Monitoring Immune Response to SARS-CoV-2

Introduction

The immune system of a COVID-19 patient produces antibodies to SARS-CoV-2 within days to a few weeks following viral infection. The immune reaction to COVID-19 produces neutralizing antibodies and generally provides immunity in the event of a second exposure to the virus and also provides a basis for vaccine development. Antibody testing is typically performed using Enzyme-Linked Immunosorbent Assay (ELISA) or related automated immuno-assays. While ELISA has high-throughput capability when automated, it requires several time and labor-intensive steps that lengthen assay time. Testing of Immunoglobulins-IgG, IgM and IgA by ELISA or other immunoassays, requires individual time-consuming assays to be run. Detection requires labeling and the use of additional reagents to report binding of the analyte to the receptor. Surface plasmon resonance (SPR) biosensors offer a label-free direct measurement platform for rapid quantitative and qualitative characterization of biologically relevant analytes. Presence of specific antibodies in the circulatory system can thus serve as a biomarker of various diseases such as microbial infection, virus infection and autoimmune disease. Here, we describe a high-throughput SPR assay to profile the immune response to SARS-CoV-2 of COVID-19 patients by measuring the IgG, IgM and IgA antibodies binding to the receptor-binding domain (RBD) of the Spike protein[1].

Key Takeaways

- Measure immune responses from patient serum to known antigens for up to 384 samples using a rapid and automated method.
- Detect multiple secondaries against the same sample to reduce assay complexity while still gaining more information.
- Profile the immune response for levels of IgA, IgG and IgM in COVID-19 patient samples.
- Get profiling results using only two microliters of patient serum.

Method

RBD Surface Preparation

RBD was immobilized onto an HC30M sensor chip (Carterra P/N 4279) using 50 mM sodium acetate, pH 4.8, and amine coupling chemistry. The sensor was prepared by treatment with a 1:1 aqueous solution of 100 mM N-hydroxysuccinimide (NHS) and 400 mM N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) for 10 minutes. The sensor was then exposed for 20 minutes to the Spike RBD his-tag (SINO biological Frankfurt, Germany). Finally, excess NHS esters were blocked with a solution of 1 M ethanolamine HCl pH 8.5 (Carterra P/N 3626) for 10 min. The sensor was then equilibrated in the running buffer composed of PBS (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) supplemented with 1% bovine serum albumin (BSA), 0.5% Casein and 0.1% Tween 20.

Isotyping of Patient Antibody Response

Serum samples were obtained from COVID-19 patients confirmed by RT-qPCR and CT-scans. They were collected within four days to 28 days after first symptoms. Control non-SARS-CoV-2 samples were obtained from anonymous donors and patients with repetitive negative RT-PCR test results.

In total 48 selected serum samples were spotted in duplicate in a single run on the HC30M RBD coupled sensor prism surface in four dilutions (1:50, 1:100, 1:200 and 1:400) to generate a 384-array. During the spotting process, the binding signals are followed for 15 minutes and each serum sample was measured in duplicate at four dilutions. The signal recorded in RU reflects the total anti-RBD antibodies bound. Without regenerating the surface, the sequential injections of solutions of anti-IgM (50x diluted, 20-S5170 GND1-D0 Fitzgerald), anti-IgG (100x diluted, 20-S1211G001-S4 Fitzgerald) and anti-IgA (100x diluted, 20-S1111G000-S4 Fitzgerald) antibodies were

performed over the 384-sera array for five minutes to identify the class of immunoglobulin bound to the surface.

Results

In the present work, we describe the development of a high-throughput assay for 384 sera for the rapid detection of the binding of SARS-CoV-2-associated antibodies of isotypes IgG, IgM and IgA in patient serum. This assay uses a biosensor derivatized with the Spike protein capable of detecting patient antibody responses directed against the RBD domain.

Forty-eight sera from patients with critical, severe, moderate, and mild symptoms and control sera were characterized in duplicate at four dilutions each as shown in **Figure 1**.

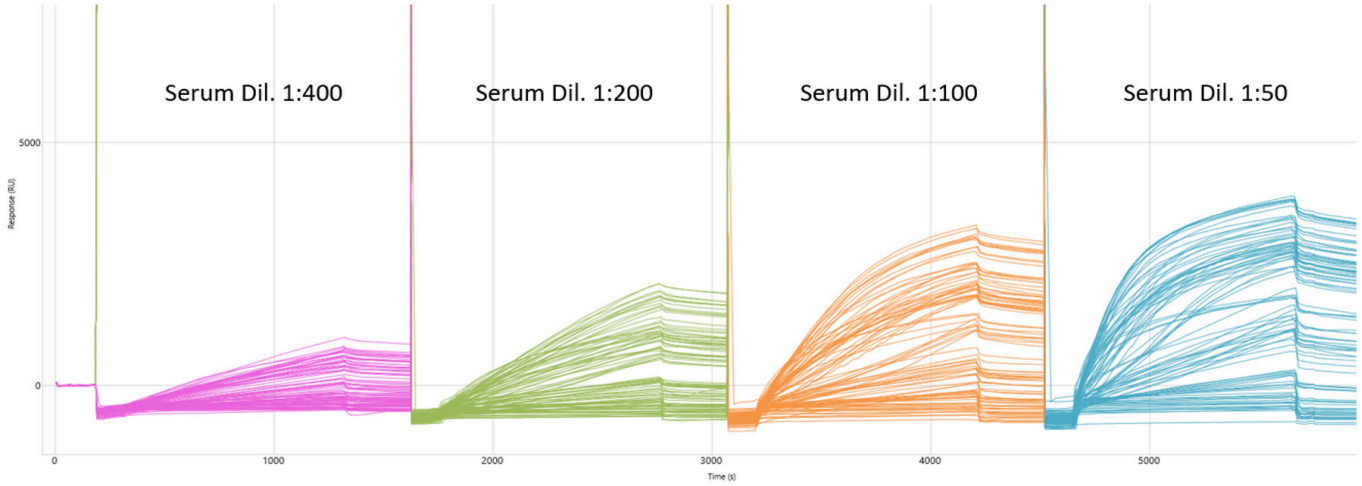

Figure 1: Sensograms of serum samples spotted at four dilutions on RBD immobilized surface, serum samples spotted at four dilutions on RBD immobilized surface.

The level of binding of the anti-isotype antibodies reflects the binding activity of anti-RBD antibodies in patient serum displayed in **Figures 2, 3, 4**.

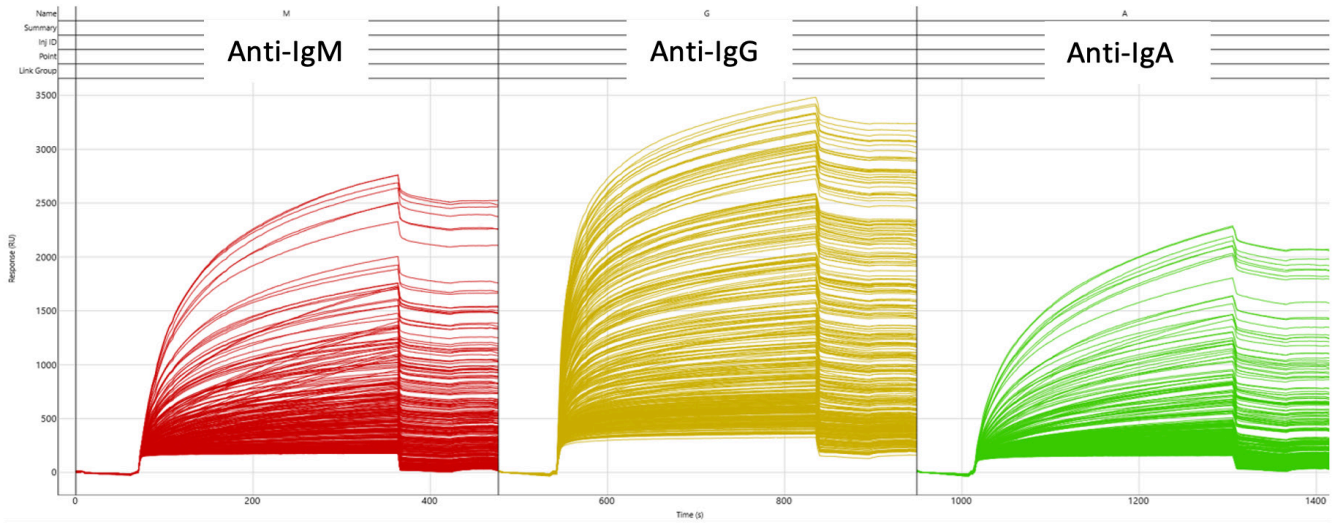

Figure 2: Sequential injections anti-IgM, anti-IgG and anti-IgA antibodies over the 384-sera array without regeneration. Each tile displays the sensorgrams recorded simultaneously for the 384 spots.

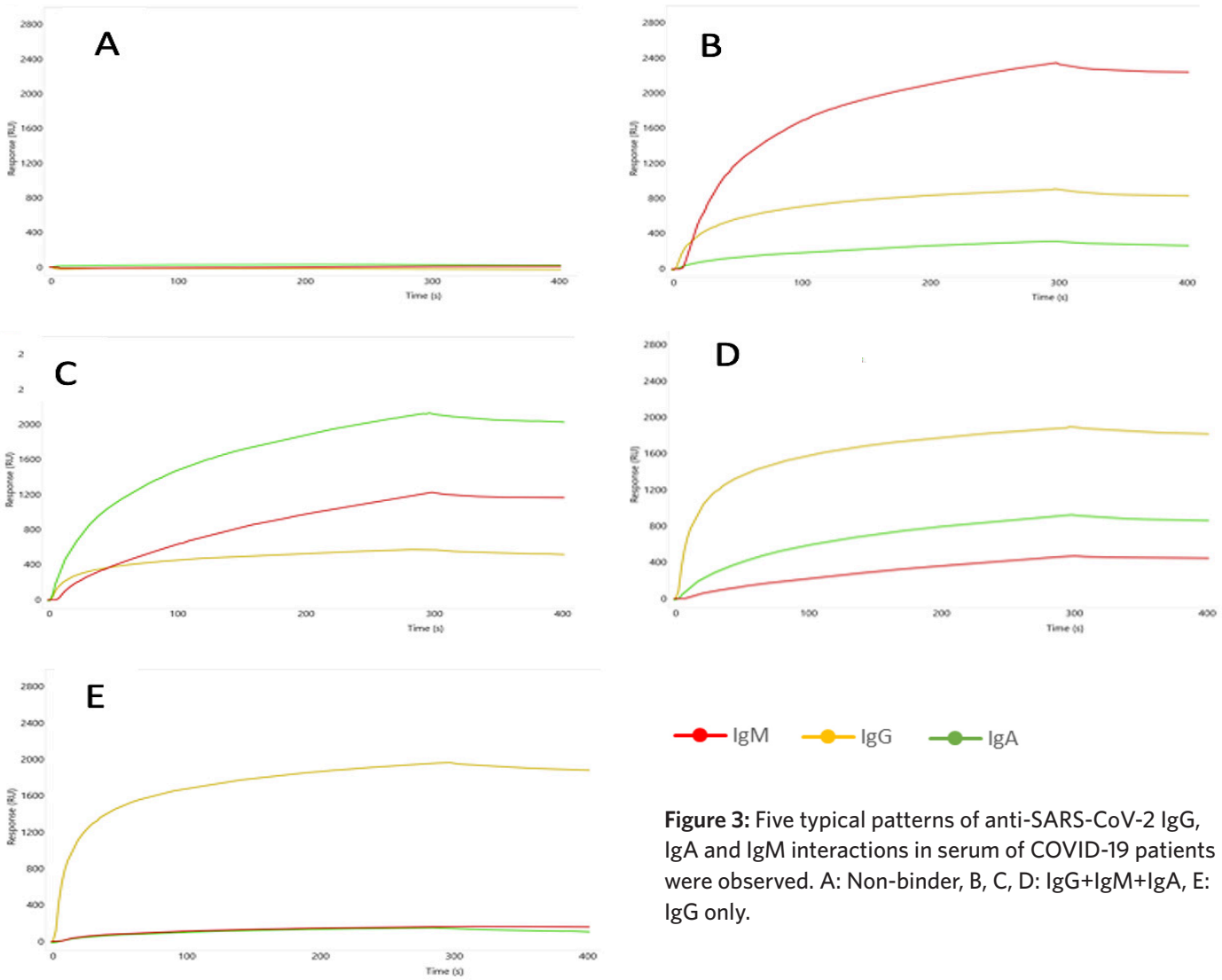

Figure 3: Five typical patterns of anti-SARS-CoV-2 IgG, IgA and IgM interactions in serum of COVID-19 patients were observed. A: Non-binder, B, C, D: IgG+IgM+IgA, E: IgG only.

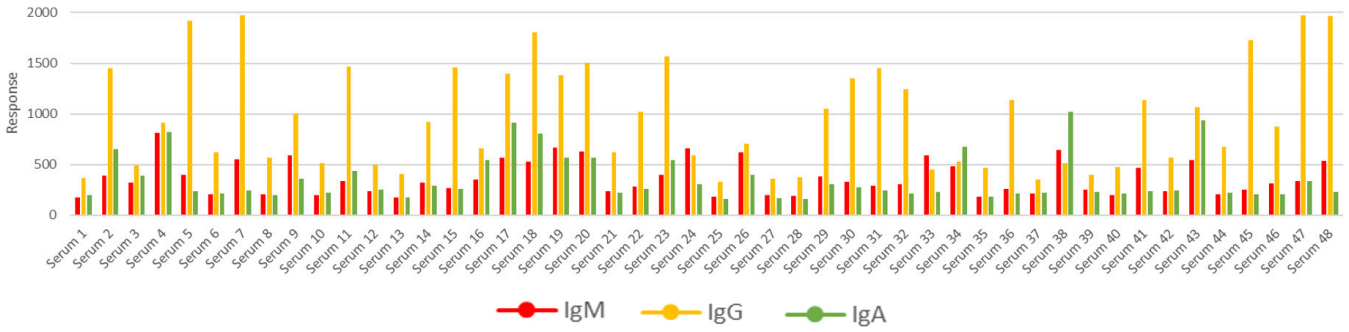

Figure 4: Levels of IgM (red), IgG (yellow) and IgA (green) for the 48 sera diluted 1:50 in running buffer. Levels of binding are determined at the end of the anti-isotype antibody injection.

An example of typical kinetics of the IgG, IgM and IgA response to SARS-associated coronavirus is shown in **Figure 5**. Such a profile was also found in the patients affected by SARS-CoV TW1, a different strain found in the first strain of the SARS virus[2]. A rise in antibody titers 10 days after symptom onset—specifically detected levels of serum IgG—was observed. Interestingly, levels of serum IgM and IgA did not show as dramatic level increases detected at the same time period[3].

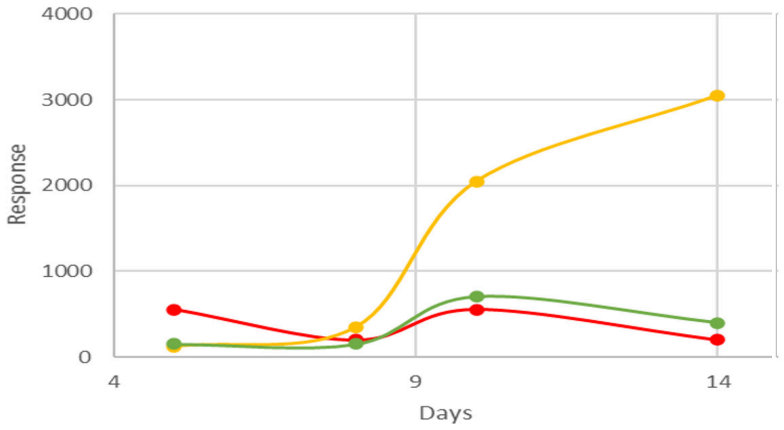

Figure 5: Typical chronological profile of antibodies in COVID-19 patient since illness onset. The levels of IgM (red), IgG (yellow) and IgA (green) as a function of days of symptoms onset.

In addition to detecting the binding of anti-RBD antibodies in COVID-19 patients, this assay is ideally suited to monitor or detect healthy people who will be vaccinated against SARS-CoV-2 in clinical trials. Results from the SPR assay described here can provide critical insights in determining the quality of the immune response after vaccination. In contrast to classical ELISA, this assay requires shorter analysis time. The relative antibody titers and the isotype of the antibodies can easily be monitored for 384 patients simultaneously in a single-step assay.

Summary

The work described here highlights the use of Carterra's LSA Platform to perform rapid high-throughput SPR screening and isotyping of SARS-CoV-2 levels in COVID-19 patients.

The approach of using the secondary isotype detection reagents adds specificity and sensitivity to the detection method as well as additional information about the patient's immune status. Inclusion of the IgA detection can potentially allow for the detection of very early cases of an immune response to respiratory infections and may be a better marker for SARS-CoV-2 than IgM[3]. The Ig serum response could prove valuable to track as there appears to be a correlation of disease pathology with the intensity of the IgA and IgG response[4], where patients with severe responses demonstrate rapid and high immune responses leading to inflammatory complications. Given this risk, understanding the extent and time course of the Ig immune response would be useful to track patient responses and inform the understanding of both safety and efficacy of clinical vaccine trials.

Also, this platform may be applicable to measure and distinguish other infectious agents using crude samples. This suggests the potential versatility of the LSA platform to perform analytical characterizations and potentially clinical diagnostics.

References

1. Schasfoort R, Weperen Jv, Amsterdam Mv, Parisot J, et al. Presence and strength of binding of IgM, IgG and IgA antibodies against SARS-CoV-2 during CoViD-19 infection. Preprint from *Research Square* (2020). doi: 10.21203/rs.3.rs-52460/v1
2. P.-R. Hsueh, L.-M. Huang, P.-J. Chen, C.-L. Kao, and P.-C. Yang. Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. *Clin Microbiol Infect*. (2004): 10(12): 1062–1066. doi: 10.1111/j.1469-0691.2004.01009.x
3. Kelvin Kai-Wang To, Owen Tak-Yin Tsang, Wai-Shing Leung, Anthony Raymond Tam, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis.* (2020): 20(5):565-574. doi: 10.1016/S1473-3099(20)30196-1
4. Yu H-qiong, Sun B-qing, Fang Z-fu, et al. Distinct features of SARSCoV-2-specific IgA response in COVID-19 patients. *Eur Respir J* (2020) in press. doi. org/10.1183/13993003.01526-2020

carterra®
info@carterra-bio.com
825 N 300 W Suite C309
Salt Lake City, UT 84103
www.carterra-bio.com