Human serum IgG antibody profiling for assessment of SARS-CoV-2 infection and COVID-19 vaccination

N. Venkateswaran, J. Walker, J. Sarwar, W. M. Nelson, K. Venkateswaran ; Tetracore, Inc., Rockville, MD

ASM MICROBE
JUNE 9-13 2022 | WASHINGTON, DC

Background: About two and half years after the onset of the COVID 19 pandemic, SARS-CoV-2 (CoV2) is still causing a significant burden on public health globally. Vaccines have now proven to be an effective tool to curb the spread of this virus. In this scenario of infection and vaccination, it is imperative to understand the extent of the immune response at both individual and population levels. Assessment of circulating IgG antibodies to multiple antigens, both wild type, and variants, can provide a snapshot of immune signature in infected and vaccinated individuals.

Methods: We developed multiplex assays based on Luminex® xMAP® technology to estimate circulating IgG antibodies to multiple recombinant proteins from wild-type and variant CoV2 and other human coronaviruses (HCoV). The 7-plex assay has three wild-type CoV2 specific antigens, CoV2 Receptor Binding Domain, Nucleocapsid Protein (NP), and a hybrid RBD-NP protein for detecting human IgG antibodies. Another expanded 36-plex assay has 32 viral antigens. These assays include four internal controls to monitor each step of assay performance. We tested 242 serum samples (102 negative, 87 infected, and 53 vaccinated) using the 7-plex panel and 234/242 samples using the 36-plex panel. Longitudinal samples from 6 infected and six vaccinated subjects were tested.

Results and conclusions: The 7-plex assay shows high sensitivity and specificity for detecting CoV2 IgG antibodies from infected samples. The IgG response to NP proteins in infected and vaccinated serum samples helps differentiate vaccinated from infected. The 36-plex expanded panel includes ious spike and NP antigens from four common-cold HCoV. Although pre-pandemic negative samples showed prevailing IgG responses to ious endemic HCoV, we only observed the CoV2 specific antibodies in the sera from the pandemic, indicating recent human population exposure to this virus. Every individual displayed a unique antibody signature. We did not see significant cross-reactivity of the IgG to HCoV antigens with CoV2 antigens. IgG response to 19 different CoV2 antigens, including ten variant proteins, was unique to the subjects. Vaccine-induced IgG antibodies were reactive with both wild-type and variant antigens. The antibodies to variant proteins are more reactive in vaccinated than naturally infected individuals.

Table 1: Three immobilized proteins from SARS-CoV-2 in 7-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 RBD-NP hybrid	CoV2-RBD-NP	36

Table 2: Thirty-two immobilized proteins from SARS-CoV-2 and other human corona viruses in 36-plex assay

#	Microsphere coupling functionality	ID	Region
1	SARS-CoV-2 Receptor Binding Domain	CoV2-RBD	25
2	SARS-CoV-2-Nucleocapsid Protein	CoV2-NP	28
3	Sars-CoV-2-Trimeric Spike protein	Cov2-S Trimer	29
4	SARS-CoV-2 S1-N-terminal domain (NTD)	33-CoV2-S1-NTD	33
5	SARS-CoV-2-S1 Protein	S1-CoV2	44
6	SARS-CoV-2-Nucleocapsid Protein	CoV2-NP	63
7	SARS-CoV-2 RBD-NP hybrid	CoV2-RBD-NP	36
8	SARS-CoV-2 Receptor Binding Domain	CoV2-RBD	26
9	SARS-CoV-2 Receptor Binding Domain	CoV2-RBD	30
10	SARS-CoV-2 variant RBD-L452R, E484Q	RBD-Delta	22
11	SARS-CoV-2 variant RBD-Y453F Mink	CoV2-RBD Mink	27
12	SARS-CoV-2 variant RBD-N501Y	RBD-Alpha	38
13	SARS-CoV-2 variant RBD-K417N	RBD-Beta	39
14	SARS-CoV-2 variant RBD-K417T, E484K, N501Y	RBD-Gamma	72
15	SARS-CoV-2 variant RBD-E484K	RBD-Beta	43
16	SARS-CoV-2 variant S1 D614G	CoV2-Alpha	34
17	SARS-CoV-2 variant S1-HV69-70del, N501Y, D614G	CoV2-S1 Alpha	35
18	SARS-CoV-2 variant S1-H69del, V70del, Y144del, N501Y, A570D, D614G, P681H	CoV2-S1-Alpha	37
19	SARS-CoV-2 variant S1-K417N, E484K, N501Y, D614G	CoV2-S1-Beta	42
20	S1 – SARS-CoV	S1-CoV	45
21	S1– HCoV-229E	S1-229E	46
22	S1– HCoV-NL63	S1-NL63	48
23	S1 – HCoV-HKU1	S1-HKU1	51
24	S1 – HCoV-HKU1(isolateN5)	S1-HKU1(N5)	55
25	S1– HCoV-OC43	S1-OC43	56
26	S1-MERS –CoV	S1-MERS	57
27	NP – MERS-CoV	NP-MERS	61
28	NP – SARS-CoV	NP-SARS	62
29	NP – HCoV-229E	NP-229E	64
30	NP – HCoV-NL63	NP-NL63	65
31	NP – HCoV-HKU1	NP-HKU1	66
32	NP – HCoV-OC43	NP-OC43	67

Table 3: Four controls included in both the 7-plex and 36 plex assays for monitoring the assay performance

#	Microsphere coupling functionality	ID	Region
1	IC (Instrument control)	IC	47
2	NC (Non-specific binding control)	NC	54
3	ScG (Human IgG Sample control)	ScG	52
4	FC (Fluorescent Reporter control)	FC	53

Table 4: Details of the serum samples used in this study

Description	Number of Serum samples	Number of individuals	2 or more time points	Median age (years)	Male	Female	Un-known
Infection	87	68	6	46	34	30	4
Vaccination	53	32	6	-	11	9	12
Negative	102	102	-	36	23	79	-
Total	242	202	12	-	68	118	16

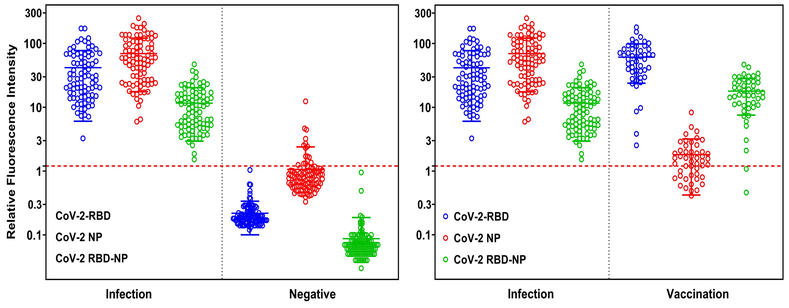

Figure 1: Comparison of IgG response to three different antigens in 7-plex assays, Infection Vs Negative; and Infection Vs Vaccination

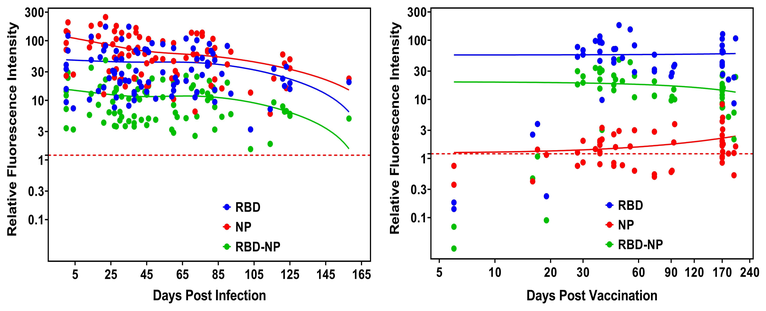

Figure 2: We observed waning of IgG antibody more prominently in infected individuals over a period of 6 months compared to vaccinated individuals.

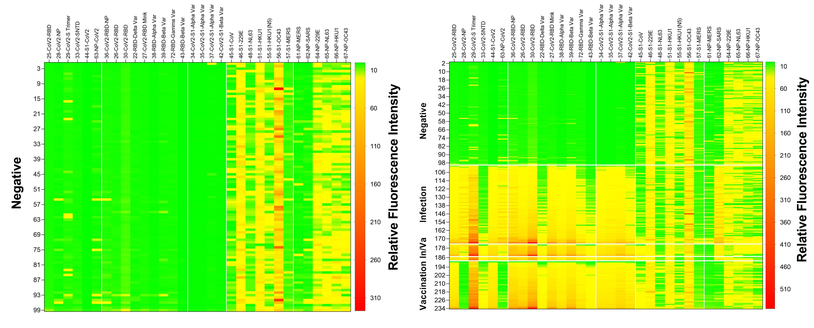

Figure 3: a) Data from 99 negative samples collected from 2017-2018 show remarkably low reactivity to CoV-2 antigens. Most reactivity was observed to nucleocapsid antigens of human corona viruses in the negative samples. Reactive antibodies to both S1 and NP antigens of HCoV-OC 43 were observed in most of the samples tested in this study. b) Expanded panels indicate that humans have experienced SARS-CoV-2 exposure only during recent pandemic.

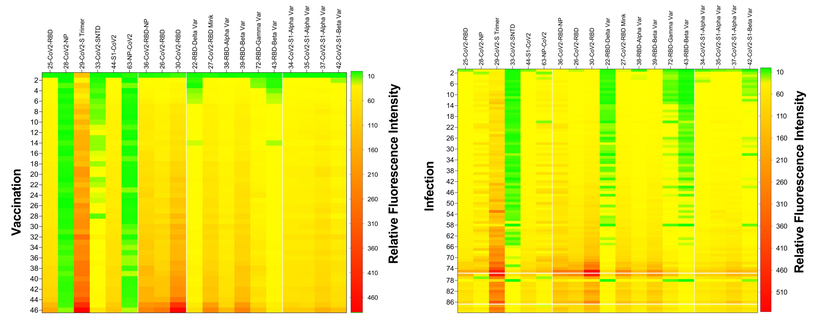

Figure 4: Response to the trimeric S protein, S1-NTD and S1 antigens is higher in vaccinated individuals compared to PCR positive subjects. The fully vaccinated individuals showed reactive antibodies to all the variant proteins.

Concluding remarks:

1) 7-plex assay provides a useful tool to detect and differentiate antibody response after infection and vaccination. It can be useful for detection of exposure to COVID 19 both at individual and population level.
2) The expanded panels provide 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 prevailing common colds causing human corona viruses.
3) Utilization of expanded panels in conjunction with 7-plex panel can be very useful in retrospective studies to reconstruct the evolution of virus and its interaction with human immune system.