Enzo Biochem

COVID-19 Antibody Screening Program

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BACKGROUND:

Utilizing serological testing to screen for antibodies provides a valuable complement to molecular testing in addressing the COVID-19 pandemic. The current global health crisis has shined light on systemic issues inherent in our healthcare system. One issue that has arisen has been the bottleneck for diagnostic testing. While many believed there was an overcapacity of testing capabilities in the system, the opposite has proven to be the case. Clinical laboratories must provide testing to the entire market, but closed diagnostic systems offered by the leading diagnostic companies have limited their ability to freely expand testing capacity and options. These closed platforms provide little or no flexibility with regard to their workflow, forcing laboratories to use only pre-programed assays and preventing the ability to develop new assays during public health emergencies. A second issue presented through this period is constraints within the supply chain from key suppliers. This limitation further complicates laboratories' ability to make testing easily available to patients. A lack of connectivity within the market between assay development and translation to testing presents a challenge in bringing robust testing to the market quickly.

With so much still unknown about SARS-CoV-2 compounded by its uneven effect on individual patients, additional patient-specific information becomes vital in patient management. To better understand a patient's disease timeline, serological testing in tandem with molecular testing provides the clinician with a full data set. Clinical testing is a critical element in the national response to broaden our understanding of the reach and scope of exposure to SARS-CoV-2. Molecular tests detect the presence of viral genome and are the standard for diagnosing COVID-19. Serological assays detect immunoglobulins against the SARS-CoV-2 virus. The emphasis of patient management in the coming months will include serological testing as it enables the identification of an individual's convalescent

period. Serological testing may also assist in determining the number of individuals who have been infected, support screening, and aid in vaccine development.

ENZO APPROACH:

Enzo continues to expand its offerings in the fight against coronavirus through a multi-step approach. To complement the molecular diagnostic AMPIPROBE® SARS-CoV-2 Assay, Enzo has developed a SARS-CoV-2 IgG ELISA Kit. Both molecular and serological tests are issued under the FDA's Emergency Use Authorization (EUA).

A concerning number of commercial serological tests being distributed were determined to have poor performance characteristics when tested. As a result, the FDA has issued new guidance in serological testing.[1] Accurate results from immunoglobulin antibody testing is a critical component to create a safe return-to-work environment and end state mandated lockdowns. Point-of-care serological lateral flow immunoassays tests provide rapid results for the detection of immunoglobulin antibodies, but they may do so at the expense of accuracy and sensitivity, resulting in unreliable results. Furthermore, such assays are individual tests, which are performed manually and cannot support the large scalability needed for population testing.[2-5]

Enzo's ELISA assay is a qualitative assay, optimized to provide accurate and sensitive detection of IgG antibodies to SARS-CoV-2 in human serum, with high throughput testing capabilities for the clinical laboratory setting.

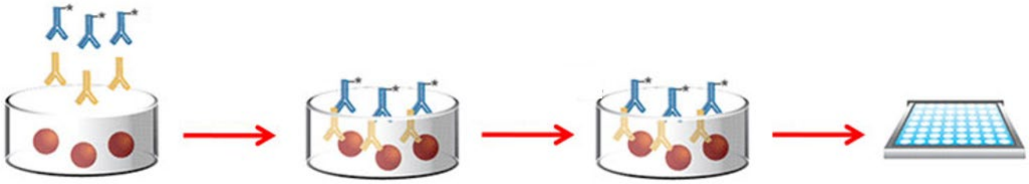

Figure 1 Indirect ELISA with a two-step binding process involving a specific SARS-CoV-2 antigen and a HRP conjugated anti-human IgG secondary antibody.

Clinical performance of the SARS-CoV-2 IgG ELISA Kit was evaluated using a PCR comparator with serum samples demonstrating a positive percent agreement (PPA) and negative percent agreement (NPA) with 96.5% specificity and 100% sensitivity. Antibody assays must be reliable and have high specificity without cross-reactivity to related viruses.

Various competitor assays detect total immunoglobulin with no differentiation between immunoglobulin classes. Enzo's assays differentiate between immunoglobulin types, which allows clinicians to identify the different stages of infection. The Enzo ELISA kits are qualitative immunoglobulin assays for the detection of IgG (completed), IgM (in-development) and IgA (in-development) specific antibodies. The timing of specific immunoglobulin production and seroconversion plays a pivotal role in clinical testing and interpretation. During the early stage of infection, the IgM antibodies are produced. As the levels of IgM drops, IgG levels rise and remain detectable during late stage infection and through recovery. During this seroconversion, the levels and detection of specific immunoglobulins allow for the identification of early infection (IgM) transitioning to post infection (IgG).

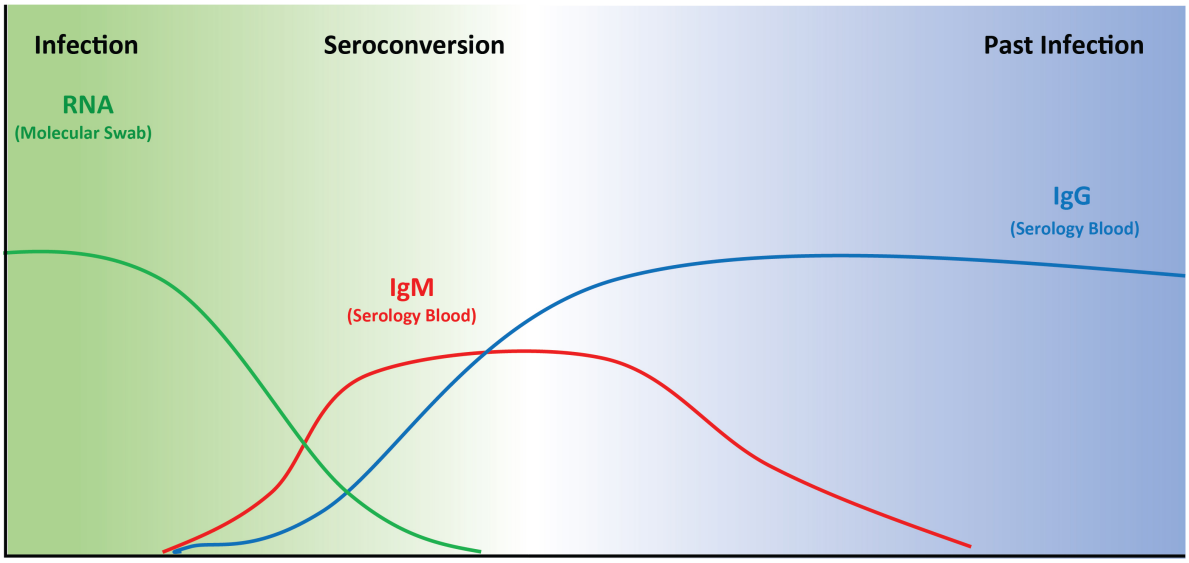

Figure 2 Immunoglobulin seroconversion.[6-14] IgM is produced first in response to infection, circulating in the blood, and accounting for 10% of human immunoglobulins. Abundant in serum, nasal mucus, and saliva IgA accounts for 10-15% of human immunoglobulins. IgG is the most abundant antibody in the blood, accounting for 70-75% of human immunoglobulins.

The presence of IgM and IgG antibodies coupled with RT-PCR results demonstrate the possible clinical scenarios that can be encountered. Combining molecular and serological testing can improve the understanding of the clinical course of COVID-19.

RT-PCR Result	IgM Result	IgG Result	Clinical Significance
+++	-	-	Window period of infection
++	+	+/-	Early stages of infection
+	+	+	Active stage of infection, early seroconversion
+	-	+	Late stage of infection
-	+	-	Early stage of infection, with false negative RT-PCR
-	-	+	Post infection and has recovered
-	+	+	Recovery stage of infection, with false negative RT-PCR

Figure 3 Clinical significance of molecular and serological testing[6-14]

With the ever-changing landscape, new information on the COVID-19 pandemic continues to emerge from various government agencies. Extensive testing can help broaden our understanding by increasing the sample size. Case studies observing the immunoglobulin seroconversion of SARS-CoV-2 specific IgG, IgM, and IgA antibodies provide as valuable resources to monitor and evaluate the specific immunoglobulin class responses. Studies demonstrate adding IgA along with IgM and IgG may help improve the diagnosis of SARS-CoV-2 at early stages.[15-16]

Monitoring of inflammation cytokine levels have been suggested as an important diagnostic tool for determining early therapy for patient management. This uncontrolled release of cytokines, which causes a "cytokine storm", is associated with respiratory decline and failure. Enzo offers multiple ELISA based Research Use Only assays - IL-1 (beta), IL-6, IL-8, TNF-alpha, and Gamma Interferon for the monitoring of cytokine storm.

From the first line of defense in identifying infection with our AMPIPROBE® SARS-CoV-2 Assay to patient management with our SARS-CoV-2 IgG ELISA Kit, Enzo's comprehensive solutions to COVID-19 testing addresses current market challenges. Together, these tests not only benefit the delivery of healthcare to the individual patient but also permit healthcare bodies and government agencies to track and screen the COVID-19 pandemic at the population level. Thus, molecular and antibody monitoring provides both an economic and clinical benefit in the fight against COVID-19.

Enzo is providing a comprehensive end-to-end solution including molecular sample collection, extraction, and detection, as well as serological immunoglobulin testing. Enzo continues to launch new assays to respond to the current challenges plaguing the healthcare market including those presented by COVID-19.

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