Quantitative Multiplex SARS-CoV-2 IgG Antibody Test for Measuring Vaccine-Induced Immune Response

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Background: COVID-19 vaccination has been vital to control the pandemic; however, several studies have reported a waning immunity to vaccines over time. Circulating IgG antibody levels are a good indicator of vaccine-induced immunity. The development of reliable methods for quantifying circulating IgG in serum and plasma is critical to monitoring the durability of the immune response. Currently available serological tests provide a qualitative result for the presence or absence of antibody reactivity to a single antigen in a sample. It is essential to quantify the antibody levels against more than one antigen of the SARS-CoV-2 virus to understand the kinetics of antibody response. We have developed a multiplex assay to simultaneously quantify antibody responses to 4 different antigens of SARS-CoV-2 in a single test.

Methods: This multiplex assay utilizes Luminex® xMAP® technology, where we immobilized four recombinant proteins, namely, the receptor-binding domain of S1(RBD), nucleocapsid protein (NP), S1 protein (S1), and trimeric Spike protein (trimer) on internally coded magnetic microspheres (Table 1). This assay includes four internal controls to monitor each step of assay performance. Using the WHO quantification standard (NIBSC lot # 20/136), we have quantified IgG response to the four antigens, RBD, NP, S1, and trimeric spike protein. We assessed 101 serum or plasma samples from 45 vaccinated individuals immunized by different vaccines, including Moderna mRNA-1273,Pfizer/BioNTech BNT162b2, Janssen (Johnson & Johnson), Ad26.COV2.S (Table 2) . The time range of sample collection varies from 7– days post vaccination to 499 days post vaccination. Five individuals had one or more samples post booster shots. This sample set includes 15 samples from vaccine breakthrough infection cases from 7 individuals. Two samples from an individual who received vaccination 33 days post infection are also included in this study. We also had 81 negative samples that were not vaccinated and collected between 2017-2018. First a screening of all the samples was performed using 1 in 400 dilution of the samples. The samples that were above the limit of quantification were reflex tested using 1 in 4000 dilution.

Results: Samples from uninfected or unvaccinated individuals showed negative to a low level of reactivity in the multiplex assay. We determined the threshold levels for each of the four antigens based on the uninfected sample set from 2017-2018. Varying levels of IgG antibody response were measured in vaccinated samples to RBD, S1, and trimer, with very little reactivity to nucleocapsid protein. Samples for natural infection showed significant levels of IgG antibody response to nucleocapsid protein, and these levels diminished over time faster than the three spike protein antigens.

Conclusion: This multiplex quantitative IgG test provides a valuable tool to quantify the circulating IgG antibodies in case of infection and vaccination. This assay may measure vaccine immunogenicity and assess the durability of immune response after vaccination.

Table 1: Immobilized proteins from SARS-CoV-2 in 8-plex assay

#	Microsphere coupling functionality	ID	Region
1	SARS-CoV-2 Receptor Binding Domain	CoV-2-RBD	25
2	SARS-CoV-2-Nucleocapsid Protein	CoV-2-NP	28
3	SARS-CoV-2-Spike Trimer Protein	CoV-2-Trimer	29
4	SARS-CoV-2-Spike S1 Protein	CoV-2-S1	33
5	IC (Instrument control)	IC	47
6	NC (Non-specific binding control)	NC	54
7	ScG (Human IgG Sample control)	ScG	52
8	FC (Fluorescent Reporter control)	FC	53

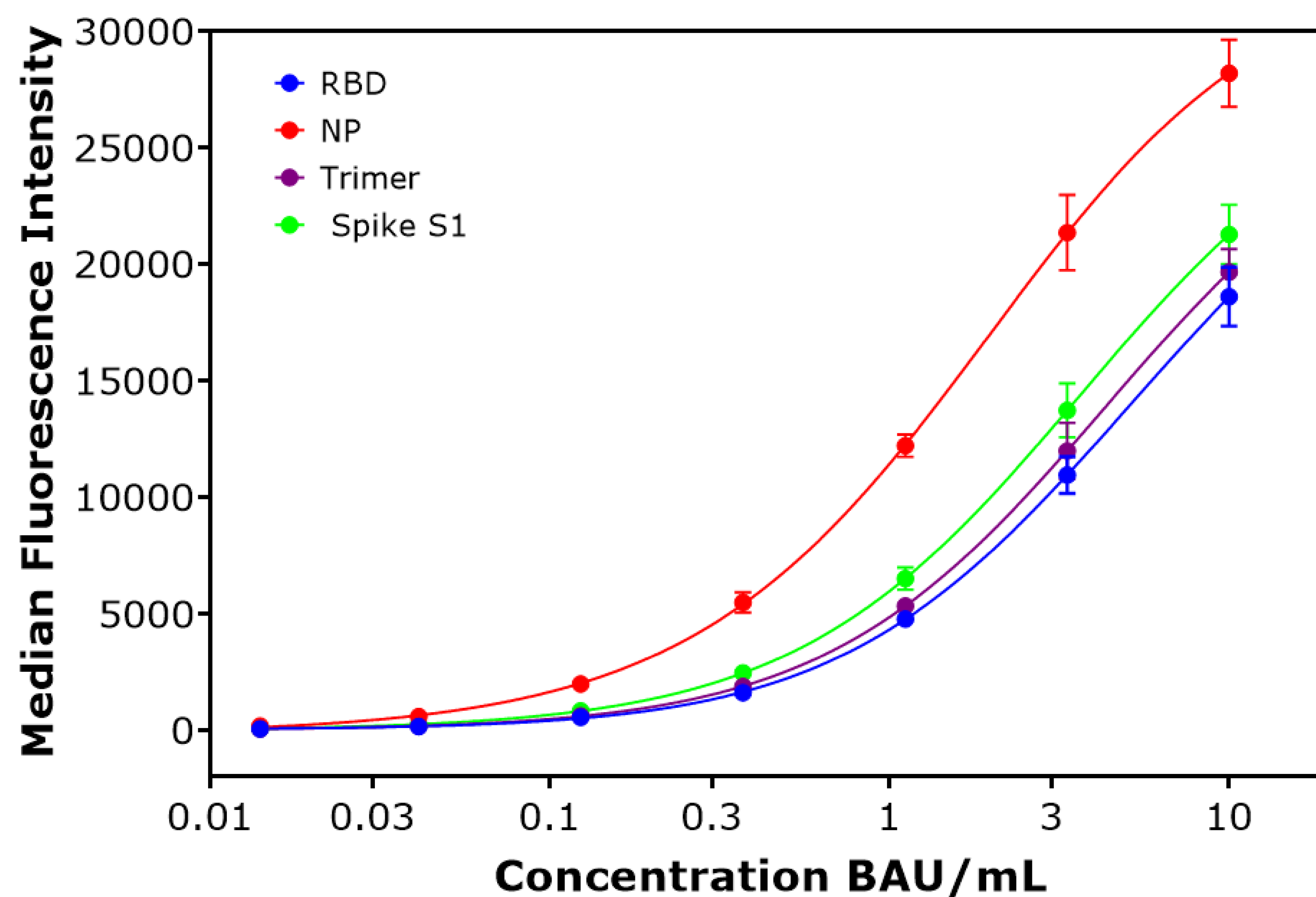

Figure 1: NIBSC Lot Number 20/136 WHO standard was used at various dilutions for titration and for generating the standard curve. The lyophilized standard when rehydrated in 250 µL of PBS is assigned 1000 binding antibody units (BAU)/mL. This sample was diluted in assay buffer 100 fold to get the highest standard of 10 BAU/mL. This standard was 3 fold serially diluted 6 more times to have the standard curve ranging from 10 BAU/ML down to 0.014 BAU/mL. The binding antibodies in samples to the four different antigens used in this multiplex quantitative assay were calculated using this standard curve. The asymmetric 5PL regression analysis was performed to calculate the BAU/mL in unknown samples.

Table 2: Details of the samples used in this study

Description	Number of samples	Number of individuals	2 or more time points	Male	Female	Un-known
Vaccination	101	45	17	15	15	15
Negative/ Pre-vaccine	85	81	-	14	66	1
Vaccine Breakthrough	15	7		4	2	1

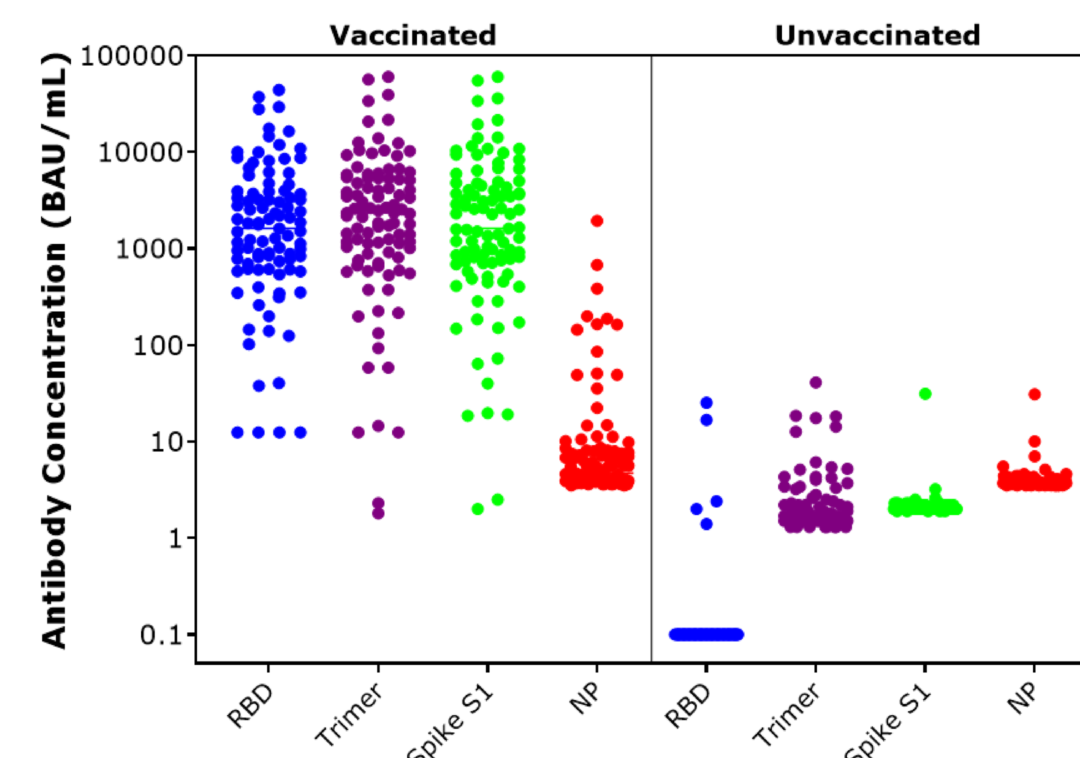

Figure 2: Distribution of antibody responses to four different antigens in vaccinated, breakthrough infection and negative samples

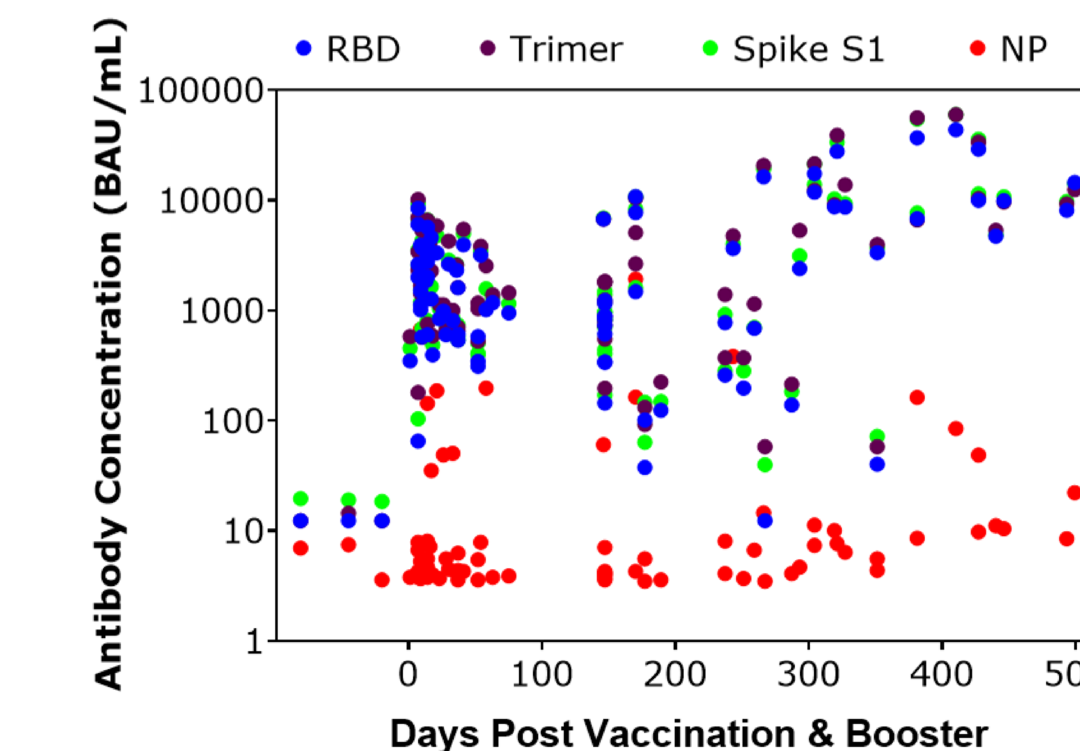

Figure 3: The antibody responses in vaccinated individuals prior to the vaccine, after vaccination with spike protein vaccines and booster over time show that antibody responses to all three spike proteins are seen to a varying degree. The nucleocapsid protein antibodies arise only after the breakthrough infections.

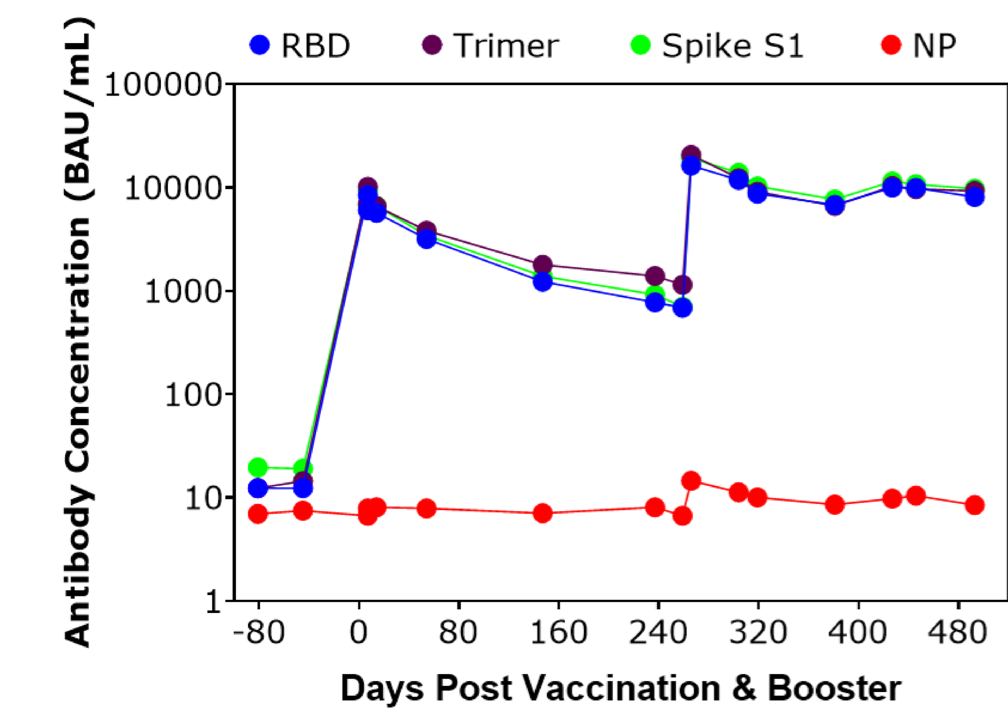

Figure 4: The longitudinal antibody responses in an vaccinated individual 81 days prior to the vaccine, up to 488 days after vaccination with spike protein vaccines and two boosters. This figure shows waning antibody responses to the three spike proteins after the initial two dose regimen. After the third and fourth booster dose the antibody levels are maintained at a high level.

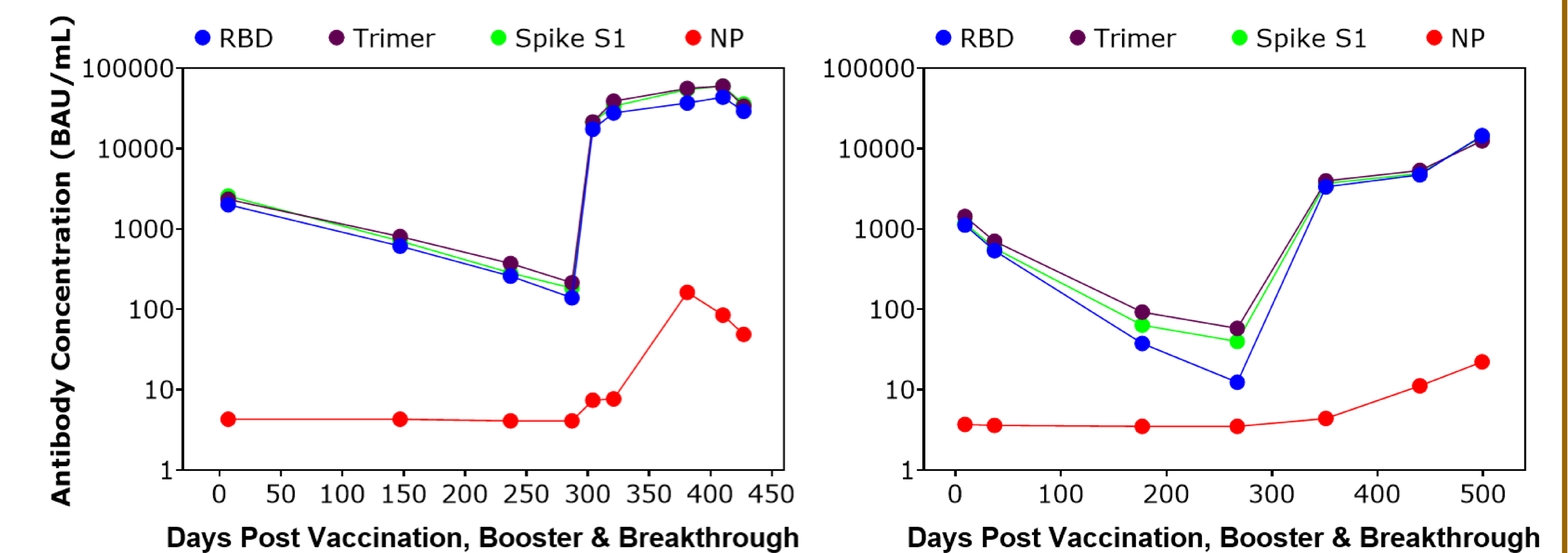

Figure 5: The longitudinal antibody responses in two individuals with break through infection up to 499 days after vaccination with spike protein vaccines and booster. Both these cases had break through infection after first booster. In both these cases a decline in antibody responses to three spike proteins was observed. After the third booster dose there were breakthrough infections and a rise in nucleocapsid antibody response was observed that confirmed the exposure to the virus after the vaccine.

Concluding remarks:

1) 8-plex assay provides a useful tool to detect and differentiate antibody response after infection and vaccination. It can be useful for quantitation of IgG responses post vaccination or infection at both individual and population levels.
2) Individuals with breakthrough infections and previous infection followed by vaccination showed IgG responses to nucleocapsid protein of the virus which is not quantifiable in case of uninfected but vaccinated individuals.
3) This 8-plex panel provides a useful quantitative tool to monitor and assess the long term durability of circulating IgG antibodies after vaccination and infection.