms and rapidly increased to 100%. In contrast, RNA detectability decreased from 66.7% in samples collected before day 7 to 45.5% between days 15-39. The authors concluded that combining RNA and antibody testing significantly improved virus diagnosis. Specificity was estimated at over 99% by testing serum from healthy individuals obtained before the outbreak of SARS-CoV-2.

The value of combining antibody and RNA PCR testing was confirmed in a further report which stated that the detection rate increased to 98.6% for combined testing compared to 51.9% for a single PCR. (Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease. COVID-19. Li Guo, Lili Ren, Siyuan Yang et al Clin Infect Dis. 2020 Mar 21. pii: ciaa310. doi: 10.1093/cid/ciaa310. [Epub ahead of print]). The authors also commented on the cross reactivity of the antibodies with SARS-CoV -1 but not other coronaviruses.

Utility of antibody testing

1. Diagnosis of infection. Antibody testing is useful in the diagnosis of acute infection especially as time increases from onset. It may add to the diagnostic rate in patients with negative PCR.
2. Screening of contacts and reduction of quarantine. Currently contacts of patients diagnosed with the disease are asked to remain in quarantine. This is a particular problem for health care providers many of whom may be unnecessarily removed

from work due to a shortage of testing. Antibody testing adds a diagnostic test which may reduce time lost.

3. Possible identification of immune populations. PCR becomes negative as the virus is lost from an individual. A negative PCR does not indicate if the person has been infected and may be immune. Antibody testing may allow mass testing of the population and identification of individuals who have recovered from infection. This clearly will become important as restrictions are removed and populations begin to resume normal life. Antibody testing will also allow ongoing surveillance of the population to determine population immune status and will allow detection of both potentially immune and susceptible.

Quality control

There are number of concerns with regard to antibody testing. The performance characteristics of tests may vary. It is important to know the sensitivity, specificity, and reproducibility of each test method. Most importantly, we do not yet know if the presence of antibodies is associated with immunity from reinfection and the duration of any immunity conferred.

Current Practice in China

This section summarizes our experience in Xijing and three other institutions in China. The antibody assay we are using in the Xijing Hospital of Fourth Military Medical University is a product of Beijing Wantai Biological Pharmacy, which has been approved in early March by National Medical Products Administration of China and received a European Conformity (CE) certificate. The product covers 28 provinces with over 200,000 tests done in China, and 23 countries with 35,000 tests done over the world.

This is a chemiluminescent immunoassay based on double-antigen sandwich principle for specific antibody capture in the serum or plasma. The antigens used in the system are recombinant proteins containing the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2. Total antibodies are detected by applying 2 RBD proteins as the immobilized and HRP-conjugated antigen. The IgM μ chain capture method (IgM-ELISA) is used to detect the IgM antibodies. The assay takes 29 minutes to give the first result and the speed of detection can reach 200 tubes per hour if using automatic device of Caris200, Huawei Medical LLC, Beijing, China.

The sensitivity and specificity of the kit is 94.8% and 99.7% respectively according to a previous study of 386 patients with confirmed COVID-19 and 1859 healthy controls.

Relationship of PCR and antibody positivity following infection.

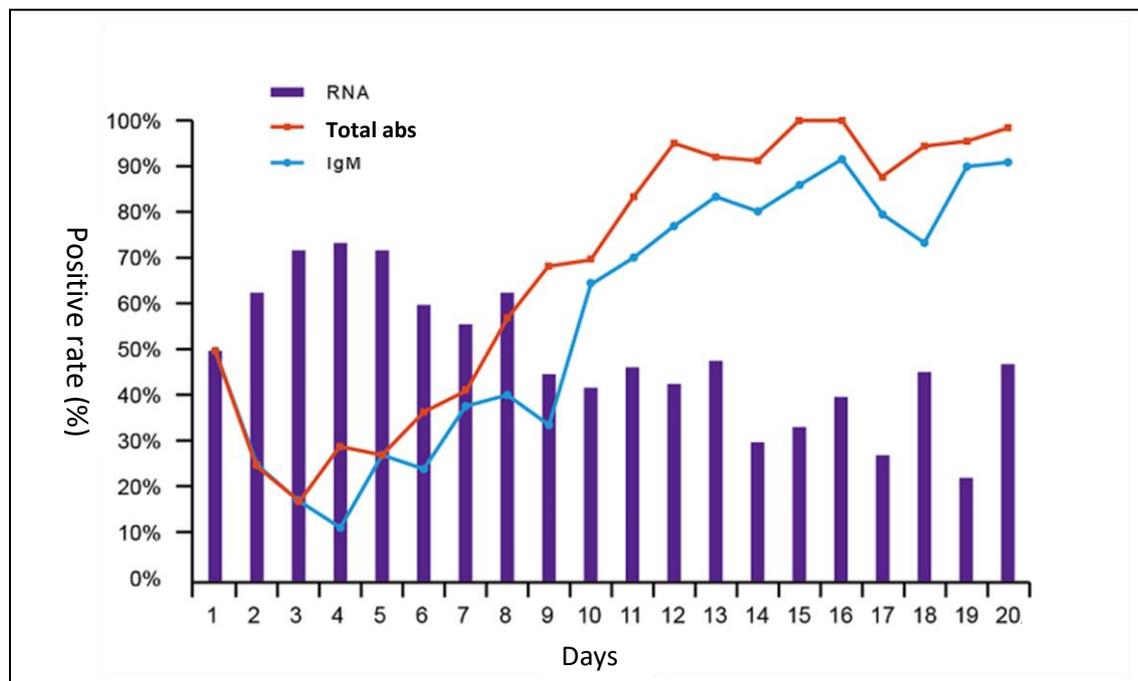


Figure 1. Positivity of PCR RNA obtained by oropharyngeal and/or nasal swabs, Total antibodies and IgM antibodies by days from onset of symptoms in SARS-CoV-2 in patients with COVID-19 (Data on file of Beijing Wantai Biological Pharmacy, submitted to National Medical Products Administration of China). N=380

The IgM positivity (not quantification) curve does exactly track the total antibody curve in the initial 20 days. We know from other published study that the IgG response is different, about 19% in the first 7 days since onset and rise to 80% by 30 days (Clin Infect Dis. 2020 Mar 28. pii: ciaa344. doi: 10.1093/cid/ciaa344.). We assume the COVID patients had not previously been exposed to SARS-CoV-2 as this virus is new to humans. One possible explanation could be the cross-reactivity to other coronavirus although the assay claimed a 99% specificity with over 1800 healthy subjects as controls. The fall to 50% in antibody response from first day of onset to days 3-4 post onset may not mean anything as there were too few chances to have patients' blood taken for antibody analysis at day 1 of onset.

Of concern, 40% of patients had detectable RNA in swabs 20 days post onset. We do not know how this relates to the risk of them transmitting infection but, if verified, this finding has implications with regard to the duration of isolation of affected patients.

In practice, the kit demonstrates a detection rate of 30%-40% for patients with COVID-19 within the initial 7 days, 70% at 8-10 days, and 100% through 12 days. In contrast to the antibody detection, the PCR assay in the same cohort of COVID-19 patients showed 60%-70% of positivity within the initial 7 days and dropped to 40%-50% since then

(Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, Wang X, Yuan J, Li T, Li J, Qian S, Hong C, Wang F, Liu Y, Wang Z, He Q, Li Z, He B, Zhang T, Fu Y, Ge S, Liu L, Zhang J, Xia N, Zhang Z. Clin Infect Dis. 2020 Mar 28. pii: ciaa344. doi: 10.1093/cid/ciaa344.).

Role of antibody testing in PCR negative patients

| Days of onset | RNA negative | Ab positive in RNA negative |
|----------------------|---------------------|--|
| ≤3 days | 7 | 2 |
| 4-7 days | 28 | 15 |
| 8-14 days | 57 | 56 |
| ≥15 days | 30 | 30 |

Table 1. Days since onset of symptoms in PCR negative patients and antibody status at different time points of COVID-19 in clinically confirmed patients (Data on file of Beijing Wantai Biological Pharmacy, submitted to National Medical Products Administration of China)

As shown above, the antibody assay is complementary to the PCR assay to identify those false negative patients at early phase of infection. In the first eight days post infection antibodies added an approximately 50% diagnostic gain in PCR negative patients. After eight days, antibodies were detected in nearly 100% of PCR negative patients.

Approved indications of the antibody assay are:

1. Additional testing of suspect patients with negative nucleic acid test of SARS-CoV-2.
2. Antibody titering for patients recovered from COVID-19.

Potential Indications.

1. Initial diagnosis of COVID-19. First test for suspect or clinically diagnosed patients with COVID-19. If the total antibody is positive, no matter what the result of the PCR assay, the patient is managed as confirmed case. If the total antibody is negative, nucleic acid assay is performed and antibody is rechecked in a week's time.
2. As a screening test for the asymptomatic patients with close contact.
3. As a check on the immune status of a confirmed patient positive with a positive PCR. A positive antibody result may indicate that an appropriate immune response has been activated. According to recently published studies, a high titer of antibodies may be associated with increased severity of patients with COVID-19, indicating strong immune response in the severe patients.

4. As a test for asymptomatic subjects at the end of 14 days quarantine. If the total antibody is positive, a nucleic acid assay is recommended, and the close contacts should be traced and watched.

We have started the SARS-CoV-2 antibody assay in my hospital two weeks ago mainly for screening for COVID-19 in every patient prior to his admission to hospital. We also perform this assay to screen patients before elective endoscopy. It becomes a useful complement to the PCR and reduces the heavy burden on the clinical lab doing PCR testing by doing 400-500 tests of SARS-CoV-2 antibody assay every day.

As of March 16, 2020, eight different kits from seven biopharma companies of SARS-CoV-2 antibody assay have been approved by the National Medical Products Administration of China. Among them, there is one kit for total antibody and IgM by chemiluminescence, 2 kits for IgG or IgM by magneticluminescence, 5 for total antibody or IgM/IgG by colloidal gold technique. They are used in many institutes or hospitals in China for research or clinical purpose. Comparative data on test characteristics and performance is awaited.