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A Method to Expand the xMAP® SARS-CoV-2 Multi-Antigen IgG Assay for Serological Isotype Profiling

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Introduction

Since its initial identification in December of 2019, SARS-CoV-2 quickly accelerated from local community spread to an international outbreak.[1,2] COVID-19, the disease caused by SARS-CoV-2, was declared a pandemic by the World Health Organization (WHO) in March 2020, and caused more than one million deaths as of December 2020.[3] In order to identify cases and track the spread of the disease in both symptomatic and asymptomatic individuals, a variety of viral detection assays were developed. These assays test for the presence of SARS-CoV-2 genomic material, viral antigens, or antibodies present in human serum resulting from an immune response to the virus.

Luminex's xMAP® SARS-CoV-2 Multi-Antigen IgG Assay is a serological assay that utilizes a multiplex microsphere format to determine SARS-CoV-2 exposure and the antibody immune response to infection. The assay detects IgG antibodies in serum or plasma that bind to the viral nucleocapsid antigen, the spike 1 domain of the spike antigen (S1), and the receptor binding domain (RBD) portion of the spike protein.

The serological antibody response during acute infection is clearly linked to viral infection outcomes, as seen with the original SARS coronavirus,[4] influenza,[5] and HIV.[6] While the majority of the serological immune response in humans is of the IgG isotype, IgM and IgA antibody responses also represent a significant portion of the response to viral infection.[7,8] IgA titers have been found to be significantly higher in severe COVID-19 patients, and remained higher for a longer time than what has been seen in milder cases.[9] Serological IgM and IgA responses may also be predictive of survival, with anti-spike IgM and IgA enriched in surviving donors, and nucleocapsid-specific IgM and IgA at higher levels in individuals who died.[10] These outcomes may be related to the early neutralizing response to SARS-CoV-2 infection, which shows closer correlation to IgA than IgG or IgM.[11]

The current understanding of the IgM and IgA response to SARS-CoV-2 infection is more limited than that of the IgG response. Having the capability to monitor the early onset of IgM and IgA responses, as well as measure their decay on a large set of samples quickly, is a necessity for future epidemiologic studies to more fully understand the COVID-19 antibody response. This study describes the modification of the on-market xMAP SARS-CoV-2 Multi-Antigen IgG assay for evaluation of IgM and IgA responses, with demonstration of the chronological evolution of the three isotypes in donor samples.

Methods

Sample preparation for IgG analysis was performed as described in the Luminex xMAP SARS-CoV-2 Multi-Antigen IgG Assay package insert.[12] For IgM and IgA detection, the assay detection reagent was modified and depletion of serum IgG was performed. For IgM, the assay detection reagent was replaced with 1:1,600 R-Phycoerythrin Goat Anti-Human IgM (Jackson ImmunoResearch Laboratories, Cat. # 109-116-129) in assay wash buffer. For IgA, the assay detection reagent was replaced with 1:1,600 R-Phycoerythrin, Goat Anti-Human Serum IgA (Jackson ImmunoResearch Laboratories, Cat. # 109-115-011) in assay wash buffer.

For IgM and IgA detection, serum samples were first diluted 1:20 in assay wash buffer. Samples were then diluted 1:20 in assay wash buffer containing 1:50 Goat Anti-human IgG (Meridian Bioscience, Cat. # L15406G) to yield a final dilution of 1:400. Samples were incubated for 30 minutes at room temperature to allow for depletion of serum IgG. 50 µL of 1:400 diluted serum samples were added to individual wells in a 96-well assay plate. Assay microspheres

were thoroughly mixed by vortexing and 50 µL was added to each sample well, followed by incubation at room temperature in the dark for 60 minutes with plate shaking. The plate was then washed twice with assay wash buffer using a magnetic plate separator. 50 µL of 1:1,600 anti-IgM or anti-IgA detection reagent was added to the washed beads, followed by incubation at room temperature in the dark for 60 minutes with plate shaking. Following incubation, an additional two washes were performed, and the beads were resuspended by pipetting with 100 µL of wash buffer. Samples were then run on a FLEXMAP 3D® instrument using the standard FMMultiCoV2 protocol.

A variety of assay conditions were tested, including sample dilutions of 1:100, 1:200, and 1:400 and detection reagent dilutions of 1:400 and 1:1,600. Non-IgG depleted samples were compared to varying IgG depletion conditions.

Optimal conditions for IgM and IgA detection were determined to be a 1:400 sample dilution with 1:1,600 detection reagent, and depletion of IgG using 1:50 anti-human IgG for 30 minutes. For serum samples more concentrated than 1:400, incomplete depletion of IgG or lessened incubation time for IgG depletion may lead to undesired effects, including IgG bead loss, heightened IgG median fluorescence intensity (MFIs), and incorrect antigen MFIs.

Example Use Case

To confirm the ability of the modified xMAP SARS-CoV-2 serology assay to detect IgM and IgA, 20 pre-December 2019 samples (from 20 unique donors) and 66 samples with positive RT-PCR SARS-CoV-2 tests (from 40 unique donors) were tested for IgG, IgM, and IgA responses. Nine donors were sampled at multiple time points. Samples ranged from 1 to 116 days post-symptom onset. 100% of pre-pandemic controls were called negative for IgG response by the standard xMAP SARS-CoV-2 IgG assay, and 94% of molecular-positive samples from 21 days post-symptom onset were called positive for IgG response.

Most samples showed a significant response to SARS-CoV-2 antigens compared to pre-pandemic negative controls. IgG titers rose for all 3 antigens until they peaked at days 29–41, with samples at day 60 and beyond retaining moderately high titers comparable to week 1–2 after the onset of illness **(Figure 1)**. The population average IgM response reached a maximum during days 8–14 for nucleocapsid antigen, and days 15–21 for the RBD and S1 antigens. Significant loss of binding was seen after day 60, declining to levels more comparable to negative samples. The IgA response peaked during days 8–14 for nucleocapsid, with an RBD/S1 peak at days 29–41. Nucleocapsid and spike antigen binding returned to pre-pandemic control levels past 60 days, with low binding retained for the RBD.

While a significant amount of serum IgG was seen for all antigens at 60+ days post-symptom onset, only a small RBD response was retained for IgM and IgA. The majority of samples remained SARS-CoV-2 IgG-positive until at least 60 days post-symptom onset, with one donor reaching peak anti-RBD IgG titers at day 50. IgM and IgA titers began to decline around week 3 from symptom onset, with very low remaining levels by week 10.

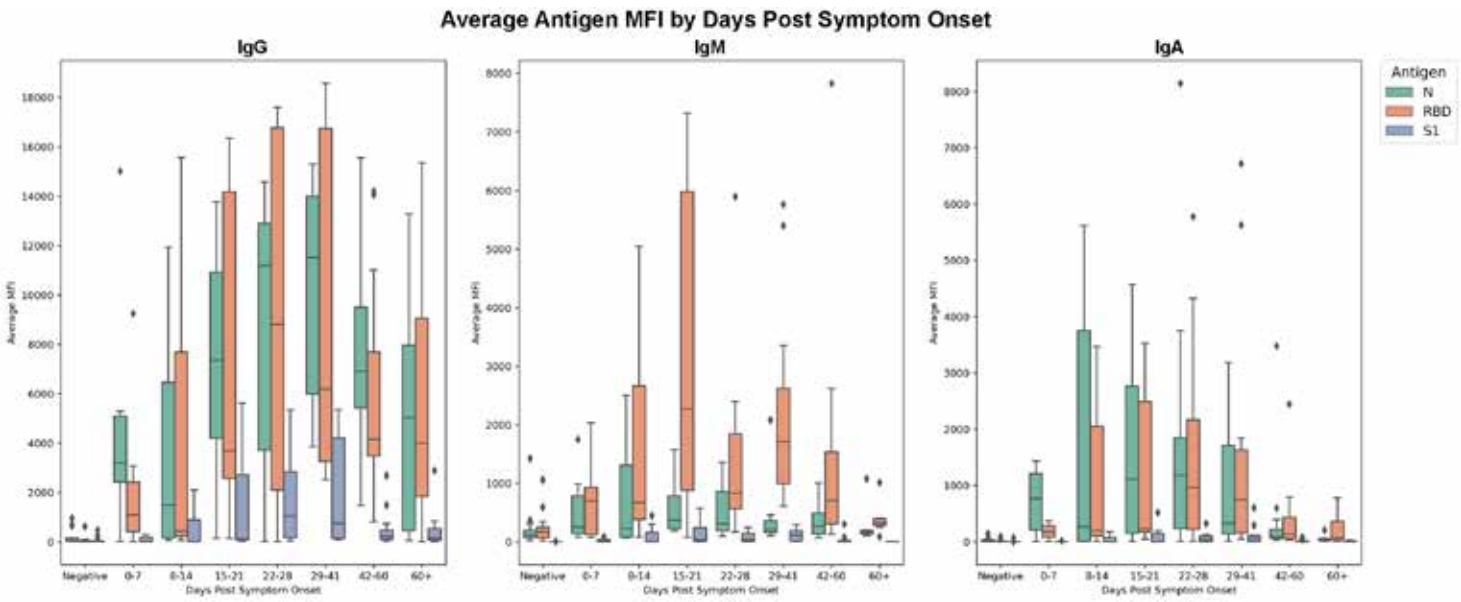

Figure 1. Average MFI measurements of IgG, IgM, and IgA against SARS-CoV-2 nucleocapsid (N), spike receptor binding domain (RBD), and spike 1 (S1) antigen by days post-symptom onset and in pre-pandemic negative controls. Number of samples per bin – negative: 20, 0–7: 7, 8–14: 7, 15–21: 9, 22–28: 10, 29–41: 11, 42–60: 15, 60+: 7.

Among serum samples from nine donors with multiple dates post-symptom onset, individual IgM and IgA peaks often occurred prior to IgG peaks. For the nucleocapsid response, 100% of the donors had an IgG peak occurring later or at the same time as IgM and IgA (67% of donors peaked after IgM and IgA). For the RBD response, 89% of donors had an IgG peak occurring later or at the same time as IgM and IgA (44% of donors peaked after IgM and IgA). For the S1 response, 100% of donors had an IgG peak occurring later or at the same time as IgM and IgA (56% of donors peaked after IgM and IgA).

Summary and Conclusion

While serological IgG levels are commonly measured to qualify ongoing or previous exposure to SARS-CoV-2, IgA and IgM collectively make up around 20% of total circulating antibody in the blood, and reflect different stages of the immune response during COVID-19 progression. Characterizing the role of these isotypes in acute infection and recovery is imperative to forming a full understanding of the differential outcomes to the virus.

This article outlines a usable method for the quantification of IgA and IgM antibodies that bind to SARS-CoV-2 antigens, using an alternative detection reagent along with the depletion of IgG. The modified assay demonstrated a similar serological response to what was seen with IgG, with the expected earlier onset of IgM and IgA, followed by a faster return to pre-infection levels compared to the more prevalent isotype, as exhibited both in a tested population and in individual donors across multiple time points post-onset of symptomatic disease. This alteration of the xMAP SARS-CoV-2 assay allows for the rapid and high-throughput quantification of the IgM and IgA responses to viral antigens in order to further academic understanding of COVID-19.

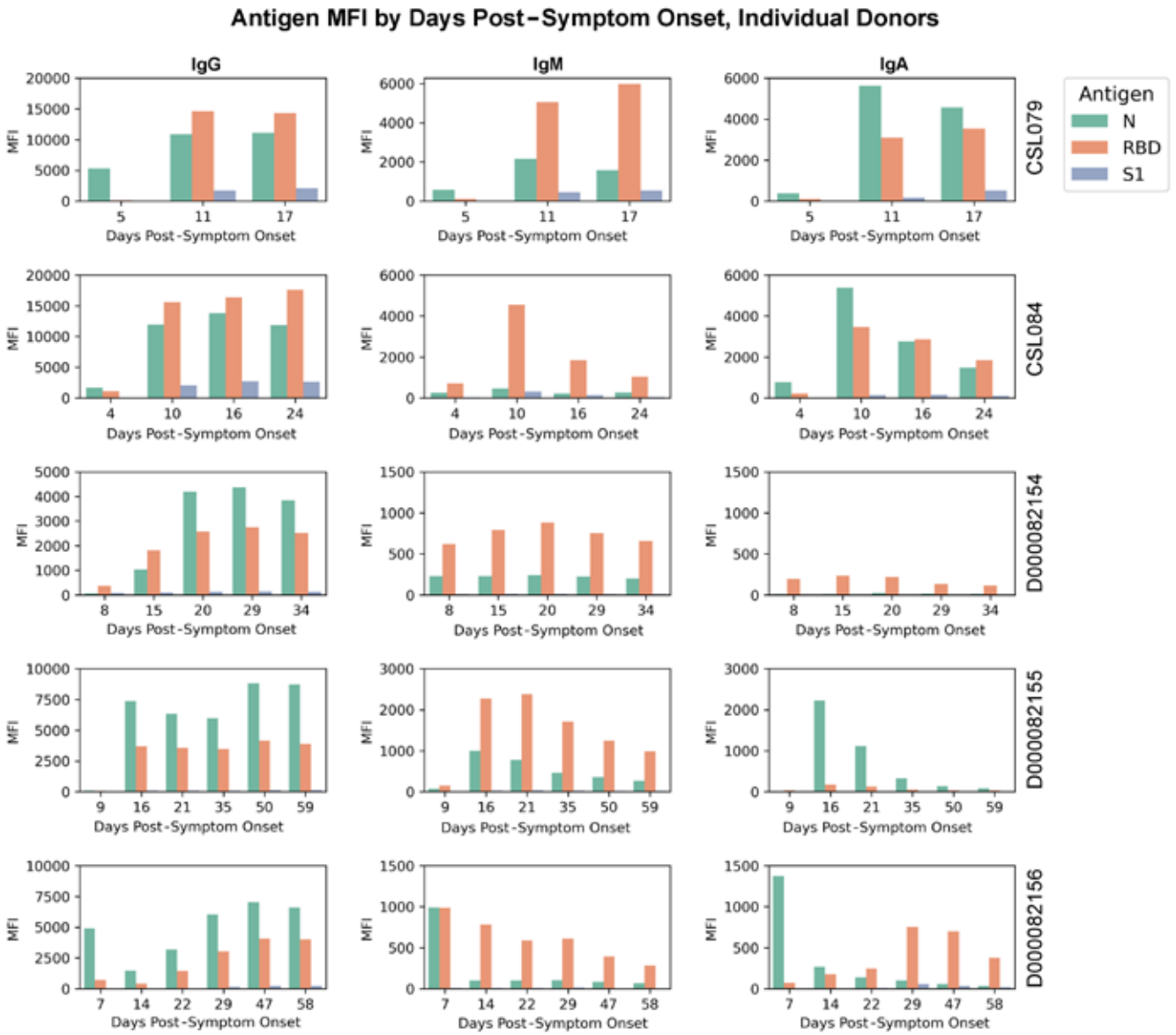

Figure 2. Individual donor SARS-CoV-2 antigen MFIs at sequential time points post-symptom onset. Rows represent individual donors, with columns presenting IgG, IgM, and IgA responses respectively.

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