A MULTIPLEXED ASSAY TO SIMULTANEOUSLY DETECT BINDING AND NEUTRALIZING ANTIBODIES AGAINST SARS CORONOVIRUS-2 AFTER INFECTION AND VACCINATION

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Background: The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused a significant burden on humanity's physical, psychological, and economic health over the last two and a half years. As we enter the third year of this pandemic, several vaccines and therapeutics have become available that target the spike protein S1 receptor binding domain (RBD) of the virus spike glycoprotein. The spike glycoprotein is a homo trimer of transmembrane protein consisting of binding S1 and transmembrane S2 domains. The SARS-CoV-2 enters host cells via the binding of RBD to the angiotensin-converting enzyme 2 (ACE2) receptor. Recent research has demonstrated that antibodies elicited against the RBD of the S1 domain after infection can cause inhibition of RBD-ACE2 binding and neutralize the virus. RBD is also a key target for the vaccine-induced circulating antibody response.

Methods: We have developed and evaluated a multiplexed assay to simultaneously detect binding and neutralizing antibodies against SARS-CoV-2 after infection and vaccination in serum and plasma samples. We immobilized the ACE-2 and trimeric spike proteins on magnetic microspheres to capture the antibodies from serum or plasma samples. The measurement of RBD and antibody binding is performed by biotinylated RBD protein and streptavidin-phycoerythrin conjugate. We have also included four internal controls microspheres to monitor the assay performance (Table 1).

We tested 208 samples from 161 unique individuals (Table 2). These include 79 PCR negative serum and plasma samples, 46 PCR positive samples collected from day 0– 372 days after onset of symptoms or a positive result, 67 uninfected vaccinated sera samples from 37 subjects, and 16 samples from 9 subjects with vaccine breakthrough infections (Table 3).

Results:

- Titration of positive control serum indicates that dilution of 1/50—1/800 give 10 and above percent inhibition and RFI of 10 and above (Figure1) . The cross sectional study with 208 samples was then performed at 1/50 dilution based on this data.
- Percent inhibition on ACE II binding microspheres was below 10% in all 79 negative samples. An empirical 15% inhibition was selected as cut-off for this study which will be confirmed by more testing. Using 15 % inhibition as the cut off 7of 20 samples collected aft 0-7days after PCR positive, 21 of 23 at 14-30 days, and only 1of 3 after >30 days showed positive neutralizing antibodies. 14 of 16 samples from vaccine break through infection showed >15% inhibition and the other 2 of16 showed 14% inhibition. Neutralizing antibodies and binding antibodies were higher after vaccination versus infection. (Figure 2)
- Longitudinal testing of samples from a single subject at 12 different time points after 4 days to 256 days of two dose Moderna vaccine followed by two boosters of Pfizer vaccine was showed that both neutralizing and binding antibody titers fall and then rise after the booster (Figure 3).
- Longitudinal study using 67 samples from 37 subjects shows that neutralizing antibodies and binding antibodies fall over time. Only 50 % of samples collected after 4 months of vaccination (11 of 22) showed positive neutralizing antibodies (Figure 4).

Conclusions:

- This multiplex assay can be performed in less than 3 hours to screen for neutralizing and binding antibodies in a sample.
- Future work is planned to evaluate the assay with more SARS-CoV-2 negative samples from other disease states.

Table 1: The microspheres and their functionality in the multiplex assay

#	Microsphere region	Immobilized protein	Functionality
1	27	Human ACE II	Human ACE II binds Receptor Binding Domain (RBD) S1 protein of the virus
2	29	SARS-CoV-2 Trimer	SARS-CoV-2 spike protein trimer, antigenic moiety targeted by most vaccines
3	45	Instrument control	Assures instrument performance in each well
4	64	Fluorescent Reporter control	Assures addition of fluorescent reporter in each well
5	65	Biotin Control	Assures addition of biotinylated RBD in each well
6	66	Non-specific binding Control	Indicates matrix effect

Table 2: Details of 208 samples tested in this study

Sample type	Number of samples	Unique individuals
Negative	79	79
Vaccine	67	37
Infection	46	36
Vaccine breakthrough infection	16	9
Total	208	161

Table 3: Details of various positive infected and vaccinated samples tested in this study

Infection			Vaccination	
Days post hospitalization or PCR positive	Number of samples	Vaccination Breakthrough Infection	Time after vaccination	Number of samples
0-7	20	-	0 - 1 month	26
8-30	23	8	1 - 3 month	9
31-90	2	4	4 - 6 month	16
> 90	1	2	7 - 9 month	6
Unknown	-	2	11 - 14 month	10
Total	46	16	Total	67

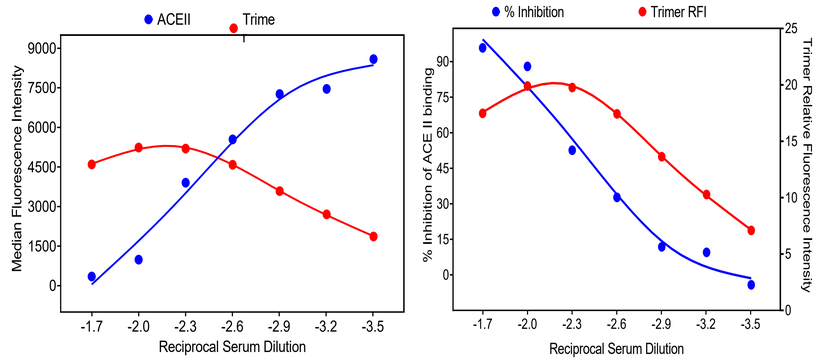

Figure 1: Titration of positive control serum– serial dilution of serum from 1/50 to 1/3200; Left — MFI values observed on both ACEII and Trimer coupled microspheres; Right — Percent inhibition of RBD binding to ACE II coupled microspheres and relative fluorescence intensity (RFI) on trimer coupled microspheres with respect to negative control.

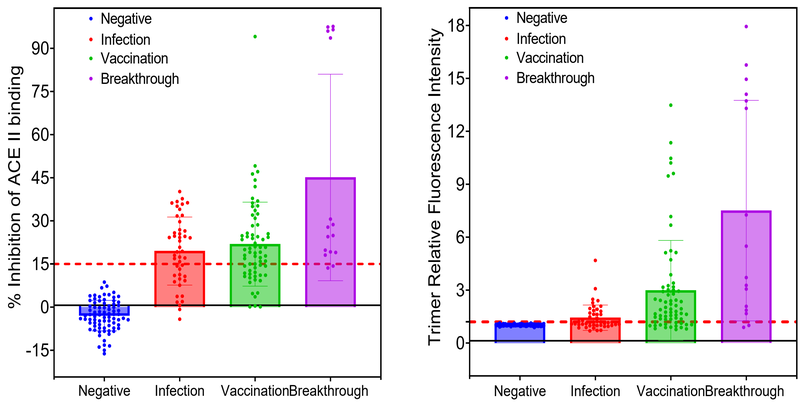

Figure 2: We observed that there was higher by antibodies generated after inhibition after vaccination than after infection. Higher inhibition is also directly correlated with a rise in binding antibodies to trimer.

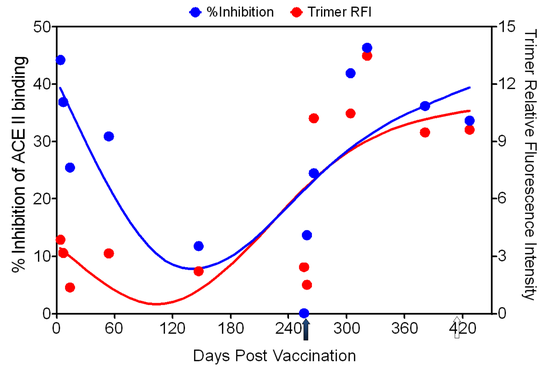

Figure 3: Longitudinal study on a vaccinated subject shows that percent inhibition of RBD binding to ACE II and antibody binding to trimer both decrease over time post vaccination. Subject received boost after 256 days of vaccination and both parameters went higher again. The boost also seems to have maintained the inhibition and binding ability of antibodies longer. Two arrows on X-axis indicate first boost at day 256 & second boost at day 417.

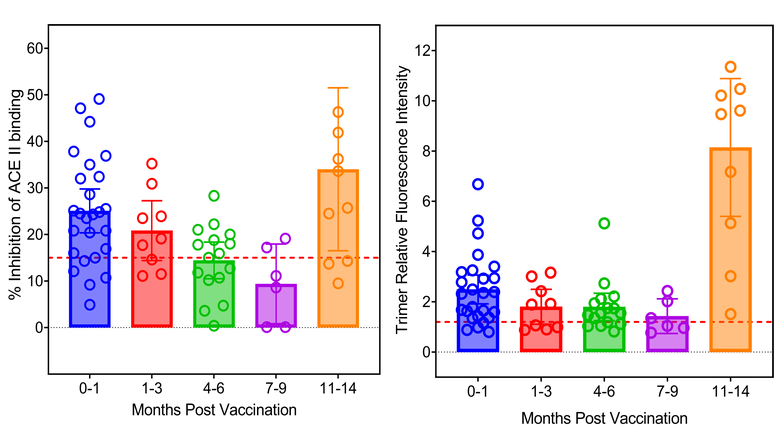

Figure 4: Longitudinal study using 67 samples as described in Table 2 shows that percent inhibition of RBD binding to ACE II and antibody binding to trimer both decrease over time post vaccination. The 10 samples in 11-14 month group were from individuals who received booster.