Development and Evaluation of a Multiplex Serological Assay for Assessment of Circulating IgG Antibody Response to SARS-CoV-2 Antigens after Vaccination

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Background: World Health Organization (WHO) declared a COVID 19 pandemic caused by SARS-CoV-2 virus on March 11th 2020 and since then the clinical relevance of serological assays has been debated in the scientific community. After a year and a half, this virus is still causing a significant burden on public health worldwide. Vaccines have now proven to be a handy tool to curb the spread of this virus. In this scenario of infection and vaccination, it is imperative to understand the durability of immune response post vaccination at both individual and population levels. The assessment of circulating IgG antibodies to multiple antigens, both wild type, and variants, can provide a snapshot of immune signature in vaccinated individuals. We developed and evaluated a multiplex panel of antigens to determine the presence of circulating IgG after vaccination.

Methods: A 36-plex panel of immobilized antigens on magnetic microspheres was developed and evaluated in this study. This panel was based on Luminex xMAP technology. This 36-plex expanded panel includes 19 different wild-type and variant proteins from SARS-CoV-2, 13 different S1 spike, and nucleocapsid proteins from other human coronaviruses. We assessed 94 serum or plasma samples from 38 vaccinated individuals immunized by different vaccines, including Moderna mRNA-1273 (51 samples), Pfizer/BioNTech BNT162b2 (41 samples), Janssen (Johnson & Johnson) 2Ad26.COV2.S (2 samples). We also included 111 negative samples that were not vaccinated and 87 of these were collected between 2017-2018. Another 4-plex panel comprising of four CoV2 viral antigens, namely, receptor binding domain (RBD), Spike Trimer, Spike protein subunit 1 (S1), and nucleocapsid protein (NP) was used for quantitation of binding antibodies in unknown samples after vaccination and infection. The WHO standard NIBSC Lot # 20/136 was used as the reference standard.

Results: Multiplex assay showed unique antibody reactivity profile for each individual subject tested. Data indicate a positive response to SARS-CoV-2 Trimer, Spike N-terminal domain (S-NTD), RBD, and S1 proteins following vaccination and a negative response to nucleocapsid proteins (Figure 1). Almost all the negative samples tested showed a positive circulating IgG response to various human coronaviruses causing common colds (Figure 2). We also observed that the mutation at E484 in RBD most severely affects the antibody response. Lowest antibody response was observed in RBD mutants from, beta, gamma and delta variants which have mutation at E484K and delta variant has mutation E484Q. Antibody response to single mutations N501Y and K417N are not significantly different from wild type RBD after vaccination. The triple mutant with K417N,E484K and N501Y also shows decreased antibody response after vaccination compared to wild type (Figures 3 and 4). The pattern of reactive antibodies to 17 antigens from CoV2 and its variants after Pfizer and Moderna vaccines are not very similar in this data set (Figure 5). Longitudinal samples from three subjects that include samples from vaccine break through infection after booster show a rise in antibody response to nucleocapsid protein after the break through infection (Figure 5).

Conclusions: The expanded 36-plex panel provides a useful tool to monitor and assess the long term durability of circulating IgG antibodies in case of infection and vaccination with respect to the background of naturally occurring antibodies to against common cold causing human corona viruses. Utilization of expanded panels in conjunction with 8-plex quantitation panel can be very useful in retrospective studies to reconstruct the evolution of virus and its interaction with human immune system.

Table 1: Details of the serum and plasma samples used in this study

Description	Number of samples	Unique subjects	2 or more time points	Male	Female	Un-known
Vaccination	94	38	20	30	36	28
Negative	111	110	1	24	87	0
Total	205	148	21	54	123	28

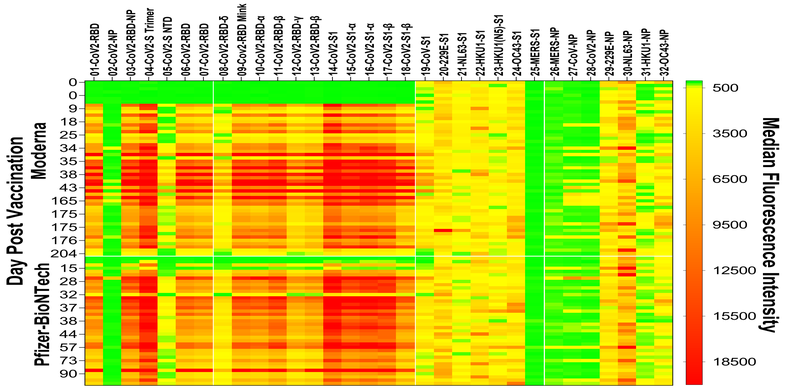

Figure 1: Antibody reactivity (Median Fluorescent intensity, MFI) to the CoV-2 and other HCoV protein antigens is shown in the Heat Map in 94 samples from 38 vaccinated individuals immunized with either of the three vaccines, Moderna mRNA-1273 (51 samples) or Pfizer/BioNTech BNT162b2 (41 samples) or Janssen (Johnson & Johnson) 2Ad26.COV2.S (2 samples). All the samples showed positive differential antibody reactivity to all the variant proteins.

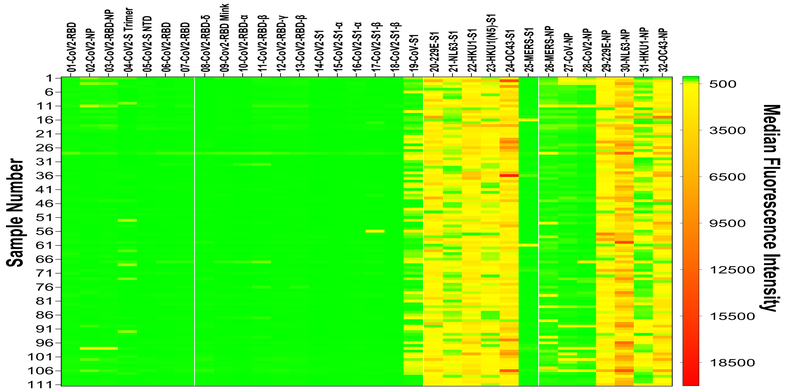

Figure 2: Antibody reactivity (MFI) to the CoV-2 and other HCoV protein antigens is shown in the Heat Map from 87 negative samples collected prior to COVID19 pandemic and 24 PCR negative samples collected during the pandemic show much lower reactivity to CoV-2 antigens. Most reactivity was observed against nucleocapsid (NP) antigens of HCoVs NL63 and 229E. Reactive antibodies to both S1 and NP antigens of HCoV-OC 43 were observed in most of the samples tested in this study. There was no reactivity to SARS-CoV and MERS antigens observed in this set of negative samples.

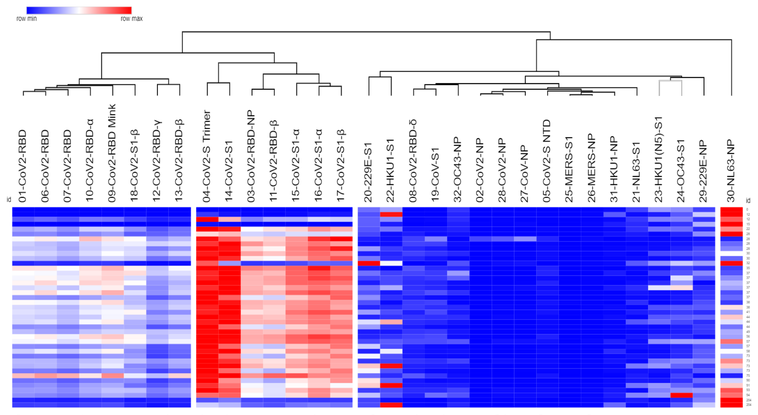

Figure 3: Hierarchical Cluster analysis of antibody profile after Pfizer vaccine in 41 samples collected between day 0 to day 204 shows that reactivity to various spike RBD wild type, α, β, and γ variant proteins cluster together, separated distinctly from CoV2 S1, Trimer, and NP proteins. Other HCoV, MERS and CoV antibody responses cluster with S-NTD and RBD-δ. The antibodies to NL63 NP protein are seen to be higher in all unvaccinated and vaccinated sample and cluster separately. Hierarchical cluster analysis was performed using Morpheus, a versatile visualization and analysis software; https://software.broadinstitute.org/morpheus

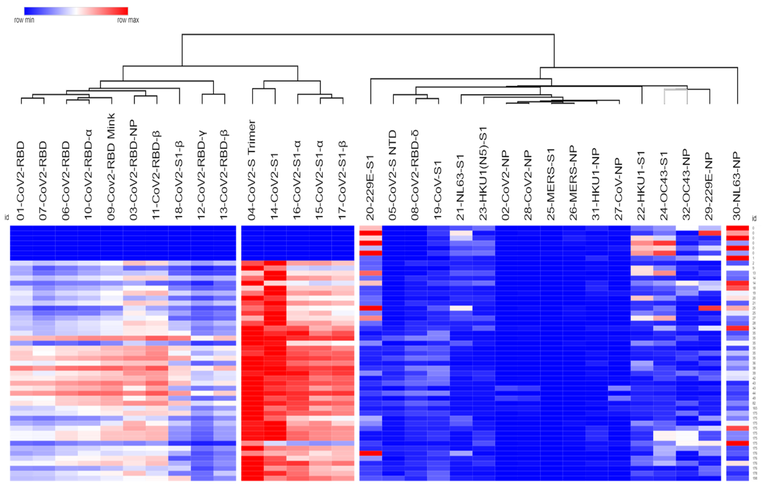

Figure 4: Hierarchical Cluster analysis of antibody profile after Moderna vaccine in 51 samples collected between day 0 to day 198 displays that reactivity to various CoV2 spike RBD wild type, α, β, and γ variant proteins cluster together, separated clearly from CoV2 S1, Trimer, and NP proteins. Other HCoV, MERS and CoV antibody responses cluster with S-NTD and RBD-δ. The antibodies to NL63 NP protein are seen to be higher in all unvaccinated and vaccinated sample and cluster separately. The time course clearly indicates a marked increase in antibody response to RBD and S1 proteins after second dose but it starts to wane over time. Antibody reactivity to CoV2 wild type protein is relatively higher than CoV2 variant proteins.

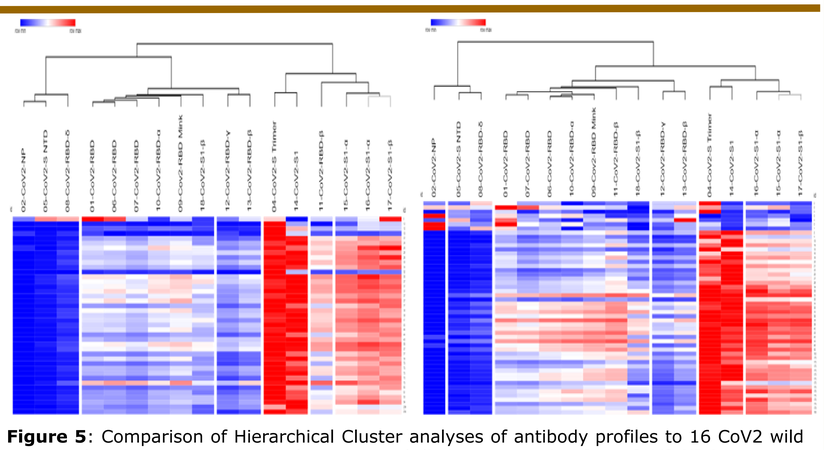

Figure 5: Comparison of Hierarchical Cluster analyses of antibody profiles to 16 CoV2 wild type, and variant spike proteins demonstrated distinct separation from CoV2 NP in samples after Moderna and Pfizer vaccination. Antibody reactivity to RBD proteins are more robust after Moderna vaccine compared to Pfizer vaccine in this data set. Both vaccines elicit minimum response to RBD-δ in comparison to other CoV2 RBD proteins. Higher reactivity to RBD protein directly related to S-NTD reactivity.

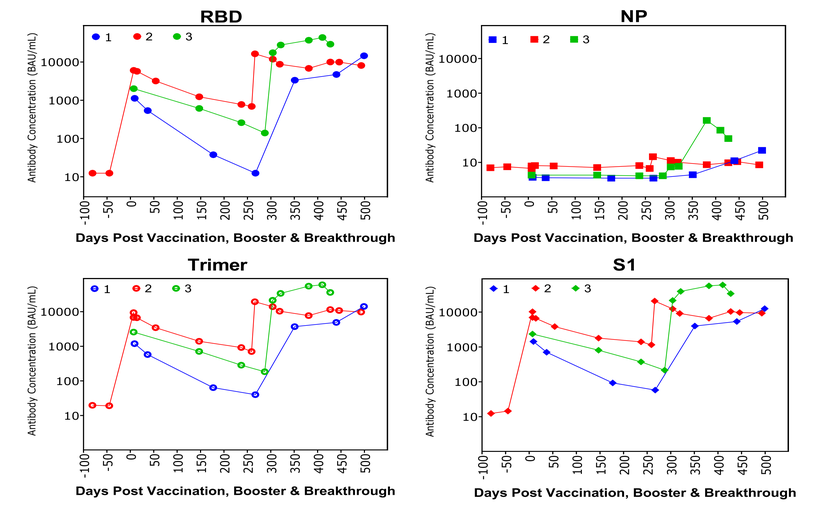

Figure 6: Multiplex assay was used to quantify IgG antibodies in binding antibody units (BAU) to CoV2 RBD, S1, Trimer and NP proteins. Testing longitudinal serial samples from 3 different individuals are shown in this Figure. Antibody reactivity to CoV2 RBD, S1 and Trimer proteins is robust after first two dose regimen of vaccine but waning is rather rapid. Increased antibodies were detected following booster and remained higher. Two individuals had breakthrough infections and increased levels of NP antibodies were measured in these sample after breakthrough infection.

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