Serological Testing as Aid in Diagnosis and Management of Symptomatic and Asymptomatic COVID-19 Patients

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SARS CoV-2 is the virus responsible for COVID-19 disease. It is highly infectious and is spreading fast, currently at pandemic proportions, with more than 25 million people infected globally and over 850,000 deaths.[1] The infection can be asymptomatic or symptomatic with variable severity: patients could initially present without cough or radiologic abnormalities; display mild cough and fever; or develop a more severe form of the disease that leads to bilateral pneumonia and sometimes to death.[2] To overcome the challenges of these non-specific symptoms, in addition to the clinical picture and chest computer tomography (CT) scan images, laboratory parameters should be considered to inform the clinical decision. COVID-19 diagnosis relies heavily on laboratory tests, mainly on nucleic acid detection of the SARS CoV-2 virus in respiratory secretions of the infected patients, using a Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test.[3] In this article, we will discuss how:

Antibody detection could contribute significantly to increasing diagnosis accuracy of recent or prior COVID-19 infections in both symptomatic and asymptomatic patients, in addition to antigen (virus or specific viral proteins) detection.

WHAT DO SEROLOGICAL TESTS MEASURE AND WHEN?

Serological tests target the main viral antigens of SARS-CoV2: the spike and the nucleocapsid proteins.

A serological test measures the antibodies produced by the body in response to antigens. The immune system recognizes these antigens as foreign and in defense, it triggers seroconversion or production of antibodies, which become detectable some time post exposure. In the case of SARS-CoV-2, antibodies are produced mainly against the spike (S) protein and the nucleocapsid (N) protein:

- **The spike (S) protein** is essential for viral infection, and antibodies binding to specific epitopes on S protein have *a neutralizing role* for viral infection by blocking virus-receptor interaction .
- **The nucleocapsid (N) protein** has a role in viral replication but unlike S protein, has *no ability to elicit neutralizing antibodies*, however, it may induce specific antibody and cellular immune responses.[5,6,7]

The period between exposure to the virus and detectable antibody levels is variable and influences sensitivity of the serological tests. As the acute infection progresses, the sensitivity of the serological tests is expected to rise. During seroconversion, the antibodies levels rise, transitioning from undetectable in the early stages of infection, to detectable in the later stages. The time required for antibodies to become detectable varies with each patient and severity of disease; antibody responses may be more easily detectable in symptomatic severe cases (hospitalized patients) than in mild or asymptomatic infections.[8,9,10] These clinical factors influence the sensitivity of a serological test, which is expected to be lower in the early stages of infection (<7 days from onset of symptoms) than in later stages (>14 days).[8]

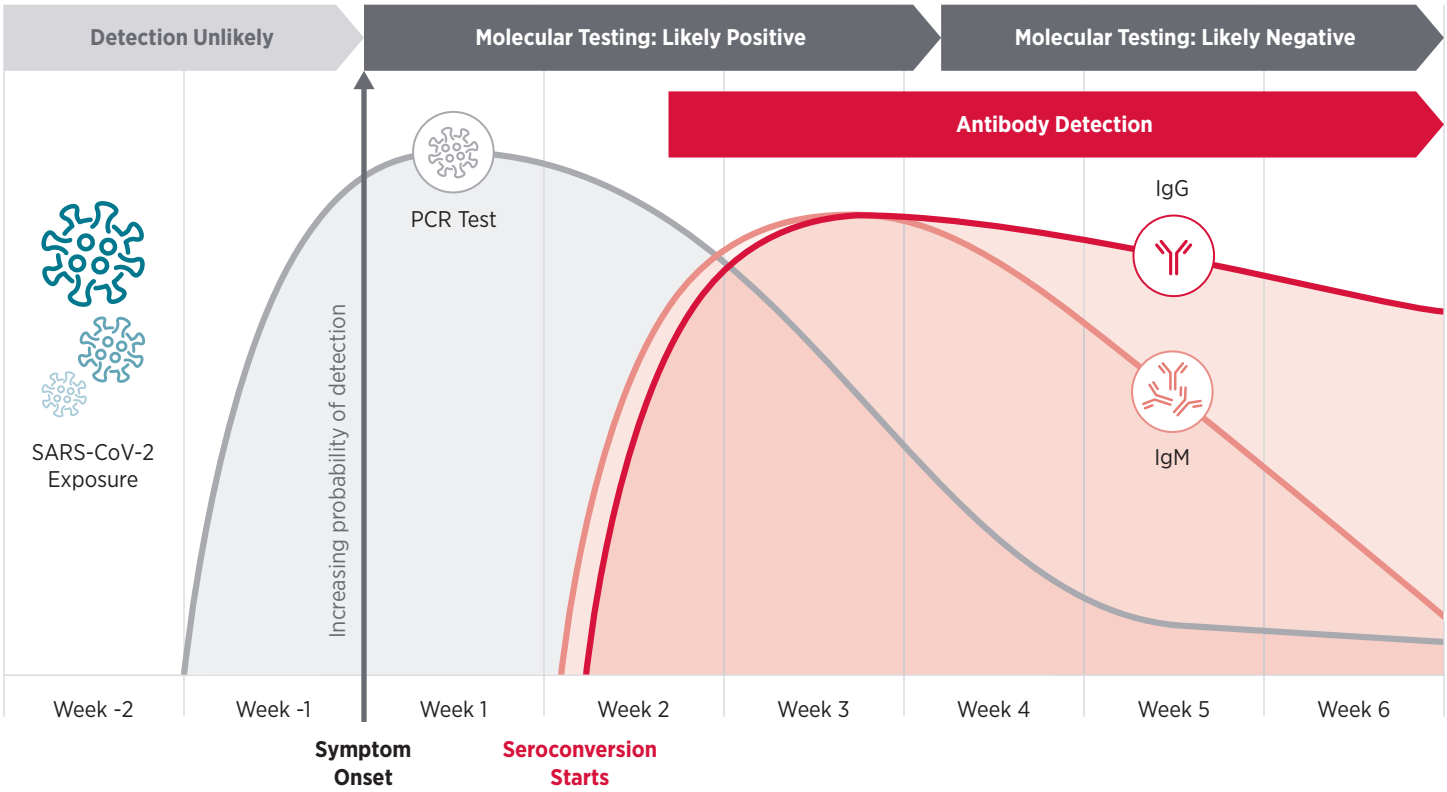
The main antibody isotypes involved in SARS-CoV-2 serological response are IgA, IgM, and IgG, which can be detected together in a Total antibody assay.

- **IgA** antibodies are involved in defense mechanisms against viruses, and infections with respiratory viruses can induce efficient IgA responses in secretions as well as in sera. IgA antibodies are valuable diagnostic markers: they show stronger and more persistent elevations than IgM, and they can be detected as early as the first week after onset in mild COVID-19 or later in severe disease, with a peak observed at 3 weeks. They bind multiple epitopes on the spike protein, and therefore may be crucial for neutralizing viral particles of SARS-CoV-2.[2,11,12]
- **IgM** antibodies emerge days/a week post-symptom onset as an early response to novel antigens and decrease significantly after 18 days.[12,13] Seroconversion of IgM is expected to occur earlier than IgG, however, in SARS-CoV-2 it can also occur at the same time, or even later.[13]
- **IgG** is characterized by a longer half-life and lower molecular weight than IgM, thus having the ability to provide long-lasting protection and effective tissue penetration. Although IgG seroconversion is expected to occur 2–3 weeks post-symptom onset, in SARS-CoV-2 infection it could also occur earlier, leading to significant overlap in IgG and IgM seroconversions as shown in Figure 1.[12,13,14]

The use of a total antibody test increases the sensitivity for COVID-19 detection due to the following factors:[11,12,13,14,15]

- The significant overlap between antibody isotopes
- The presence of the IgA antibodies in the early stages of infection
- The lack of chronological order of IgM and IgG antibody appearance in SARS-CoV-2

Figure 1: Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection



Adapted from Interpreting Diagnostic Tests for SARS-CoV-2, N Sethuraman, et al, *JAMA*, published online May 6, 2020.
Graph is illustrative and should not be used as a primary reference.

WHAT ARE THE CURRENT CHALLENGES WITH DIAGNOSING COVID-19?

RT-PCR sensitivity limitations could lead to a significant number of false negative results, repeat testing, and delayed patient quarantine management and treatment.

RT-PCR is considered the gold standard method for diagnosing COVID-19, since viral culture is not a feasible option for rapid diagnosis as it takes 3–5 days and requires special biosafety level 3 (BSL-3) containment. An RT-PCR test is highly specific (barring technical errors or sample contamination), however, the sensitivity could be significantly impacted in both symptomatic and asymptomatic patients due to low viral load, host immunity, nucleic acid isolation method, as well as the sample quality, source, and timing of swab collection in relation to the disease stage.[3,10,14,16] As seen in Figure 1, the sensitivity of the RT-PCR decreases in the late stages of the disease as the antibody levels increase.[14]

In a study of 205 patients with confirmed COVID-19 infection, RT-PCR positivity was as low as 63% in nasal swab specimens and 32% in pharyngeal swab specimens; bronchoalveolar lavage specimens had the highest sensitivity (93%), followed by sputum (72%).[14,17] Owing to the analytical performance of the RT-PCR test or to the clinical presentation, a study found that RT-PCR missed 15/51 patients with COVID-19 diagnosis; in this study, the sensitivity of the CT scan was higher than the RT-PCR (98% vs 71%).[18]

Patients with high suspicion of COVID-19 should therefore not be prematurely cleared from quarantine by one negative result of RT-PCR testing; in addition to the clinical picture, chest CT images and other laboratory parameters should be taken into account for diagnosis and further management.[3,19]

RT-PCR AND SEROLOGY TESTS TO DIAGNOSE COVID-19: WHAT DOES THE CLINICAL EVIDENCE SAY?

The combination of serology tests and RT-PCR, particularly for patients with high suspicion for COVID-19 but RT-PCR negative, increases the diagnostic accuracy of COVID-19 acute infection and mitigates RT-PCR false negative rates.

A study showed that combined sensitivity of a PCR test together with an IgM ELISA test was higher than the PCR test (98.6% vs 51.9%) alone. During the first 5.5 days, PCR had a higher positivity rate than the IgM ELISA test, whereas the IgM ELISA test had a higher positivity rate after day 5.5 of illness. The conclusion of the authors was that *"the humoral response to SARS-CoV-2 can aid in the diagnosis of COVID-19, including subclinical cases."* [14,16]

Combining RT-PCR and Total antibody tests significantly improved the sensitivity of diagnosis for COVID-19 patients, even in the early phase, 1–7 days post-symptom onset.

A second study showed that combining RT-PCR (RNA in Table 1) and Total antibody (Ab in Table 1) tests significantly improved the sensitivity of diagnosis for COVID-19 patients, even in the early phase, 1–7 days post-symptom onset (Table 1). In addition, the Total assay (Ab) sensitivity exceeded RT-PCR in the second week from symptom onset (89.6% compared to 54%) and at later stages (100% compared with 45%). Combined, the sensitivity reached 97% at 8–14 days post-symptom onset and 100% at 15–39 days post-symptom onset (Table 1).[20]

Table 1: Sensitivity of diagnosis for COVID-19 over time

		RNA		Ab		IgM		IgG		RNA + Ab	
Days After Onset	**n**	Sensitivity (%, 95% CI)	n+	Sensitivity (%, 95% CI)	n+	Sensitivity (%, 95% CI)	n+	Sensitivity (%, 95% CI)	n+	Sensitivity (%, 95% CI)	n+
Total	173	67.1% (59.4, 74.1)	112[a]	93.1% (88.2, 96.4)	161	82.7% (76.2, 88)	143	64.7% (57.1, 71.8)	112	99.4% (57.1, 71.8)	172
1–7	94	66.7% (55.7, 76.4)	58[a]	38.3% (28.5, 48.9)	36	28.7% (19.9, 39.0)	27	19.1% (11.8, 28.6)	18	78.7% (69.1, 86.5)	74
8–14	135	54.0% (44.8, 63.0)	67[a]	89.6% (83.2, 94.2)	121	73.3% (65.0, 80.6)	99	54.1% (45.3, 62.7)	73	97.0% (92.6, 99.2)	131
15–39	90	45.5% (32.0, 59.5)	25[a]	100.0% (96.0, 100.0)	90	94.3% (87.2, 98.1)	83[b]	79.8% (69.9, 87.6)	71[c]	100.0% (96.0, 100.0)	90

a. There were 7, 11, and 35 patients that had not performed RNA testing during the 1–7 onset day, 8–14 onset day, and 15–39 onset day, respectively.
b. Two patients missed IgM tests due to inadequate plasma samples.
c. One patient missed IgG tests due to inadequate plasma samples.

Adapted from Antibody Responses to SARS-CoV-2 in Patients of Novel Coronavirus Disease 2019, J Zhao, et al.

DO THE GUIDELINES INCLUDE RECOMMENDATIONS ON THE USE OF SEROLOGY TESTS WITH RT-PCR TESTS FOR DIAGNOSING COVID-19?

Serological tests, especially a Total antibody assay which has a higher sensitivity compared to an IgM or an IgG assay, could greatly enhance the sensitivity of the RT-PCR test for diagnosing recent infection starting from the second week post-symptom onset (Table 1).[20]

This is emphasized by the Centers for Disease Control (CDC), which states that:

"Serologic testing can be offered as a method to support diagnosis of acute COVID-19 illness for persons who present late."

"For persons who present 9–14 days after illness onset, serologic testing can be offered in addition to recommended viral direct detection methods such as polymerase chain reaction or antigen detection tests. During this time period, the sensitivity of nucleic acid detection is decreasing, and the sensitivity of serologic testing is increasing."[21]

In children presenting with late complications of COVID-19 illness, such as multisystem inflammatory syndrome, "serologic testing should be offered as a method to help support a diagnosis."[21] A study showed that the majority of patients with Kawasaki disease (70%) tested positive for SARS-CoV-2 infection by RT-PCR, antibody testing, or both; 58/186 were antibody positive and RT-PCR negative, with the antibody test providing the only link between the Kawasaki disease and SARS-CoV-2 infection.[22] The study incorporated serology testing as one of the inclusion criteria for case definition.

The antibody test provides the only link between the Kawasaki disease and SARS-CoV-2 infection.

WHAT ADDITIONAL CHALLENGES MAY SEROLOGICAL ANTIBODY TESTING INFORM? HOW CAN SEROLOGY TESTS HELP IDENTIFY INFECTIONS?

Serological testing can help identify asymptomatic infection in close contacts with RT-PCR negative results.

The asymptomatic infection poses a special challenge in the prevention of COVID-19, as an asymptomatic individual with infection will become a transmission source unless contained and quarantined. In a study surveying a cohort of 164 close contacts, the percentage of asymptomatic infection was as high as 6.1%. Symptoms screening and nucleic acid testing missed 4.3% (7/164) of patients with COVID-19 infection in this cohort (Figure 4).[13] Serology testing confirmed the presence of IgG and/or IgM antibodies in those 7 patients.[13]

Another study assessed IgA and IgG levels in 109 symptomatic/asymptomatic healthcare workers subjects exposed to COVID-19 patients. The asymptomatic/PCR negative group (17/109) contained very few S1-specific serum IgA-positive and no IgG-positive subjects, which underlines the advantage of a Total antibody assay versus an IgG in the population with RT-PCR negative results and high suspicion of COVID-19. In the symptomatic/PCR negative group, there were four out of 71 (6%) participants with positive IgA and IgG values, likely representing individuals with a mild SARS-CoV-2 infection, which speaks to the situation where a serology assay aids in the diagnosis of COVID-19 and could expedite isolation and further patient management. As expected, the acute symptomatic/PCR positive group contained more seropositive individuals, with 8 out of 21 (38%) subjects having positive IgA and IgG titers for S1 of SARS-CoV-2 at the time of sampling.[23] In this last group of patients, serology (Total antibody or IgG only) would not only confirm diagnosis but the high titers could indicate a more severe disease.

Serology has a role in diagnosing COVID-19 recent or prior infections.

The studies presented demonstrate the role of the serology testing (Total antibody or IgG assay) in enhancing RT-PCR sensitivity for COVID-19 diagnosis. They could be used:

1. As a reflex test after a negative RT-PCR result in patients with high suspicion of infection.[8]
2. As the first test in the algorithm, to expedite symptomatic patient triage and quarantine management in the hospital in case the RT-PCR test is not available.[8]

Cheng, et al, describe the advantages and limitations for these two approaches in the excerpt below from the table in their article, *Possible Use Cases for Antibody Detection Tests:*[8]

Table 2: Advantages, limitations, and considerations for antibody detection tests

Use Case Diagnosis	Advantages	Limitations	Considerations
Aid diagnosis of suspect cases, especially when PCR-negative but radiography or CT is suggestive	+ May improve overall sensitivity of diagnosis + Diagnosis of patients presenting late or for postinfectious syndromes (low viral load) + Diagnosis of patients when lower respiratory tract sampliing not available	- Unlikely to catch early stage infection (<7 d) - May not detect asymptomatic cases - Negative test cannot rule out infection - IgM appears early, but is less specific	! Total antibody may have best sensitivity ! Should be confirmed by PCR, where possible ! Rising titers and seroconversion can improve clinical sensitivity and specificity
Aid diagnosis of suspect cases when PCR is not available	+ May improve overall sensitivity of diagnosis + Diagnosis of patients presenting late or for postinfectious syndromes (low viral load) + Diagnosis of patients when lower respiratory tract sampliing not available + Could enable decentralized or community testing in settings where the availability of PCR testing is limited	- Unlikely to catch early stage infection (<7 d) - May not detect asymptomatic cases - Negative test cannot rule out infection - IgM appears early, but is less specific	! Total antibody may have best sensitivity ! Should be confirmed by PCR, where possible ! Rising titers and seroconversion can improve clinical sensitivity and specificity

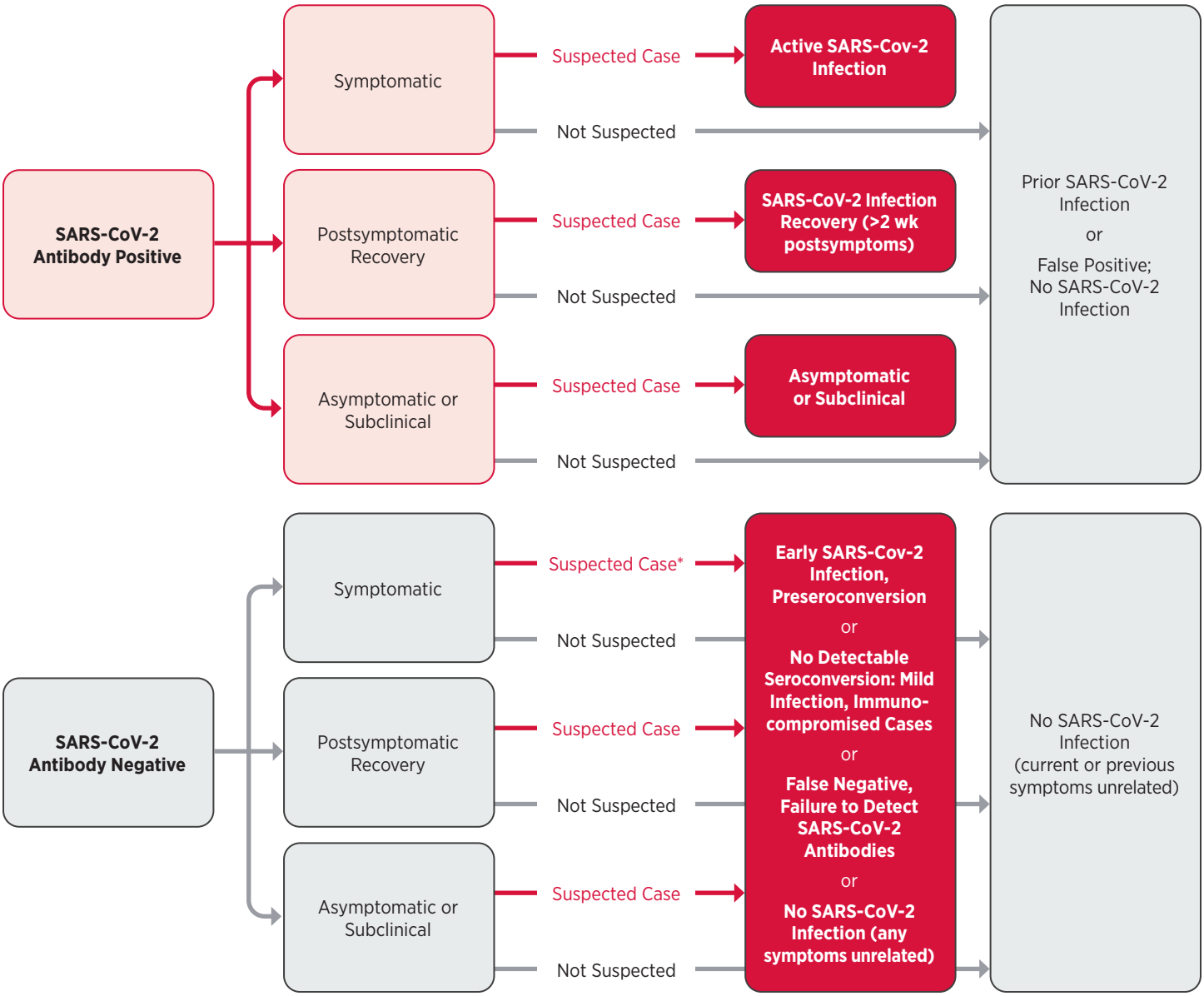
3. A combination of Total and IgG only assay by performing Total assay first for diagnosis sensitivity and reflex a reactive result to IgG only assay to detect specifically if the patient has IgG antibodies.
4. An indication of prior COVID-19 infection in patients with Kawasaki disease (Total antibody or IgG only).
5. As an add-on to the RT-PCR test request to aid in diagnosis in assessing disease severity.

Interpretation of the serology results.

In a review of serological tests recently published in the Annals of Internal Medicine, the authors suggest the following interpretation flows for an antibody positive or negative result.

The complex interpretation of an antibody positive or negative result is represented below and includes different scenarios.[8]

Figure 2: Most likely interpretations, according to clinical context, for antibody detection tests



* Includes high exposure, high risk, hot spots, and contact tracing.

Adapted from Serodiagnostics for Severe Acute Respiratory Syndrome–Related Coronavirus-2, M Cheng, et al, *Annals of Internal Medicine*, published online June 4, 2020. Chart is illustrative and should not be used as a primary reference.

In conclusion, it has been consistently shown that antibodies can be detected early in the course of illness and that serology tests have a significant role in COVID-19 diagnosis when used with additional laboratory tests and clinical investigations.

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