DIAGNOSTICS

Serological approaches for the diagnosis of SARS-CoV-2 infection

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OVERVIEW ON THE IMMUNE RESPONSE TO PATHOGENS

The immune system uses a complex and intricate set of pathways and processes to enable humans to fight off pathogens. As is well documented in scientific literature, both innate and adaptive responses constitute the human immune system. The former is a hard-wired response in the host that recognizes molecular patterns of both pathogens and toxins. The adaptive response is driven by a genetic element that rearranges to drive a very specific antigen binding array of molecules that are foreign to the host. The human response to viruses uses both the innate and the adaptive arms in its attempt to rid the host of the invading pathogen.[1] The humoral response is a component of the adaptive immune response that allows for antibodies to bind to foreign invading pathogens, marks the pathogens and their toxins for phagocytosis and recruits further phagocytic cells to the site via the activation of the complement system and eventually prevents the pathogen from infecting target cells. As macrophages invade the area and bind the antibodies, they trigger a wave of cytokines that induce a more widespread systemic response. In viruses that invade the mucosal and respiratory systems, IgA plays a role in the affected portions by early binding to the virus triggering some of the initial less specific activation of the immune system. As the response matures the introduction of IgM molecules drives a larger wave of cytokine production by the invading white blood cells. As the immune response reaches full maturity more specific and higher affinity IgG production helps the host clear the pathogen from interstitial areas and removes infected cells displaying viral antigens on the surface.

DIAGNOSTIC APPROACHES

Clinicians and laboratorians have worked on studying viral infections to identify and characterize the pathogens and then aid the treatment of the illnesses these viruses have caused. Utilizing direct detection methods such as nucleic acid amplification of viral RNA or DNA, Polymerase Chain Reaction (PCR), or immunoassays for the detection of viral antigens, usually ensures the best sensitivity for early detection of the viral infection. Tests for human antibodies to the virus can also be used as additive or surrogate markers to aid the diagnosis of infection but will always have a lag due to the time required for the human body to mount its full-blown humoral response. On the other hand, as time passes from when the viral infection has occurred, disease management and treatment will rely more on the indirect methods that evaluate and measure the human response to the infection. In clinical practice, it is important to know at what stage of the disease the patient is in, how fast and strong an immune response has been mounted, and what types of antibodies are being generated (IgG and/or IgM). For these purposes, test methods that separately look at IgM and IgG in the blood can help address those needs and make the clinical interpretation easier.

THE IMMUNE RESPONSE TO SARS-CoV-2

The SARS-CoV-2 virus is a beta corona virus with similarities to other respiratory viruses such as MERS, and SARS-CoV-1. Viral particles bind to the surface of the human cell and after entering the cell replicate (RNA) and then get exocytosed. To mount an antiviral response, the innate immune system recognizes molecular structures that are produced by the invasion of the virus. Laboratory evidence of clinical patients showed that a specific T-cell humoral response against SARS-CoV-2 is important for the recognition and killing of infected cells, particularly in the lungs of infected individuals. Then, this infection induces the generation of IgG antibodies that are initially targeting the N protein and can be detected by serum as early as day 4 after the onset of disease and with most patients seroconverting by day 14.[2]

The time course of viral and host immunity biomarkers during SARS-CoV-2 infection is described in **Figure 1.** In the late incubation period and early stages, only the viral RNA, and to a lesser extent the viral antigen, may be detected by molecular or serological techniques, respectively, that are carried out on samples taken usually from the upper respiratory track of patients. After a few days the humoral immune response starts to develop, overlapping the direct identification of the pathogen. While RNA detection is the mainstay for the diagnosis of SARS-CoV-2 and allows for the diagnosis of a confirmed COVID-19 case, RNA levels drop quickly and after a few weeks RNA and/or antigen are usually not detectable any more[3, 4, 5], especially when RNA is tested for on nasopharyngeal swabs instead of endotracheal aspirate.[3] In those instances, the laboratory support to the diagnosis may be provided only by serological assays that detect the specific antibody responses.[4, 5]

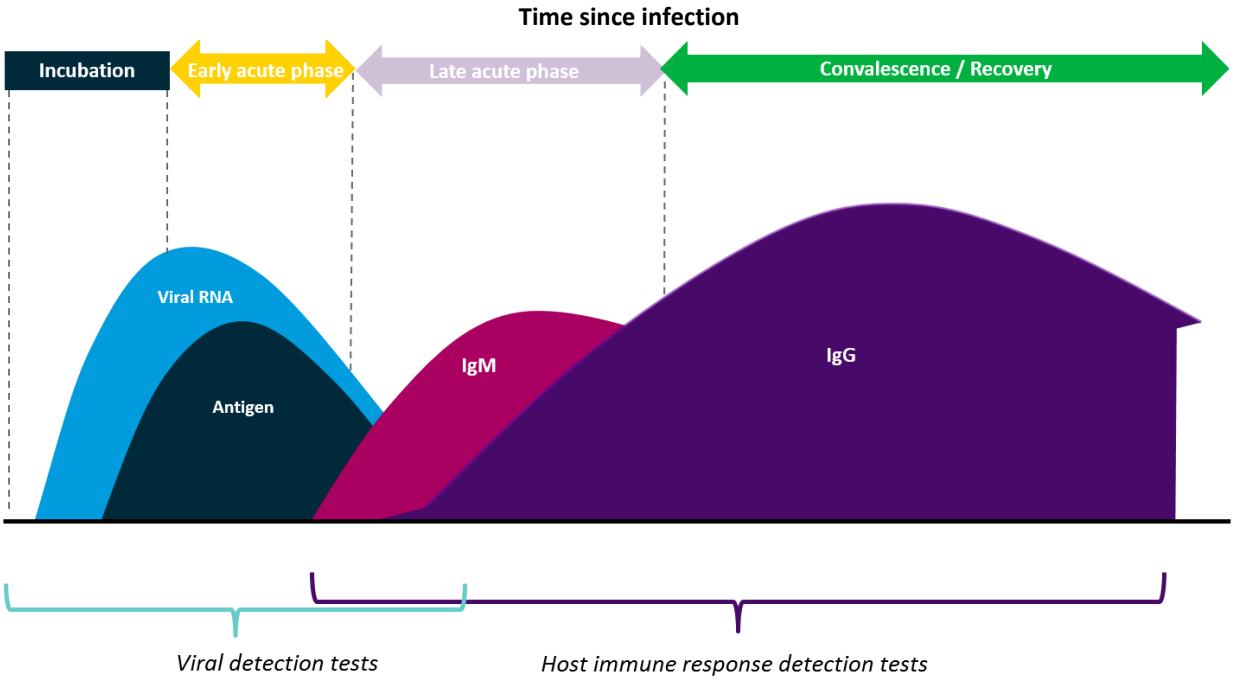

Figure 1: Kinetics of viral and host biomarkers in SARS-CoV-2 infection

Rationale for testing IgG / IgM vs. total Ig

The possible combinations of viral and host biomarkers in SARS-CoV-2 infection and the likely interpretation criteria are indicated in Table 1.

- In acute phase, **only** the separate assessment of the IgM and IgG response may provide disease stage vs. later stages.[6] This allows laboratorians and physicians to dissect the immune response and provide clear actionable answers **(Table 1)**.
- Combination assays may provide earlier detection, but this is not lost since the two separate assays can still be run from the same sample at the same time on the random-access analyzers. Currently available seroconversion data do not bring conclusive evidences on the time of appearance of IgM vs. IgG antibodies: Tan et al[4] have reported IgM to be detected earlier, but by the time the detection of viral RNA fell under 50% of cases IgG positivity rates were higher and would have guaranteed a better diagnostic yield **(Figure 2).** Conversely, on a much higher number of patients Long et al[7] have not observed an earlier detection of IgM compared to IgG and also found higher rates of IgG positivity **(Figure 3)**. It shall be mentioned that the cumulative

results on IgM and IgG positivity described above represent an average of many different profiles and the individual response may vary[7], as shown in the two examples on **Figure 4**.

- Having separate assays provides a continuum of data from infection all the way through to recovery and will be of value when testing is carried out in asymptomatic individuals that are not tested or test negative for viral RNA. A combined assay will not allow the assessment if the person has been infected recently and may still harbor the virus.
- Epidemiological studies and public health studies in general will need a positive IgG response to determine that the infection is not recent. [6,8] This type of information cannot be determined by a total Ig assay alone.

Table 1

TEST RESULTS			GENERAL INTERPRETATION*
PCR	IgM	IgG	
+	-	-	Patient may be in the initial period of infection when antibodies are not yet produced or are under the limit of detection
+	+	-	Patient is in the active phase of infection has started to develop an immune response with antibody production
-	+	-	Patient may be in the early stage of infection. PCR result may be false-negative or IgM false positive.
+	+	+	Patient is still in the active phase of the infection; immune response has progressed.
+	-	+	Patient may be in the late stage of infection or has developed a recurrent infection.
-	+	+	Patient may be in the late or recovery stages of infection or PCR false negative
-	-	+	Patient may have recovered or has been infected in the past.

* Test results must be considered along with other clinical data available to the physician.

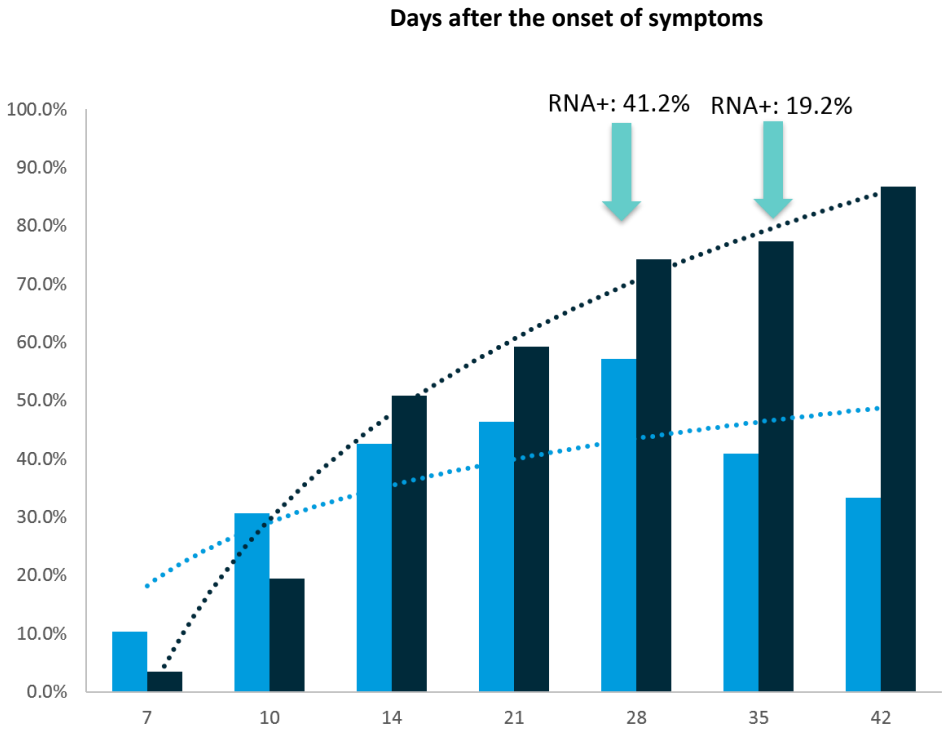

Figure 2: Positivity rates for IgM and IgG antibodies to SARS-CoV-2 in 67 patients with COVID-19. Light Blue=IgM; Dark Blue=IgG. Data from: W. Tan et al. Viral Kinetics and Antibody Responses in Patients with COVID-19. medRxiv preprint doi: https://doi.org/10.1101/2020.03.24.20042382

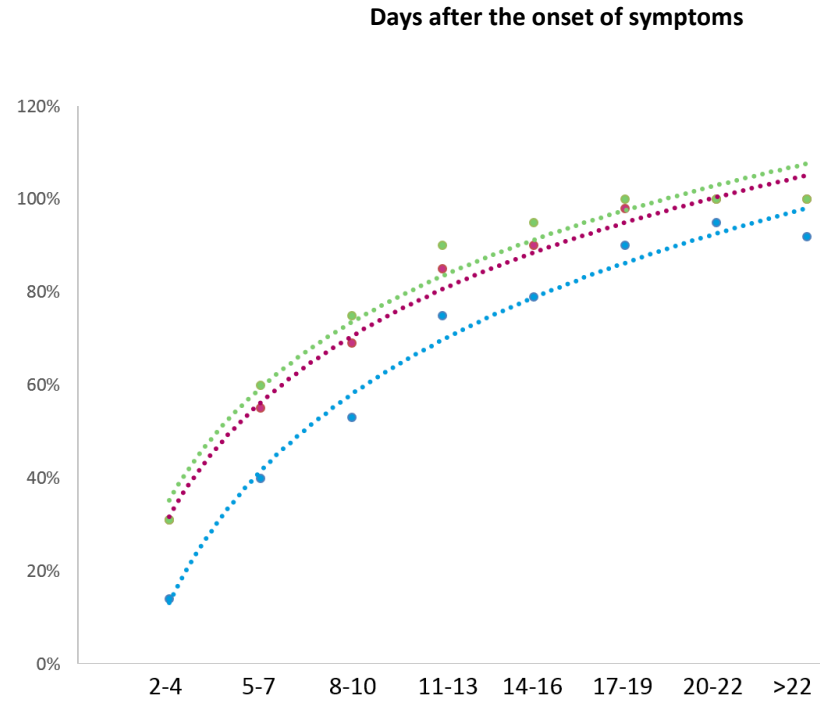

Figure 3: Positivity rates for IgM and IgG antibodies to SARS-CoV-2 in 285 patients with COVID-19. Blue=IgM; Magenta=IgG; Green= IgM and/or IgG. Data from: Q-X Long, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nature Medicine 2020; https://doi.org/10.1038/s41591-020-0897-1

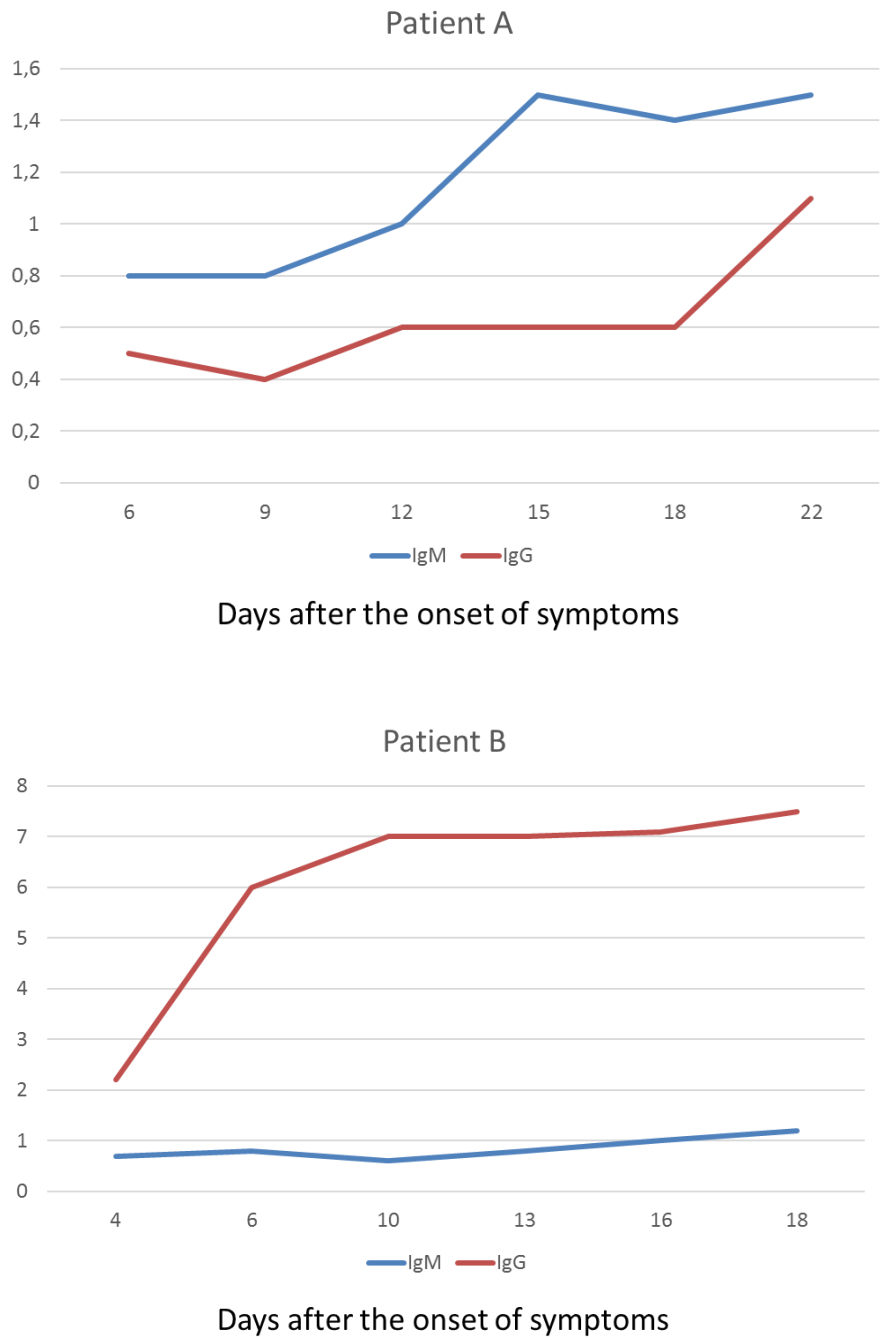

Figure 4. Seroconversion profiles from two patients with COVID-19 disease whose samples have been assayed for SARS-CoV-2 IgM and IgG antibodies at different time points from the onset of symptoms. In patient A, an earlier IgM response has been recorded, while in patient B, IgG appeared first. The positivity threshold is 1 for both assays. Data from: Q-X Long, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nature Medicine 2020; https://doi.org/10.1038/s41591-020-0897-1

KEY POINTS ON THE RATIONALE FOR SEPARATE IgG, IgM TESTING VS. A TOTAL Ig

1. Serology will give the treating physician insights to help enable appropriate and timely actions by providing greater understanding as to the stage of infection. Using a total Ig assay could confuse the picture on the patient status.

a. A PCR positive, IgM negative, and IgG negative individual may be in early stages of infection and should be quarantined and insure healthcare workers in contact with the person have appropriate personal protective equipment.

b. A PCR negative but IgM positive may indicate a missed (false negative PCR) this may call for a retest on PCR or management as if in early stage of infection.

c. A PCR negative, IgM negative and IgG positive is indicative of a past infection

d. IgM presence alerts the physician that there is likely an acute infection, suggesting that clinical action should be taken (treatment, patient isolation/quarantine, or return visit). May also be used at locations where PCR is not readily available

The continuum of data from infection to recovery helps enable better patient assessment and management. Understanding the magnitude of IgM vs IgG in a patient at different infection stages may help inform a patient's progress towards clearing the virus. Appearance of IgM and IgM titer magnitude may also provide an indication of the cytokine storm initiation. Finally, having an IgM vs. an IgG assay targeting different viral proteins can lend itself to confirmatory algorithms.

2. Recency of infection to help enable more accurate epidemiology studies.
An assay targeting the whole antibody response by all Ig classes cannot reliably differentiate recent infections from more distant ones and may generate confusion on potential active infections vs. past infections, engendering the need for further testing steps on positives. Epidemiologists and healthcare workers will need to further elucidate the type of immune reaction that is occurring when a positive signal for a total antibodies assay has been detected. In addition, total antibody results may lead to the misclassification of individuals in terms of time of infection. As further data develops, it is IgG which will eventually be used to predict immunity and protection, if longer term immunity is found to occur for SARS CoV-2.

CONCLUSION

Serological testing helps to enable accurate diagnosis of SARS-CoV-2 infection in both symptomatic and asymptomatic cases. While current evidence suggests that testing for IgM-class antibodies may not always guarantee a greater yield compared to testing for IgG in the acute stages, being able to evaluate the different Ig classes allows for a better interpretation of the different clinical stages and inform decisions on further testing need in people with symptoms or at high risk of exposure. Since the IgG serological response is long-lasting [1] the evaluation of an IgG antibody response appears well suited for the purpose of population screening to assess the burden of infections and possibly to evaluate incidence and the efficacy of prevention measures.[6,8] Finally, should immunity be conferred by specific antibodies be demonstrated for SARS-CoV-2, antibody tests together with the direct virus detection will be essential tools in the development of de-escalation strategies in which mobility and contact restrictions can be removed.[6]

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