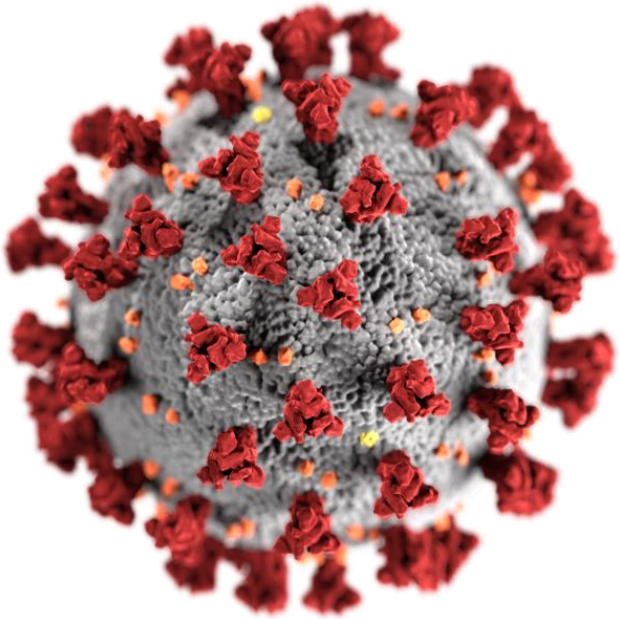

Severe Acute Respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody detection tests

Tech Note

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Summary

While nucleic acid detection of SARS-CoV-2 in respiratory samples remain the mainstay of diagnosis of COVID-19 infection, the rapidly growing pandemic has created a supply chain problem for the availability of various components of the performance of RT-PCR tests.

Additionally, RT-PCR tests are resource-intensive with high Turn around time. Anecdotal evidence also points to an extremely high false negative rate due to sample collection errors and also false positive results because of the inherent error-prone nature of the enzyme 'Reverse transcriptase' used to convert the viral RNA into cDNA that is amplified in RT-PCR tests.

On the other hand, well designed and evaluated antibody tests that detect Total antibodies to SARS-CoV-2 infection are more sensitive than RT-PCR (that detect the viral genome first) after 8 days post infection, when the virus gets eliminated, and therefore a combination of RT-PCR and Rapid Total Antibody test (RTAT) based on Double antigen sandwich assay can provide accurate diagnosis of COVID-19 patient for clinical diagnosis and epidemiological testing. A follow-up sample (longitudinal sample) should be re-tested in cases of indeterminate results by RT-PCR and total antibody test, if corresponding clinical symptoms persist. A sensitive and specific rapid test such as Rapid Total Antibody test (RTAT) based on Double antigen sandwich assay may also provide a basis for the identification, isolation and presumptive treatment of RT-PCR negative asymptomatic seroconverted individuals to limit the virus spread also. It may be remembered that RT-PCR can give false negative results and false serological tests are known to occur too, so a combination of RT-PCR in

follow up samples along with Rapid total antibody test (RTAT) is a prudent approach to detect the SARS-CoV-2 infection.

A diagnostic algorithm is also included in this tech note, portraying the use of Rapid Antibody tests for diagnosis and epidemiological use. The tech note is a summation of references collated directly from the referenced journals, in the bibliography section of this tech note.

Background

"In December 2019, a novel coronavirus causing severe acute respiratory symptoms emerged in Wuhan, China. The World Health Organization (WHO) termed the disease, coronavirus disease 2019 (COVID-19), and the causative virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Accurate diagnosis of COVID-19 is essential, not only to ensure appropriate patient care but also to facilitate identification of SARS-CoV-2 infected people, including asymptomatic carriers, who need to be isolated to limit the virus spread. The WHO recommends nucleic acid detection of SARS-CoV-2 in respiratory samples for the diagnosis of COVID-19. Unfortunately, in the face of the rapidly growing epidemics worldwide, an increased demand for diagnostic tests has led to a critical shortage in operational material for respiratory sample collection and within the molecular diagnostic workflow. This impedes rapid large scale testing, a necessity for controlling the epidemic. Moreover, the heterogeneity of respiratory sample material and anatomical location of sample collection, for example throat swab, saliva or endotracheal aspirate, affect the sensitivity of SARS-CoV-2 viral nucleic acid testing. Overall, there is an urgent need to identify alternative diagnostic means." [1]

"Antibody testing, either using enzyme-linked immunosorbent assay (ELISA) or point-of-care (POC) lateral flow immunoassays, may overcome some of these challenges. SARS-CoV-2-specific antibodies can be detected in the serum of **approximately 40% of COVID-19 patients as early as seven days after the onset of symptoms, with seroconversion rates rapidly increasing to >90% by day 14.**

In recent studies, antibody testing has been shown to be more sensitive than viral nucleic acid detection after approximately eight days of COVID-19 illness duration. While the combination of PCR and antibody tests is optimal for accurate diagnosis, **antibody detection will be particularly relevant for the later stages of infection where the virus has been eliminated**. In addition to the diagnostic value of antibody testing, it will identify individuals who developed immunity after infection that may protect against subsequent re-infection, as well as define and monitor the extent of virus spread and a population's herd immunity on a societal level." [1]

"Antibody testing may therefore be relevant in the following settings:

i) **diagnosis of patients who seek medical attention after more than seven days after the onset of symptoms;**
ii) **contact tracing;**
iii) **determining potential immunity and risk of infection; and**
iv) **sero-epidemiological studies to understand the extent of COVID-19 spread**." [1]

Sero-response in the SARS-CoV-2 infections (COVID-19)

In the classical infectious serology model, that is well understood through various "older infections" and "viral agents", it is generally understood that in the first phase of the infection, i.e. Day 0 of infection, free virus (antigen) may be found in the blood

stream, and as the immune response develops, it is the IgM class of antibodies that start to increase in concentration. After some delay, it is the IgG response to the viral agent that takes over to give an exponential peak in terms of concentration.

So, before seroconversion, it is the antigen that could be detected, during acute phase the IgM antibodies can be detected, and in the later phase, the IgG antibodies can be detected to provide indirect proof of exposure to and infection with the viral agent.

Of course, it is understood that host immuno-competence, the viral dose at the time of exposure also play an important role in the generation of the immune response. Hence, patient-to-patient variation is expected. However, broadly, the understanding is that, presence of IgM antibodies only, signify an acute phase of infection, whereas, detection of IgM and IgG classes both, may indicate an ongoing infection moving towards convalescence. Whereas, presence of only IgG class of antibodies indicates the recovery phase. It is also widely expected that, the IgG antibodies to the viral agent are protective in nature, preventing future re-infection from the same viral agent.

“In SARS-CoV-2 infections (COVID-19), in a large proportion of patients, the appearance **of IgG antibody precedes IgM**” [6].

In a significant proportion of patients, these antibodies appear almost simultaneously, and more importantly, **the IgA isotype appears first with very high titres**.” [5]

“In addition, we observed that IgA levels in serum correlate positively with COVID-19 severity.” [7]

Thus from the seroresponse to COVID-19 infections, it is clear that the detection of Total Antibodies (IgM + IgG + IgA) for screening of patients is of critical value as the test cannot afford to miss all the three isotypes, and therefore detection and differentiation of IgM and IgG isotypes only, has limited clinical value.

However, most rapid test manufacturing companies believe that the classical model of seroresponse holds true for detection of COVID-19 infections, and have therefore designed their products as the usual IgM/ IgG detection and differentiation tests.

Therefore the correct approach to detect all three isotypes is to design a Rapid Total antibody test (RTAT) based on a Double-Antigen sandwich assay where the SARS-CoV-2 specific antibodies are sandwiched and captured between the conjugated antigen and the antigen striped on the test-line. Since this system detects all three isotypes, a.k.a. Total Antibodies (IgM + IgG + IgA) the test is enabled to detect the Total antibodies 2 or 3 days earlier than the IgM or IgG based tests. This tests not only scores in early detection, but also scores in higher sensitivity and specificity.

Furthermore, some latest references also mention the relevance of IgA antibodies in the serodiagnosis of COVID-19 infections

“The concentrations varied widely among different patients. Median 75 concentration of IgA and IgM reached peaks at 16-20 days after illness onset at 8·84 μg/mL and 7·25 76 μg/mL, respectively, while median concentration of IgG peaked during 21-25 days after illness onset at 77 16·47 μg/mL. [7]

“The combination of IgA/IgG or IgA/IgM/IgG provides improved diagnostic reliability as compared to conventional IgM/IgG combinations. [7]

“Although IgM reached its peak at early stages, its detecting sensitivity is lower than that of IgA and IgG. Our data suggest that IgM has the lowest diagnostic power among the three types of antibodies for diagnosing SARS-CoV-2. Adding IgA into a diagnostic kit that contains IgG and IgM improves the serologic testing power at both early and late stages.” [7]

"Therefore, we highly recommend the use of RBD-specific IgA/IgG or IgA/IgM/IgG combinational serological test supplementing nucleic acid detection to provide a more accurate diagnosis of COVID-19." [7]

Antibody detection relative to the duration of illness

"To evaluate the sensitivities of the assays at different stages of COVID-19 disease, case sera were grouped according the duration of disease: early phase, 7 to 13 days after the onset of disease symptoms; middle phase, 14 to 20 days after the onset of disease symptoms; and late phase, ≥21 days after the onset of disease symptoms. The sensitivities of the assays ranged from 40 to 86% for the early phase samples, 67 to 100% for the middle phase samples, and 78 to 89% for the late phase." [1]

	Early Phase	Middle phase	Late phase
COVID -19 disease phases	7 to 13 days after symptoms	14 to 20 days after symptoms	≥ 21 days after symptoms
Sensitivities of assays	40 to 86%	67 to 100%	78 to 89%

"The clinical sensitivity of IgM for early diagnosis of COVID-19 is currently unclear. SARS-CoV-2-specific IgM does not consistently appear before its IgG counterpart, with some studies reporting detection of SARS-CoV-2 spike protein-specific IgG before IgM. While all the POC tests evaluated in this study are capable of detecting both SARS-CoV-2 IgM and IgG antibodies, **the majority detected both antibody types simultaneously, even in the early convalescent phase**, while some detected only IgG and others only IgM." [1]

There are studies which have shown that "Day 8 (after onset of symptoms) IgM antibodies appeared in 73.3%, IgG in 54.1 % and Total antibodies (IgA+IgM +IgG) in 89.6 % of the positive individuals, Similarly Day 15 after onset of symptoms, IgM antibodies appeared in 94.3%, IgG in 79.8 % and **Total antibodies (IgA+IgM +IgG) in 100 % of the positive individuals**." [3].

"The differences observed for the sensitivity and specificity of the SARS-CoV-2-specific total antibody testing and antibody type ELISAs correspond to previous reports. The Wantai Total Ab ELISA performed as reported by the manufacturer (94.5% sensitivity and 100% specificity) and a separate study (93.1% sensitivity) where the Total Ab ELISA, IgM ELISA and IgG ELISA produced by Beijing Wantai Biological Pharmacy Enterprise were compared. In the latter study, the IgM and IgG ELISAs had lower sensitivities (83% and 65%, respectively) compared to the Total Ab ELISA (93.1%)." [1]

"Since the appearance of antibodies is time dependent, diagnosis of COVID-19 by serological methods is limited to patients with a longer duration of illness. Within seven days of symptom onset or in the acute phase of disease, nucleic acid detection of SARS-CoV-2 in respiratory samples is superior to antibody detection for the diagnosis of COVID-19. However, after eight days of illness, the sensitivity of serological assays surpasses that of nucleic acid testing. Here we reported a 100% seropositivity in patients 10 days after the onset of symptoms. It is unclear whether the latter sensitivity can be extrapolated to mild COVID-19 cases, since the present study comprised severely ill adult COVID-19 patients only. However, the sensitivity of the assay may not necessarily be very affected, since reports show similar seroconversion rates for patients with mild and severe COVID-19 disease despite generally lower SARS-CoV-2 antibody titres in the former group. Conversely, studies on the dynamics and

detection of SARS-CoV-2 antibodies in children are lacking and requires urgent attention." [1]

It is also to be noted that rate of sero-conversion cannot be 'similar' in different samples (individuals). It is a variable component, specific to a given infected person and the stage of expression of the protein (antibody), based on the viral RNA genome translation, across the different classes of immunoglobulins (IgM, IgG, IgA).

RT-PCR and serodiagnostic tests have a complementary role

It is vital to understand that neither RT-PCR, nor a Rapid Total Antibody Test (RTAT) alone can give conclusive results to screening of patients for SARS-CoV-2 infections. There is a possibility that the RT-PCR test may show a negative result in a seroconverting patient (Rapid Total Antibody Test positive) and it may also be the case that a sample that shows a positive result with RT-PCR, is negative when tested with the Rapid Total Antibody Test (RTAT) as false serological tests are known to occur too.

"Many cases that were strongly epidemiologically linked to SARS-CoV-2 exposure and with typical lung radiological findings remained RNA negative in their upper respiratory tract samples. There are four potential reasons: 1) the viral loads in upper respiratory tract samples are much lower than that in lower respiratory tract samples in COVID-19 patients. 2) the releasing viral loads of patients in different stage of infection varies with a wide range. 3) the collection of high-quality swab specimen requires skillful health-workers; and 4) PCR reagents from different sources have high variance." [3]

"Table 3. Serological presence of antibodies against SARS-CoV-2 in patients with undetectable viral RNA at different time since onset of disease.

Days after Onset	No. of patients with undetectable RNA*	Detectable antibody in plasma, n (%)		
		Ab	IgM	IgG
1-3	7	2 (28.6)	2 (28.6)	2 (28.6)
4-7	28	15 (53.6)	12 (42.9)	8 (28.6)
8-14	57	56 (98.2)	45 (78.9)	40 (70.2)
15-39	30	30 (100)	28 (93.3)	22 (73.3)

* RNA was tested using throat/nasal swab sample." [3]

"Serological testing may be helpful for the diagnosis of suspected patients with negative RT–PCR results and for the identification of asymptomatic infections". [2]

"RT–PCR-based viral RNA detection is sensitive and can effectively confirm early SARS-CoV-2 infection. Our data indicate that virus-specific antibody detection for COVID-19 could be important as a complement to nucleic acid testing for the diagnosis of suspected cases with negative RT–PCR results and (2) in surveying for asymptomatic infection in close contacts. Confirming suspected COVID-19 cases as early as possible with the help of serological testing could reduce exposure risk during repeated sampling and save valuable RT–PCR tests. In our small-scale survey, seven cases with negative nucleic acid results and no symptoms, showed positive IgG and/or IgM. This highlights the importance of serological testing to achieve more accurate estimates of the extent of the COVID- 19 pandemic." [2]

Studies shows higher sensitivity when RT-PCR and Rapid Total Antibody Tests are used as companion in the diagnosis of individuals- Refer to **Table** below.

"Performance of different detections in samples at different time since onset of symptoms

	Day 1 -7	**Day 8-14**	**Day 15-39**
Viral RNA	66.7%	54.0%	45.5%
Total Antibodies (IgA, IgG, IgM)	38.3%	89.6%	100%
IgM antibodies	28.7%	73.3%	94.3%
IgG Antibodies	19.1%	54.1%	79.8%
RNA+ Total Antibodies	**78.7%**	**97.0%**	**100%**

"[3]

RT-PCR and Rapid Tests such as Rapid Total Antibody Tests (RTAT) are therefore **complementary** and **companion** tests and must be treated as such in order to get accurate results.

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