PERFORMANCE PAPER

Serology testing in patients with COVID-19 infection

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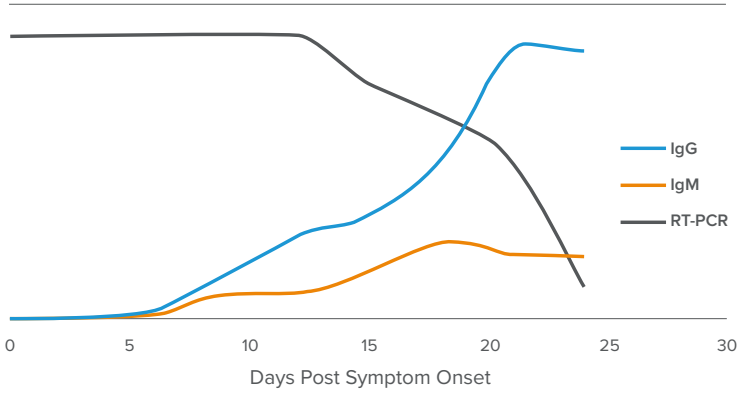
Introduction

RT-PCR for the SARS-CoV-2 virus is the primary diagnostic modality for COVID-19 infection. Patients are typically RT-PCR positive before the onset of symptoms, and may continue to shed virus from the nasopharynx for up to 21 days after symptom onset. However, specimen collection is critical to this test's sensitivity and several reports describe cases of likely false-negative RT-PCR results in patients with COVID-19 infection.[1]

In contrast, IgG and IgM antibodies to SARS-CoV-2 are typically detected within the first 7-10 days of symptom onset, although variability has been observed.[1-3] Some patients may mount an immunologic response within the first 3 days of symptoms, and asymptomatic patients and those with mild symptoms may develop low-level or undetectable antibody responses.[3] Data to date[2-3] suggest IgG and IgM become detectable in roughly the same timeframe (Figure 1).

Unlike RT-PCR, serology testing for IgG and IgM are less impacted by specimen collection technique as antibodies are homogeneously distributed in the blood.

Figure 1. COVID-19 Test Result Dynamics



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Interpretation of diagnostic test results for COVID-19 serology testing is best with individual IgM and IgG readouts, as is possible with the BioCheck SARS-CoV-2 IgG and IgM Combo Test. Clinical interpretation of IgM, IgG and RT-PCR results are shown in Table 1.

The role of serology tests in the management of COVID-19 continues to be defined. The American Clinical Laboratory Association (ACLA) has developed recommendations on the use of serology testing[4] for COVID-19.

- Serology tests should not be used to diagnose acutely ill patients.
- Serology tests can be useful for retrospective diagnosis (i.e., after 11 days of symptoms) when RT-PCR becomes negative.
- Serology tests may help identify healthcare and other essential workers who have been exposed to SARS-CoV-2. If IgG is positive and RT-PCR results are negative, it may be safe for the worker to return to work.

Table 1. *Possible Interpretation of SARS-CoV-2 serology and RT-PCR results*

RT-PCR	IgM	IgG	Interpretation(s)
+	-	-	Patient may be in asymptomatic / early stage of infection.
+	+	-	Patient may be in early stage of infection.
+	+	+	Patient in active stage of infection.
+	-	+	Patient may be in late stage of infection. Patient may have evidence of a recurrent infection, especially if a prior RT-PCR was negative. Patient may be in active stage of infection with a false-negative IgM.
-	+	-	Patient may be in early stage of infection with a false-negative RT-PCR. Patient may not be infected with a false-positive IgM. Retesting may be indicated, especially if symptomatic.
-	-	+	Patient may have had a past infection and is recovered. Patient may have a false-positive IgG.
-	+	+	Patient may be in recovery stage of infection. Patient may be in active stage of infection with a false-negative RT-PCR.

Principles of the BioCheck SARS-CoV-2 IgG / IgM Combo Test

The BioCheck SARS-CoV-2 IgG and IgM Combo Test is a chemiluminescent assay for the detection of SARS-CoV-2-specific IgG and IgM in the serum of patients who meet CDC SARS-CoV-2 clinical criteria (Box 1). The test is indicated for the diagnosis of current or past infection with SARS-CoV-2, when used in conjunction with CDC COVID-19 epidemiological criteria and clinical criteria.

The BioCheck SARS-CoV-2 IgG and IgM Combo Test is cartridge-based and performed on the MS-Fast analyzer, a fully automated system manufactured by Sophonix Co, Ltd. Up to eight specimens can be tested per module at a time, and the time to results is approximately 30 minutes. The BioCheck SARS-CoV-2 IgG and IgM Combo Test provides individual semi-quantitative readouts for each IgG and IgM. Sensitivity is improved when these two reactions are interpreted as a combination test.

Antigenic target

The antigenic target used in the BioCheck SARS-CoV-2 IgG and IgM Combo Test is the S1 spike antigen. This domain of the spike protein is the most divergent immunogen across the human coronaviruses (CoVs), including HKU1 (25% amino acid identity), OC43 (25% identity), NL63 (21% identity), 229E (24% identity), SARS-CoV (66% identity) and MERS-CoV (24% identity).

No cross-reactivity has been shown for S1-specific IgG assays and antibodies against these other human coronaviruses.[3]

Box 1. *CDC Clinical and Epidemiological Criteria for COVID-19 Testing Prioritization*

Clinical Criteria

Fever
Cough
Shortness of Breath

Epidemiological Criteria

Priority 1:
- Hospitalized patients
- Symptomatic healthcare workers

Priority 2:
- Patients in long-term care facilities with symptoms
- Patients 65 years of age and older with symptoms
- Patients with underlying conditions with symptoms
- First responders with symptoms

Priority 3:
- Critical infrastructure workers with symptoms
- Individuals who do not meet any of the above categories with symptoms
- Healthcare workers and first responders
- Individuals with mild symptoms in communities experiencing high COVID-19 hospitalizations

Performance data for the BioCheck SARS-CoV-2 IgG and IgM Combo Test

Clinical Performance Data

The BioCheck SARS-CoV-2 IgG and IgM Combo Test was validated on specimens collected from hospitalized patients with COVID-19 in Wuhan, China, the epicenter of the pandemic. One hundred and ten patients RT-PCR positive for SARS-CoV-2 were evaluated, >7 days after symptom onset. In addition, forty-three patients who were known to be seronegative for SARS-CoV-2 IgG and IgM, and who had no symptoms for the virus, were used as negative controls.

Sensitivity of the IgM was 95.5% and IgG was 99.1%. Specificity was 100% for both assays. (Table 2)

Table 2. *Clinical performance characteristics for the SARS-CoV-2 IgG and IgM Combo Test*

	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)
IgM	105/110 (95.5%, 89.1-98.3%)	43/43 (100%, 89.9-100%)
IgG	109/110 (99.1%, 94.3-99.9%)	43/43 (100%, 89.9-100%)
IgM & IgG	110/110 (100%, 95.8-100%)	43/43 (100%, 89.8-100%)

Methods

Precision

Precision data demonstrate the % CV for the IgG assay is 1.9-3.4% and for the IgM assay 3.39 – 6.69%.

Cross-reactivity

No cross-reactivity was noted between the IgG and IgM tests with specimens positive for Influenza A, Influenza B, RSV, *H. influenzae*, HBV, HCV or ANA antibodies.

Cross-reactivity with other human coronaviruses has not been specificially evaluated. However, data demonstrated that 91-100% of the human population have been exposed to and harbor IgG-specific antibodies to HKU1, OC43, NL63 and 229E.[5] None of the 43 SARS-CoV-2-negative patients yielded a positive result by the SARS-CoV-2 IgG and IgM Combo Test, indicative of a low risk for cross-reactivity with antibodies against these CoVs.

References

1. https://www.npr.org/sections/health-shots/2020/04/21/838794281/study-raises-questions-about-false-negatives-from-quick-covid-19-test
2. To *et al.* 2020. Lancet Infect Dis. Published online March 23, 2020.
3. Okba *et al.* 2020. Emerg. Infect Dis: 26:7, Early release.
4. https://www.acla.com/acla-serologic-testing-white paper/
5. Gorse *et al.* 2010. Clin and Vac Immunol. 17:1875.

IMPORTANT INFORMATION:

This test has not been reviewed by the FDA.

Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.

Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.

Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

MKT000010_A (1554)

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