“IgG Serum Antibody Responses against SARS-CoV-2 in Patients and Healthcare Workers”

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Background

The US and the world are in the midst of a pandemic caused by the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS- COV-2, clinical syndrome called COVID-19). This virus spreads through respiratory transmission and appears to be quite infectious with an R_0 of approximately 3.0. At present, there is limited data on serum antibody presence and responses from patients who have had COVID-19. Currently, most serological tests for SARS-CoV-2 provide a Yes/No response rather than a serum titer (quantity) response. Serological testing has the potential to ascertain answers to many critical questions regarding antibody protection from reinfection over time. The goal of this study is to determine the serum antibody responses to the SARS-CoV-2 receptor binding domain (RBD) and the nucleocapsid protein (NP) in 3 cohorts: Confirmed COVID+ patients, Self-reported COVID+ patients and health care workers

Methods

Clinical: Patient were enrolled after informed consent, 10 ccs of blood collected, and a questionnaire administered about demographics and COVID exposure. Serum was separated in a BSL2+ facility in full PPE, and serum was inactivated by incubation at 56°C for 30 minutes.

ELISA protocol: Immunolon 2 plates were coated with RBD (Spike) and N protein at 0.1mcg/well in 0.9M Na carbonate buffer, pH=9.5 (RayBiotech) and incubated overnight at 4 °C. Washed 3 times with phosphate buffered saline (PBS). Blocked with 100µL/well of blocking buffer (90mL Tris-buffered saline + 10mL 10% goat serum + 0.5 mL 0.5% Tween) at RT for 1 hour. For seropositivity assays, 100µL/well of the 1:100 sample serum dilution in blocking buffer were added and incubated at RT for an hour. Titer assays started at 1:10 dilution followed by 2-fold dilutions in blocking buffer until a dilution of 1:10240 and incubated at RT for an hour. Plates were washed, and goat-anti-human IgG conjugated to alkaline phosphatase (Invitrogen) was added to the plate and incubated at RT for an hour. Plates were developed using Sigma 104phosphatase substrate and read at 450nm on a Biohit BP800 ELISA plate reader.

Statistical Methods: Two-sample t-tests and two-sample z tests were used to analyze the data.

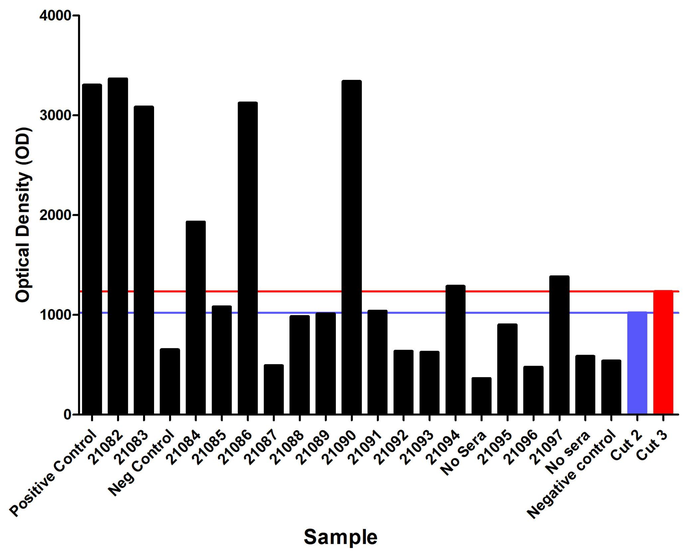
Results

Table 1: Subject Demographics

	Confirmed Positive	Self-reported Positive	Healthcare worker (HCW)	Total
Total	**32 (26%)**	**17 (14%)**	**76 (61%)**	125
Gender				
Male	17 (53%)	7 (41%)	33 (43%)	**57 (46%)**
Female	14 (44%)	10 (59%)	43 (47%)	**67 (54%)**
Race				
White	18 (56%)	11 (65%)	64 (84%)	**93 (74%)**
African American	12 (38%)	3 (17.5%)	6 (8%)	**21 (17%)**
Other	2 (6%)	3 (17.5%)	6 (8%)	11 (9%)
Age (in years)	**Mean 46**			
21-40	7 (22%)	7 (41%)	34 (45%)	48 (38%)
41-60	18 (56%)	6 (35%)	26 (34%)	50 (40%)
61+	6 (19%)	3 (18%)	16 (21%)	25 (20%)
RBD Response				
Seropositive	**27 (84.4%)**	**8 (47%)**	**3 (4%)**	**38 (30%)**
Average titer	**1:622**	**1:1154**	**1:1097**	**1:716**
Intermediate	4 (12.5%)	3 (18%)	13 (17%)	20 (16%)
Seronegative	1 (3.1%)	6 (35%)	60 (79%)	67 (54%)
NP Response				
Seropositive	**25 (78%)**	**6 (35%)**	**2 (3%)**	**33 (26%)**
Average titer	**1:1774**	**1:6400**	**1:1280**	**1:1989**
Intermediate	2 (6%)	3 (18%)	12 (16%)	17 (14%)
Seronegative	1 (3%)	7 (41%)	61 (80%)	69 (55%)

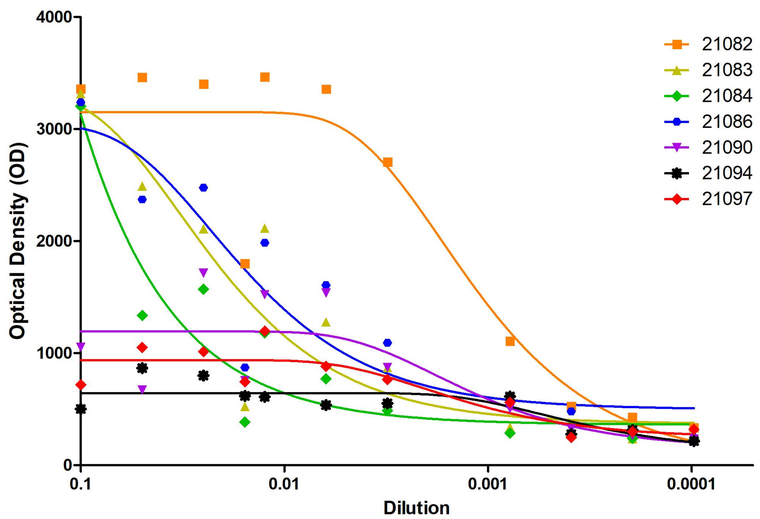
RBD positivity rates higher than NP but not statically significant. Confirmed positive higher rates than Self-reported and higher rates than HCW ($p<0.001$). Titers in the seropositive not statically significant in the 3 groups or vs. NP.

Figure 1: SARS-CoV-2 Nucleoprotein IgG ELISA Results



450nm wavelength absorbance of subject samples’ IgG reactivity to NP measured by optical density (OD). Cut 2 (blue) and Cut 3 (red) were determined as 2 to 3 standard deviations above the average negative control, respectively. OD values above Cut 3 were characterized as seropositive. Those close to the cut point visually were characterized as “intermediate”.

Figure 2: SARS-CoV-2 Nucleoprotein IgG End Point Dilution Titer



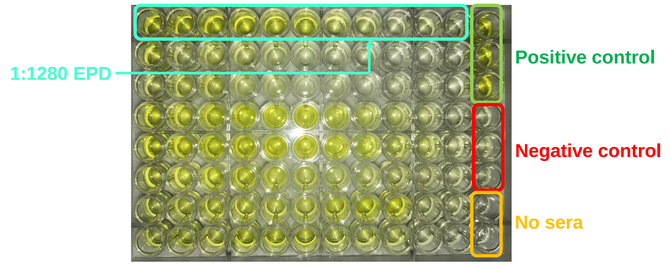
Subject samples’ titer IgG reactivity to NP measured by optical density (OD) at 450nm absorbance. 21082 shows a classic strong seropositive titer result (see Figure 2): plateau then a fast decay with an EPDT of 1:10240. 21084, however, had a relatively strong response in seropositivity but had a low EPDT of 1:640 – an intermediate subject. Cut point was determined as 3 standard deviations above the average negative control.

Table 2: Concordant and Discordant RBD and NP Responses

	RBD Seropositive	RBD Seronegative
NP Seropositive	29	1
NP Negative	1	58

Boxes in purple indicate the number of samples that had the same seropositivity results for RBD and NP (98% concordance overall in definitive seropositives and seronegatives). Boxes in yellow indicate the number of samples that had discordant seropositivity results for RBD and NP.

Figure 3: Example of an ELISA antibody titer against NP



The first column of wells contains sera at a 1:10 dilution in blocking buffer, and each subsequent well in the same row is a two-fold dilution of the previous well. The top row has an EPD titer of 1:1280.

Conclusions

- High rates of seropositivity in those with confirmed COVID-19 infections.
- HCW had relatively low rates of seropositivity
  - In range of other studies (5% in the Ochsner Medical System in the New Orleans area)
- Seropositivity to RBD was greater than NP across all three groups, but this was not statistically significant.
- All three groups were statistically different for % RBD seropositivity and for % NP seropositivity ($p<0.001$ in both).
- In those who were seropositive for RBD or NP, there was no significant differences seen in the titers generated

- In the future, this study will follow these subjects over time to see how their antibody responses change. We will also analyze how their seropositivity to both RBD and NP relate to the subjects’ clinical conditions.