

Understanding Your Test Results

What tests did you do?

Antibodies are made by your body to fight infections. Usually, they are made in response to either past infection by a virus or vaccination. This study checked 3 things:

- The **SARS-CoV-2 Spike IgG** test checks the level of COVID-19 antibodies in your blood.
- The **SARS-CoV-2 Spike ACE2** test looks at how much of the original COVID-19 strain would be blocked by your antibodies.
- The **SARS-CoV-2 Omicron Spike ACE2** test estimates how much of the Omicron strain of COVID-19 would be blocked by your antibodies.

What do these results mean? What levels are “normal”?

The **SARS-CoV-2 Spike IgG** test shows the level of COVID-19 antibodies you had in your blood when you gave the blood sample. Usually your antibody levels will go up after getting a vaccine or having an infection. Having more antibodies means your body can fight infection better than having fewer antibodies. A previous study found that on average, people had antibody levels of around **1,000,000 AU/mL** 1 week to 2 months after their vaccination, and around **10,000 AU/mL** 3-4 months after a COVID-19 infection.

The **SARS-CoV-2 Spike ACE2** test estimates how much of the original COVID-19 strain would be blocked by your antibodies. A previous study found that antibodies in people 1 week to 2 months after vaccination blocked an average of **67.9%** of the virus-cell interactions, and **12.2%** of the interactions were blocked by antibodies in people about 3-4 months after natural infection.

The **SARS-CoV-2 Omicron Spike ACE2** test estimates how much of the Omicron COVID-19 strain would be blocked by your antibodies. We do not know how much antibodies against one strain of COVID-19 protect people against other strains; that is one of the things we hope to learn from this study.

Do my results mean I am immune to COVID-19?

Having antibodies does not always mean that you are immune to COVID-19. One of the goals of this study is to determine which of these three tests best predicts protection from infection and/or serious illness from COVID-19. At this time, please continue to follow the recommended public health guidelines to protect yourself and your loved ones.

How can I learn more about antibody testing?

You can find a more detailed scientific explanation of the ZAP COVID study further down this page. In addition, the CDC has resources on antibody testing at the links below:

- For patients: <https://go.ucsd.edu/3MSuHEb>
- For healthcare providers: <https://go.ucsd.edu/34ATNWK>

As we continue to learn more from this research, we hope to understand more about COVID-19, antibodies, and vaccines in the UC San Diego community. Thank you for participating in this study--your contribution has made a big impact!

For a more detailed, scientific explanation, please see below.

Two types of antibody results are being provided in the ZAP study: IgG and ACE2.

Immunoglobulin G (IgG) is a commonly reported antibody that helps fight viral and bacterial infections, and is produced by the human body after infection or vaccination. A higher level of IgG antibodies for a particular virus often means a greater degree of protection against infection by that virus.

The ACE2 neutralizing antibody test measures active antibody levels and is reported as a percentage. The reported percentage estimates the percentage of the virus that would be controlled (neutralized) by a person's immune system. A higher ACE2 value may indicate greater protection against infection. In a prior study using the same measurement as the ZAP study, ACE2 inhibition averaged 67.9% 1 week to 2 months after vaccination, and 12.2% about 3-4 months after natural infection.

What do these results mean?

Results provided measure antibody levels to proteins produced by the original strain of the SARS-CoV-2 virus (SARS-CoV-2 Spike IgG and SARS-CoV-2 Spike ACE2), and by the Omicron strain of the SARS-CoV-2 virus specifically (SARS-CoV-2 Omicron Spike ACE2).

Spike IgG: This standard antibody test measures the level of immunoglobulin G (IgG) that binds to the spike protein on the surface of the SARS-CoV-2 virus. The spike protein of the SARS-CoV-2 virus allows the virus to attach to angiotensin converting enzyme 2 (ACE2) receptors on the surfaces of cells and infect them. IgG is a common antibody that helps fight viral and bacterial infections, and is produced by the human body after infection or vaccination. A higher level of IgG antibodies to proteins for a particular pathogen is often correlated with a greater degree of protection against infection by that pathogen.

ACE2: The ACE2 neutralizing antibody test measures active neutralizing antibody levels to SARS-CoV-2. Results are reported as percentages. The value estimates the percent of the virus expected to be controlled (neutralized) by a person's immune system when faced with the virus. A higher ACE2 inhibition value may indicate greater protection against infection.

For more information about understanding antibody testing, please see:

<https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html>.

Putting results in context - How do these values relate to what is expected?

The results below show that IgG and ACE2 levels increase after vaccination. These results use the MSD assay (the same assay used in the ZAP study) in samples collected from 1 week to 2 months following vaccination.

Serum samples from 11 paired samples collected before and after vaccination from previously infected donors were examined. The average time between the positive PCR result and the last symptoms was 16.7 days. The first dose of the vaccine was administered on average 142.4 days after the last symptoms. The collection time ranged from 8 to 58 days after the first dose of the vaccine. Five post-vaccination samples were collected after the first dose, and six after the second dose. Five donors had received mRNA-1273 (Moderna) while six had received BNT162b2 (Pfizer) vaccines. The interval between doses was 21 days for Pfizer and 28 days for Moderna.

Both the IgG and ACE2 levels increased in previously infected patients after vaccination (Figure 2 from Reference 1). All samples showed low inhibitory activity against each of the variants following natural infection (mean ACE2 inhibition across all variants=12.2%, ± 2.29). After vaccination, inhibition levels were 4- to 7-times higher, and ACE2 inhibition was 67.9% ± 10.7 on average against all tested variants. Results generated using the same assays that are used for the UCSD ZAP COVID19 study are indicated in the red boxes.

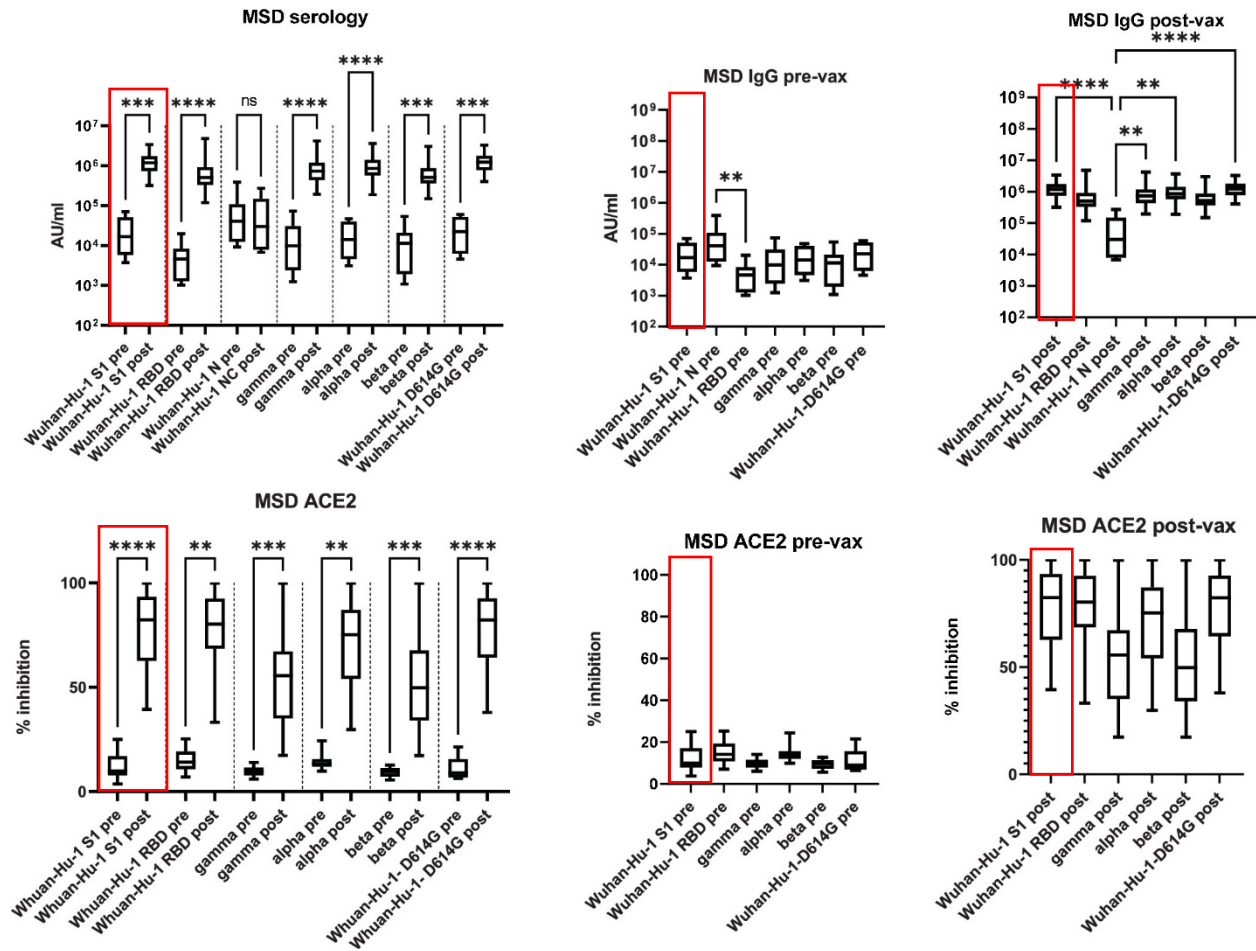


Figure 2 from Reference 1: Binding IgG and ACE2 inhibition measured on the MSD V-Plex. The AU/ml IgG increases for all samples collected after vaccination, for both S1 and RBD of the Wuhan-Hu-1 (the original SARS-CoV-2 virus), but not for N of the same virus. Antibodies against all of the tested variants increased after vaccination in the same way as for the Wuhan-Hu-1 strain. The percentage of ACE2 inhibition increased for all samples collected after vaccination, for Wuhan-Hu-1 S1 and RBS and all the variants tested. There were no significant differences among any of the variants, either before or after vaccination. (** = 0.001, *** = 0.0005, **** = <0.0001). n = 11.

Validation of assays used in the present study

How well does the MSD SARS-CoV-2 IgG assay estimate virus neutralization?

The receptor-binding domain (RBD) is part of the spike protein on the surface of the SARS-CoV-2 virus that allows the virus to attach to cells. RBD IgG levels are highly correlated with spike IgG levels. Results below show that RBD IgG levels were significantly correlated with cell-based virus neutralization levels in both previously infected and vaccinated persons. These results suggest that RBD IgG levels may indicate functional immunity to SARS-CoV-2.

This study compared antibody binding and live virus neutralization to two SARS-CoV-2 variants in patients acutely infected with SARS-CoV-2 acutely (5-19 days after symptom onset, $n = 13$), after infection (up to 8 months after onset, $n = 30$), and individuals who were vaccinated against SARS-CoV-2 (14 days after second Moderna dose, $n = 19$).

Among both acutely infected COVID-19 patients (Figure 1C) and convalescent COVID-19 patients (Figure 1K), and in vaccinated individuals (Figure 2C), RBD IgG values were highly correlated with cell-based neutralization test results.

Figure 1 from Reference 2:

Correlation with cell-based Focus Reduction Neutralization Test (FRNT) results during acute and convalescent SARS-CoV-2 infection (Figure 1C [acute, 5-19 days after symptom onset] and Figure 1K [convalescent, 1-3 and 3-8 months after symptom onset]).

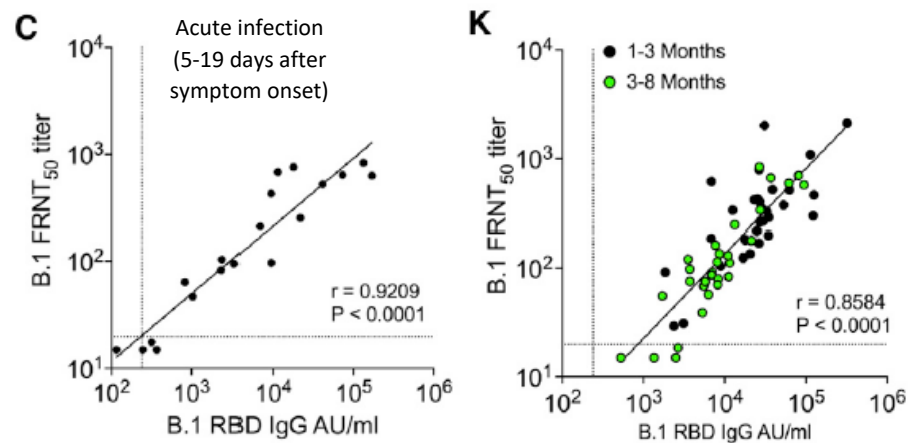
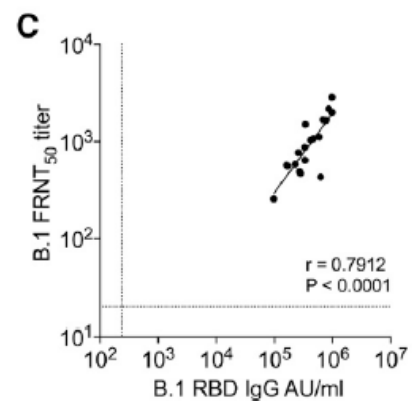


Figure 2 from Reference 2: Correlation with cell-based Focus Reduction Neutralization Test (FRNT) results 14 days after mRNA-1273 SARS-CoV-2 vaccination (Figure 2C [correlation between the MSD SARS-CoV-2 RBD IgG assay and FRNT]).



Validation of use of dried blood spots (DBS)

How well does the MSD SARS-CoV-2 Spike ACE2 assay work for finger-stick dried blood samples?

These results show that results of the MSD Spike ACE2 assay performed on dried blood spot samples, as performed in the ZAP study, are similar to results from venous blood draws, which have been used in many previous studies. Thus, results should be similar to what would have been seen if a venous blood draw had been used.

To evaluate the validity of assessing Spike ACE2 results using dried blood spots (DBS), researchers collected matched DBS and serum samples in 20 individuals previously infected with SARS-CoV-2 and 10 known SARS-CoV-2 negative individuals. Results indicated a high level of agreement in percent neutralization estimates between serum and DBS samples (Figure 2).

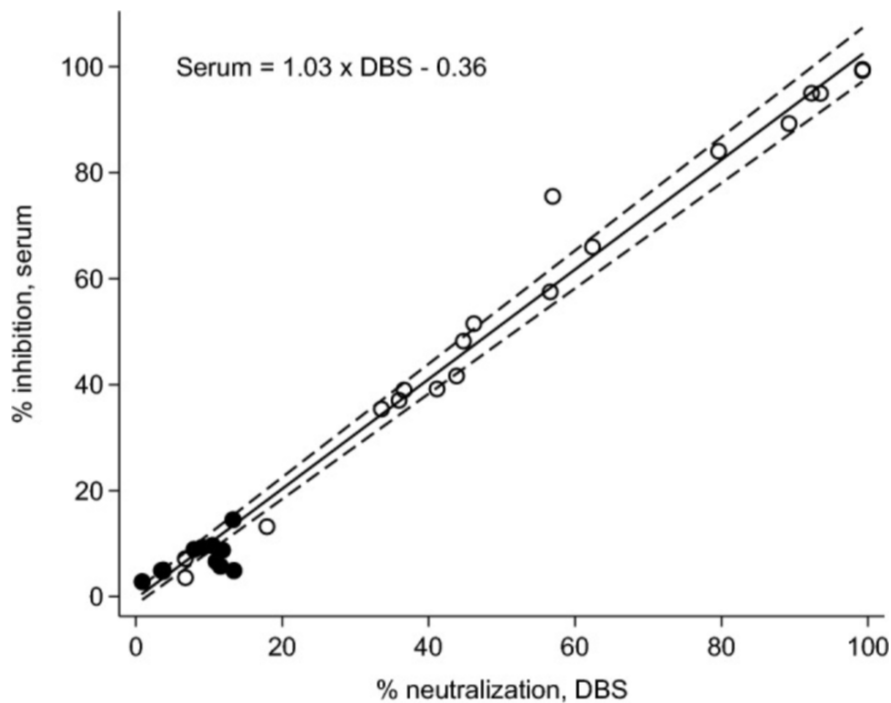


Figure 2 from Reference 3: Agreement in neutralization results for matched serum and DBS samples. Scatterplot and Passing-Bablok regression line (95% CI) demonstrating agreement in % neutralization of SARS-CoV-2 Spike-ACE2 interaction in DBS and serum samples. PCR positive samples are indicated with open circles; negative samples are indicated with black circles. The concordance correlation of absolute agreement = 0.991.

Reference 1: Di Germanio C, et al. Vaccination of COVID-19 convalescent plasma donors increases binding and neutralizing antibodies against SARS-CoV-2 variants. *Transfusion*. 2022;1–7. (<https://doi.org/10.1111/trf.16823>; PMID: 35129839)

Reference 2: Edara VV, et al. Infection- and vaccination-induced antibody binding and neutralization of the B.1.351 SARS-CoV-2 variant. *Cell Host & Microbe*. 2021; 29: 516-521. (<https://doi.org/10.1016/j.chrom.2021.03.009>; PMID: 33798491)

Reference 3: Sancilio AE, et al. A surrogate virus neutralization test to quantify antibody-mediated inhibition of SARS-CoV-2 in finger stick dried blood spot samples. *Scientific Reports*. 2021; 11: 15321. (<https://doi.org/10.1038/s41598-021-94653-z>; PMID: 34321523)