



SARS-CoV-2 and the Humoral Immune Response

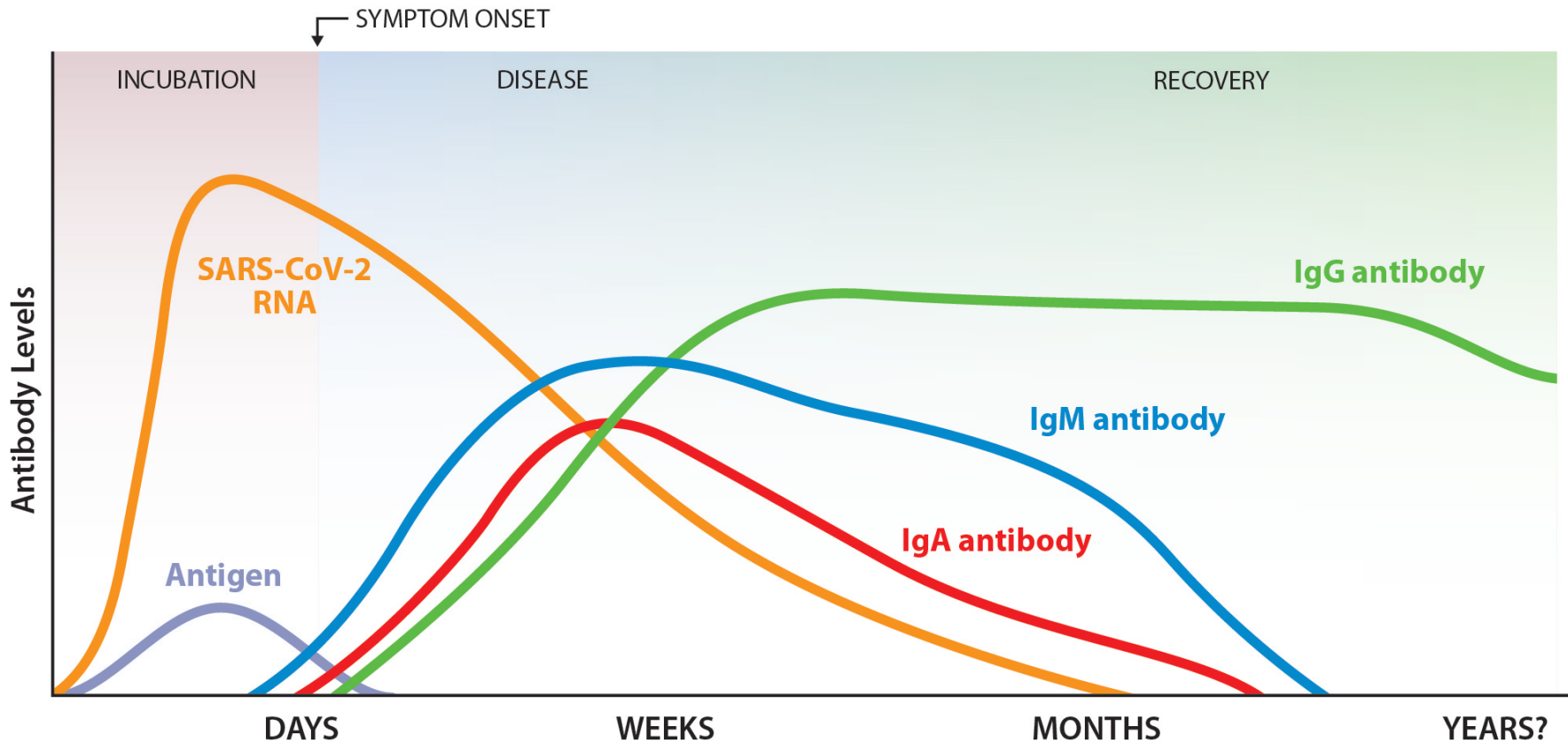
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Serologic Assays

Following natural infection or vaccination, the body develops antibodies against the virus responsible for COVID-19, SARS-CoV-2. Antibodies to two SARS-CoV-2 proteins, the spike protein and the nucleocapsid are the most frequent targets for serologic testing (1-3), which is used to determine recent or previous antibody immune response to the virus. Commercial Emergency Use Authorized (EUA) SARS-CoV-2 serologic assays are available in many formats geared towards detecting either total antibodies or specific antibody subclasses (IgG, IgM, or IgA) (4-9). A subset of antibodies to SARS-CoV-2 that have recently gained attention are neutralizing antibodies (nAbs). The detection of IgM antibodies may indicate a more recent infection; however, the dynamics of the IgM antibody response continue to be defined at present, and frequently IgG- and IgM-class antibodies develop simultaneously. Currently, there is insufficient data to support the clinical utility of standalone IgM testing. IgA based assays have been reported to suffer from lower specificity as compared to IgG-based assays, and are currently not recommended for use by either the Centers for Disease Control and Prevention (CDC) or the Infectious Diseases Society of America (IDSA). Some studies indicated a total assay may be more sensitive for most infections (4-7).

Generally, antibody response SARS-CoV-2 is characterized by two broad categories – binding and neutralizing. While binding antibodies are able to inactivate the virus through complement activation or opsonization, nAbs are able to inhibit viral replication independent of other components of the immune system by binding to regions of the virus that directly interact with host cell receptors, effectively blocking viral entry and inhibiting replication.

Understanding the kinetics of the antibody response to SARS-CoV-2 relative to time post infection or post symptom onset is a prerequisite for choosing the right serologic test for the laboratory and accurate interpretation of test results. Consensus has not yet been attained on many aspects of the SARS-CoV-2 antibody kinetics and long-term data remain incomplete. The overall kinetics of the antibody responses against SARS-CoV-2, as we currently understand them, are depicted in the following figure.



Detection of SARS-CoV-2 antibodies in the incubation phase following infection is time dependent on the functionality of the immune system. Serologic tests that are performed on samples collected too soon following infection will likely be negative. Several serologic studies published to date have demonstrated that many individuals develop an IgM/IgA response within 7-14 days of symptom onset, while the IgG response follows closely behind (10,11.) IgM/IgA levels may peak and decline earlier than IgG, often within weeks of symptom onset (12-15). Serologic assay sensitivity is much better during the convalescent phase of disease when high IgM and IgG levels are produced in the blood (three to four weeks post symptom onset). The precise kinetics of the SARS-CoV-2 immune response remain unclear. Several studies have demonstrated that the IgG antibody response in symptomatic patients is more

robust than in asymptomatic patients. Numerous studies have demonstrated that the antibody concentrations decline at varying rates and consequently the length of time that IgG Ab remains detectable is variable (16-24).

As experience with SARS-CoV-2 serologic testing begins to extend to long-term follow-up of patients, some studies have shown that up to 40% of individuals with molecular test-confirmed infections become IgG seronegative by the early convalescent phase (25). In contrast, additional studies show that in most patients have demonstrated that anti-SARS-CoV-2 antibodies declined over time, but were still detectable for months post-infection. Currently, it remains unknown what implications that waning or subsequently undetectable antibodies levels have on the durability of an effective immune response for SARS-CoV-2 (26-28). Early work on the seasonal human CoV 229E demonstrated similar IgA and IgG kinetics to those observed for SARS-CoV-2 (4), whereas the antibody response to SARS-CoV-1 peaks later (four months after infection) and remains detectable for 16 months, at which time many individuals cease to have detectable levels of antibodies. Therefore, it appears that the immune response and the level of potential protective immunity provided by measurable antibodies against CoVs may be virus specific (20).

Antibody Kinetics in Special Populations

Differences in antibody kinetics may be observed in specific sub-populations and across the spectrum of disease severity and patient immunocompetence. Studies have shown that roughly 4-10% of the population with confirmed SARS-CoV-2 infection either have an undetectable or delayed antibody response as measured by current serologic assays. It is becoming increasingly apparent that negative antibody results can be attributed to a number of factors, including time of sampling relative to days post symptom onset, assay sensitivity, assay design, and inter-individual variability (16, 29). Some evidence suggests in the US that individuals of different racial and ethnic backgrounds are affected to different degrees by SARS-CoV-2 infection. (18, 30, 31).

Serologic testing for any pathogen in immunocompromised individuals has always presented unique challenges. The current literature indicates that immunocompromised patients may be at increased risk for severe disease (32-35). While the serologic response against SARS-CoV-2 in human immunodeficiency virus (HIV)-infected individuals has not been well described, one case report suggests that Immunocompromised patients (including cancer patients) may require 30 or more days or may never generate antibody levels that are detectable as positive by this assay, and therefore caution should be used when serologic testing is performed in this population (36). Negative serology results should always be interpreted with caution in individuals who are immunocompromised. In addition, falsely negative serology results will lead to the wrong clinical decisions if used by themselves.

At least one study has demonstrated a significantly lower detection rate of SARS-CoV-2 antibodies in cancer patients compared to a control group of healthcare workers (37). In contrast, a study from another group who investigated the SARS-CoV-2 antibody response in patients with chronic lymphocytic leukemia found that hypogammaglobulinemia was negatively associated with SARS-CoV-2 IgG development (38). Limited data in organ transplant patients have shown no impaired detection of antibody response to

SARS-CoV-2 (39). Pregnant women appear to be at increased risk of developing severe SARS-CoV-2 illness, but neonates born to mothers infected with SARS-CoV-2 do not appear to be similarly affected (40, 41).

Clinical Utility and Limitations of SARS-CoV-2 Serologic Testing

In general, serologic testing may be helpful to diagnose COVID-19 in patients presenting later in their disease course (e.g., >9-14 days post symptom onset), who repeatedly test negative or indeterminate by a molecular assay and have a clinical syndrome consistent with SARS-CoV-2 infection (42). In such scenarios, it is likely that the viral load has decreased below the limit of detection by molecular testing, whereas antibody levels are increasing and ultimately detectable by serologic assays, with optimal sensitivity of serologic tests occurring at least 2-3 weeks post symptom onset (43, 17., 44, 45).

Current guidelines from the FDA do not recommend the use of serologic assays to diagnose an active SARS-CoV-2 infection (43, 46, 47-50). Of particular concern is the use of antibody tests during the first 14 days post symptom onset, as a negative test result may occur due to variability in the time to seroconversion and the sensitivity performance characteristics of the assay used. Equally important is the awareness that different serologic assays detect different antibody classes and the result may not be comparable between assays. Overall, total antibody or IgG testing may be more useful for evaluating patients presenting late in disease course due to waning IgM and IgA, but this has not been extensively evaluated (45).

Invitrox Testing and Interpretation of Results

Invitrox offers three individual tests related to SARS-CoV-2 serologic testing. The Bio-Rad Platelia assay is used for total antibody detection after natural SARS-CoV-2 infection. The Kantaro Sero-Klir assay is used to estimate an IgG antibody concentration. The GenScript cPass assay is used to estimate the presence and relative concentration of neutralizing antibodies. The latter two tests yield results for both naturally infected individuals and for vaccinated individuals, as these tests are based on detection of antibodies to the SARS-CoV-2 spike protein. Taken together, the three tests offer important insight into an individual's immune status relative to SARS-CoV-2 and also indicate whether an individual has been naturally infected with the virus.

At Invitrox, if all three tests are performed on the same sample, the results are used to derive a numerical index that provides a guide to an individual's status of humoral immunity against SARS-CoV-2. Examples of representative cases from naturally infected individuals, from vaccinated individuals, and from both naturally infected and vaccinated individuals with the scored humoral index are presented below: The total humoral index score reflects the overall level of humoral immunity as measured by these three assays. A higher score reflects a higher level of humoral immunity.

Example Test Results

Case #	Category	AGE	Gender	Matrix	Specimen Collection Date	Bio-Rad Platelia Specimen Ratio	GenScript cPass Signal Inhibition	Kantaro Sero-Klir Total IgG (AU/mL)	Total Humoral Index Score
1	Natural Infection	26	Male	Plasma	4/30/20	4.482	24%	20.1	
					Score	1	0	2	3
2	Natural Infection	40	Female	ACD Plasma	8/14/20	7.152	96%	>125	
					Score	1	3	4	8
3	Vaccination	71	Male	Serum	7/28/21	< 0.8	58%	51	
					Score	0	2	3	5
4	Vaccination	30	Female	Serum	7/28/21	< 0.8	96%	123	
					Score	0	3	4	7
5	Natural Infection + Vaccination	60	Female	Serum	7/28/21	4.783	94	>125	
					Score	1	3	4	8
6	Natural Infection + Vaccination	30	Male	Serum	7/28/21	1.269	96	80	
					Score	1	3	3	7

Key

Bio-Rad Platelia

Specimen Ratio	Score
< 0.8 (Negative)	0
> 1.0 (Positive)	1

GenScript cPass

Signal Inhibition (%)	Score
< 30 (Negative)	0
30 – 50 (Weak Positive)	1
51 – 75 (Moderate Positive)	2
76 - 100 (Strong Positive)	3

Kantaro Sero-Klir Total IgG

Total IgG (AU/mL)	Score
< 3.2 (Neg)	0
3.2 – 10 (Low Positive)	1
11 – 25 (Medium Positive)	2
26 – 125 (High Positive)	3
> 125 (Very High Positive)	4

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