Correlation of Antibody Levels with SARS-CoV-2 Virus Neutralization

Amy Jacobs, PhD[†]; Sean Riley[†]; Shazeda Chowdhury[†]; Lauren Smith[†]; Long Shen, PhD[‡]; John Rushton, PhD[‡]; Lakshmanan Suresh DDS, MS, PhD[‡]

[†]University at Buffalo – The State University of New York [‡]KSL Diagnostics

Background

- The humoral immunity is characterized by the production of antibodies by B cells as a response to antigens. Immunoglobulin IgM appear between 2-4 days of infection but has a short half-life. IgA is most abundant in mucosal surfaces but can also be found in serum and detected within the first week of symptom onset. IgG is the most abundant antibody type and provides longer-lasting immunity. IgG is seen in circulation from about 7 days onwards (Long et al. 2020). This response of immunity is also true for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). IgG titers remain stable for at least 4 to 6 months following diagnosis among COVID-19 polymerase chain reaction (PCR)-confirmed individuals, whereas IgA and IgM titers rapidly decay.
- Antibodies targeting the spike glycoprotein of the SARS-CoV-2, especially the receptor binding domain (RBD) within the S1 subunit, show the highest neutralizing capacity. The presence of neutralizing antibodies is considered a functional correlate of immunity and provides at least partial resistance to subsequent infections by virus antigen binding to prevent interaction with host cells. Although some serological assays showed a high correlation between IgG and neutralizing antibodies (Geurts Van Kessel et al., 2020), other assays have poor correlation (Focosi et al., 2020). Therefore, comparison with virus-neutralizing tests is important as part of the validation of new serological assays.
- Several laboratory-developed and commercially available assays utilizing various technology platforms are available to detect anti-SARS-CoV-2 spike antibodies. While these platforms provide a high-throughput means of detecting antibodies against SARS-CoV-2, they are unable to measure the immunological function of SARS-CoV-2-specific antibodies. In contrast, the plaque-reduction neutralization test (PRNT) quantifies levels of neutralizing antibodies capable of blocking the interaction that mediates virus entry into susceptible host cells and subsequent virus replication (Valcourt et al., 2021). For SARS CoV-2, this interaction involves binding of the receptor binding domain (RBD) of the SARS-CoV-2 spike glycoprotein with the angiotensin-converting enzyme 2 (ACE2). While the conventional PRNT is often used as the reference standard for the evaluation of virus- neutralizing antibodies, this assay is time-consuming and laborious and requires biosafety containment level 3 (BSL-3) facilities to work with the risk group-3 pathogen. As such, this is not practical for large scale community testing.

Aim

- We sought to compare the semi-quantitative titers of our laboratory-developed SARS-CoV-2 spike antibody assay against the PRNT to evaluate if there is a specific circulating antibody titer needed for optimal virus neutralization.

Materials and Methods

- The samples age groups from 18 years old and over and both sexes. All samples will be tested for PRNT. Only samples with varying IgG and IgA titers were included. IgM was not included in the study because most samples will lack IgM at 15 days post 2nd vaccination dose. The study utilized the delta strain of COVID-19 to perform the PRNT. The studies were conducted at the BSL-3 facility located in the Jacobs School of Medicine, University at Buffalo.

IgG and IgA – Sample Size 110 (5 samples/Group)
(55 Moderna, 55 Pfizer – All with 2nd dose)

Group	COI* value range
1	<0.5
2	0.5 to <1.0
3	1.0 to 2.99
4	3.0 to 5.99
5	6.0 to 9.99
6	10.0 to 14.99
7	15.0 to 19.99
8	20.0 to 24.99
9	25.0 to 29.99
10	30.0 to 39.99
11	40.0+

*COI = Cut-off Index

10 samples per group of different ages were tested at day 180 after vaccination (Sample Size 120 – 60 Moderna, 60 Pfizer)

Group	Age and Gender
1	Age 18-40, Male
2	Age 18-40, Female
3	Age 40-65, Male
4	Age 40-65, Female
5	Age 66+, Male
6	Age 66+, Female

The SARS-CoV2 virus strains studied were:

- Wild type
- Alpha (B.1.1.7)
- Beta (B.1.35.1)
- Gamma (P.1)
- Delta (B.1.617.2)
- Omicron (B.1.529)
  – Subvariant BA.1**
  – Subvariant BA.2**

**studies ongoing

- In brief, the serum samples are diluted in a 96-well plate, before SARS-CoV-2 virus is added to the diluted serum at BSL-3 and neutralization allowed to occur. The neutralized virus is then transferred onto Vero E6 cells, allowed to adsorb, overlaid with viscous medium and incubated for 24 hours. Plates are then fixed virus infected foci immune-stained, and foci counted. Well counts are then analyzed with SoftMax Pro to determine median neutralization titers of the reference and test sera (Bewley et al., 2021).

Study Results

- **IgG:**
  — There was robust virus neutralization at COI values of 20 and above, suggesting adequate humoral immunity
  — Between COI values of 10-20 there was suboptimal neutralization of virus
  — Below antibody COI values of 10 there is impaired neutralization of virus
  — The neutralization is consistent across all strains studied to date

Group Number	COI Value Range	Reciprocal of PRNT50 Average	Conclusion
1 – 5	0 – 9.99	Same as Virus Control	No Neutralization
6 – 7	10 – 19.99	115.7	Suboptimal Neutralization
8 – 10	20 – 39.99	372.0	Complete Neutralization
11	> 40	356.6	Complete Neutralization

Table: IgG COI range, PRNT averages and conclusion

- There were no significant findings for IgA

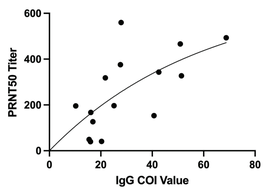

Figure 1: Plaque reduction neutralization assay (PRNT50) correlated to IgG value (COI). Viral titer of 6x103 pfu/mL (30 plaques/well) added to VeroE6 cells and incubated for 3 days before overlay and staining

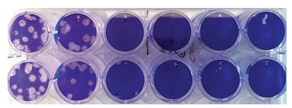

Figure 2: Example of plaque reduction neutralization test (PRNT) performed in duplicate. Overlay fixed with 4% paraformaldehyde and stained with crystal violet.

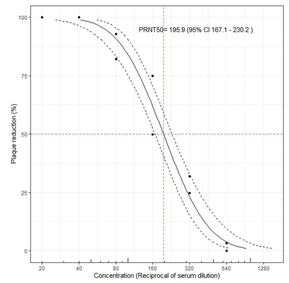

Figure 3: Representative PRNT50 analysis performed according to Bewley, et al

Conclusion

- Correlation of antibody titers with plaque reduction neutralization shows that there is 100% neutralization of the SARS-CoV-2 virus above IgG titers of 20
- IgG titers from 10 – 20 reduce effective virus neutralization by 25%
- Below IgG titers of 10, the effectiveness of virus neutralization decreases considerably
- Negative results for IgG indicate a lack of circulating antibodies that could represent a lack of immunity against the SARS-CoV-2 virus
- Additional testing with a larger cohort is needed to confirm the findings and applicability to the general population

Selected References: (1) Bewley, et al, *Nature Protocols* volume 16, pages 3114–3140 (2021). (2) Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020; 26:845e8. (3) Suresh L. Six-month longitudinal antibody kinetics of mRNA COVID-19 vaccines. *Rheumatology & Autoimmunity* 15 September 2021 https://doi.org/10.1002/rai2.12005. (4) Valcourt EJ, Manguiat K, Robinson A, Lin Y-C, Abe KT, Mubareka S, et al., 2021. Evaluating humoral immunity against SARS-CoV-2: validation of a plaque-reduction neutralization test and a multi laboratory comparison of conventional and surrogate neutralization assays. Microbiol Spectr 9: e00886-21 https://doi.org/10.1128/Spectrum.00886-21.

University at Buffalo
The State University of New York

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