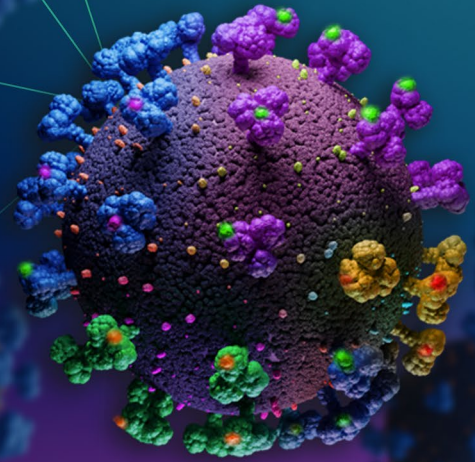


# LEVELS AND NEUTRALIZING ABILITY OF ANTIBODIES IN COVID-19 PATIENTS AND VACCINATED INDIVIDUALS



## SUMMARY

To determine the levels and neutralizing ability of antibodies from individuals with prior COVID-19 infection and vaccinated individuals, this study uses a competition ELISA assay to assess the inhibition of association of the spike protein receptor-binding domain (RBD) from the original SARS-CoV-2 virus (wild type) and the other variants with angiotensin-converting enzyme 2 (ACE-2) *in vitro*. The RBDs tested in the RBD/ACE2 inhibition assay are: wild type, Alpha (B.1.1.7, UK), Beta (B.1.351, South Africa), Gamma (P.1, Brazil), Delta (B.1.617.2, India), and Kappa (B.1.617, India).

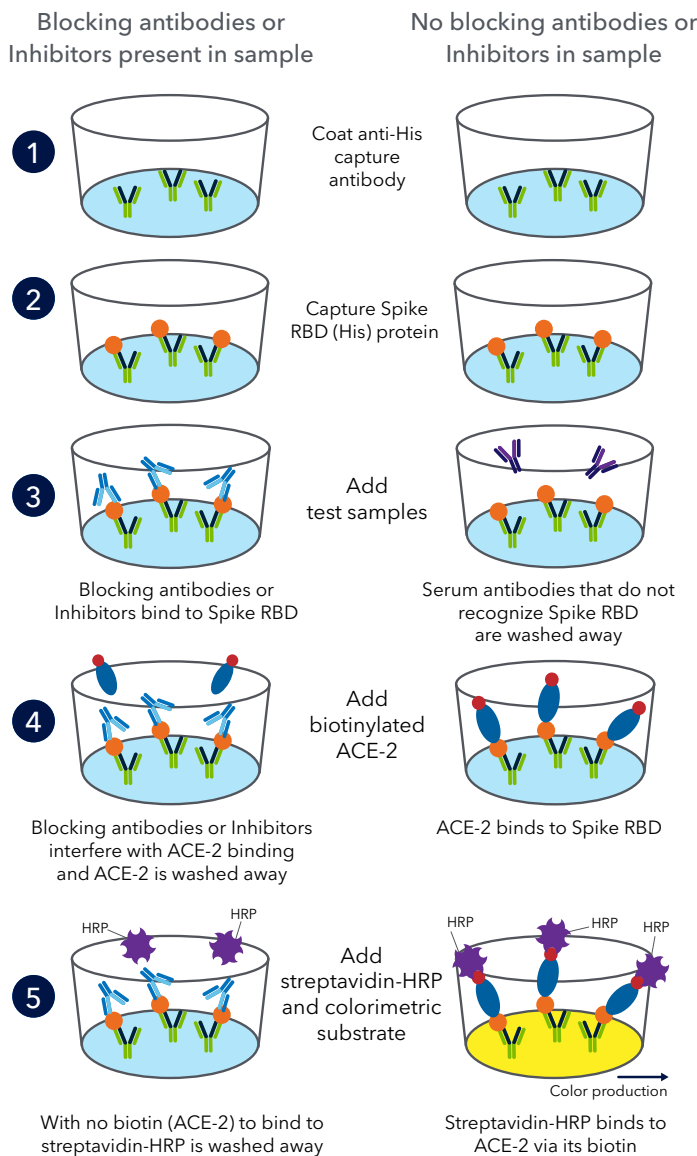
- The data suggest notable differences between the ability of sera from individuals with prior COVID-19 infection and vaccinated individuals to inhibit the association between different genetic SARS-CoV-2 variant Spike RBD and ACE-2 proteins (RBD/ACE-2).
  - a. Sera from individuals with prior COVID-19 infection inhibited RBD/ACE-2 association in the order of: wild type > Alpha > Beta ~ Gamma. The largest difference between wild type RBD and Beta or Gamma RBD is approximately 19-fold.
  - b. Sera from vaccinated individuals show ~4-fold higher ability to inhibit wild type RBD/ACE2 association than to inhibit all the variants. The inhibition ability to all the variants is similar.
- Among the vaccinated donors, only 5 of the 10 donors, after the first dose, had sufficient antibody levels to completely block the RBDs/ACE-2 association. After the second dose, all of the donors had sufficient antibody levels to completely inhibit the RBDs/ACE2 association.
- The data indicate that the neutralizing ability of sera is in the order of: completed vaccinated (2 doses) > 1 dose vaccinated > post-COVID infected individuals > unvaccinated individuals.
- This study shows the threshold of antibody levels to completely inhibit RBDs/ACE-2 associations.
- This test may be used:
  - a. As a titer assay to understand level of antibodies needed to neutralize RBDs/ACE-2 interaction.
  - b. To study antibody responses against SARS-CoV-2 variants and measure changes to inhibition of RBDs/ACE-2 associations correlated with antibody levels over time.

# INTRODUCTION

Neutralizing antibodies acquired through infection or vaccination play critically important roles in immunity. COVID-19 vaccines can prevent reinfection and attenuate severity of the disease. Genetic surveillance studies have suggested that the SARS-CoV-2 evolution includes mutations in the RBD (FIGURE 1, TABLE 1). This is important since current COVID-19 vaccine preparations use sequences of the wild type SARS-CoV-2, and do not include mutations of variants that exhibit higher transmissibility than wild type virus. The preponderance of antibody neutralizing activity from individuals with prior COVID-19 infection or vaccinated individuals targets the RBD <sup>1,2</sup>. Inhibition of RBD/ACE-2 interaction, which facilitates the entry of virus into cells <sup>3</sup> is critical to immunity by neutralizing antibodies and has been guiding development of vaccines utilizing RBD as an antigen <sup>4</sup>.

The mutations of the SARS-CoV-2 virus have resulted in increased transmissibility of variants. Several factors may account for the increased transmissibility of some SARS-CoV-2 variants including processing of the spike protein by furin-like enzymes, density of the spike protein, mutations of the proteins in the interior of the virus involved in virus replication, efficiency of viral release from infected cells, and the affinity of the RBD for ACE-2. The latter has been addressed in several reports showing variations of the affinity of RBDs of variants for ACE-2 <sup>5,6</sup>.

Here we report tests to determine the levels and ability of anti-COVID antibodies to block the RBDs/ACE-2 interaction in infected or vaccinated individuals.



## PROTEINS USED IN THIS STUDY

| PROTEINS                           | MUTATIONS           | CATALOG # |
|------------------------------------|---------------------|-----------|
| SARS-CoV2 Spike RBD                | Wild Type           | 10500-CV  |
| SARS-CoV2 B.1.1.7 Spike RBD        | N510Y               | 10730-CV  |
| SARS-CoV2 B.1.351 Spike RBD        | K417N, E484K, N501Y | 10735-CV  |
| SARS-CoV2 P.1 Spike RBD            | K417T, E484K, N501Y | 10775-CV  |
| SARS-CoV2-B.1.617.1 Spike RBD      | L452R E484Q         | 10846-CV  |
| SARS-CoV-2 B.1.617.2 Spike RBD     | L452R T478K         | 10876-CV  |
| Biotinylated Human ACE-2/Fc Avitag | Wild Type           | AVI10544  |

## ANCILLARY REAGENTS

| MATERIALS               | CATALOG # |
|-------------------------|-----------|
| Buffers and microplates | DY008     |
| His Tag Antibody        | MAB050R   |
| Streptavidin-HRP        | DY998     |

## SARS-COV-2 VARIANT INHIBITOR SCREENING KIT - NOW AVAILABLE! CATALOG # VANC00

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| VIRUS                                | RBD SEQUENCE   |
|--------------------------------------|--|
| SARS-COV-2 Spike RBD                 | RVQPTEIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVS PTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADY-NYKLPDDFTGCVIAWNSNNLDSKVG GNYNYLYRFLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV VVLSFELLHAPATVCGPKKSTNLVKNKCVNF  |
| SARS-COV-2 Spike RBD Alpha B.1.1.7   | RVQPTEIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVS PTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADY-NYKLPDDFTGCVIAWNSNNLDSKVG GNYNYLYRFLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV VVLSFELLHAPATVCGPKKSTNLVKNKCVNF  |
| SARS-COV-2 Spike RBD Beta B.1.351    | RVQPTEIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVS PTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADY-NYKLPDDFTGCVIAWNSNNLDSKVG GNYNYLYRFLFRKSNLKPFFERDISTEIQAGSTPCNGVKGFNCFYFPLQSYGFQPTNGVGYQPYRV VVLSFELLHAPATVCGPKKSTNLVKNKCVNF |
| SARS-COV-2 Spike RBD Gamma P.1       | RVQPTEIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVS PTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADY-NYKLPDDFTGCVIAWNSNNLDSKVG GNYNYLYRFLFRKSNLKPFFERDISTEIQAGSTPCNGVKGFNCFYFPLQSYGFQPTNGVGYQPYRV VVLSFELLHAPATVCGPKKSTNLVKNKCVNF |
| SARS-COV-2 Spike RBD Kappa B.1.617.1 | RVQPTEIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVS PTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADY-NYKLPDDFTGCVIAWNSNNLDSKVG GNYNYRYRFLFRKSNLKPFFERDISTEIQAGSTPCNGVQGFNCFYFPLQSYGFQPTNGVGYQPYRV VVLSFELLHAPATVCGPKKSTNLVKNKCVNF |
| SARS-COV-2 Spike RBD Delta B.1.617.2 | RVQPTEIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVS PTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADY-NYKLPDDFTGCVIAWNSNNLDSKVG GNYNYRYRFLFRKSNLKPFFERDISTEIQAGSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV VVLSFELLHAPATVCGPKKSTNLVKNKCVNF  |

FIGURE 1. Amino acid sequences of the Spike RBD proteins used in this study. Mutated residues are in orange.

## RESULTS AND DISCUSSION

**COVID-19 infected individuals.** The sera of ten individuals infected with the original COVID-19 Wuhan virus (wild-type) without vaccination were collected in March 2020. Half of the donors had sufficient antibody levels to block the interaction of wild type RBD and Alpha variant RBD with ACE-2. The ability of antibodies to block RBDs/ACE-2 association of Beta and Gamma variants was weaker than for wild type and Alpha variant RBDs. The general trend is in the order, wild type > Alpha > Beta ~ Gamma. Control sera from healthy uninfected donors did not affect the RBD/ACE-2 interaction. Values of serum dilution giving 50% inhibition (IC50) of association are shown in TABLE 2. The largest difference in IC50 between wild type and Beta or Gamma variants is 19-fold. Assay plots with sera of two COVID-19 infected individuals having the highest and lowest antibody levels (TABLE 2) are shown in FIGURE 2. Although the information about the time period from diagnosis of infection to blood draw is unavailable, it is clear that the ability of elicited antibodies to inhibit association of RBDs of variants with ACE-2 varies widely. The data agree with many reports that, in its evolution, the wild type SARS-CoV-2 virus exploits mutations in the RBD to evade immune responses<sup>7</sup>.

**Vaccinated individuals.** Ten plasma samples of donors who received the Moderna vaccine were tested (TABLE 3). Results of the serological assay suggest that three of the donors were infected with SARS-CoV-2 prior to vaccination (TABLE 3). Six of the donors did not have enough neutralizing Abs after the first dose to completely block the RBDs/ACE-2 association. After the second dose all donors showed complete blockage of RBDs/ACE-2 association, and substantially increased antibodies levels. This is in line with many reports suggesting that two doses of the Moderna vaccine are required for antibodies to confer protection against reinfection. None of the three donors infected with COVID-19 prior to vaccination (TABLE 3) had high enough neutralizing antibodies levels at the time of blood draw to inhibit RBD/ACE-2 interaction. Sera from vaccinated individuals blocked wild type RBD/ACE-2 association more efficiently than variant RBDs/ACE-2 association. All variants tested show similar IC50 in this assay, which is ~4-fold lower than the IC50 of wild type RBD/ACE-2 inhibition of association (TABLE 3). This is in stark contrast to the results with sera of COVID-19 infected individuals, which show up to 19-fold difference between wild type and some of the variants tested. Inspection of the data in TABLE 2 also suggests that antibody levels specific for the spike protein determined by COVID-SeroKlir Kantaro SARS-CoV-2 IgG Ab Kit, a Bio-Techne manufactured serological assay, which has been granted Emergency Use Authorization by the FDA, correlate with the ability of antibodies to inhibit RBD/ACE-2 interaction.

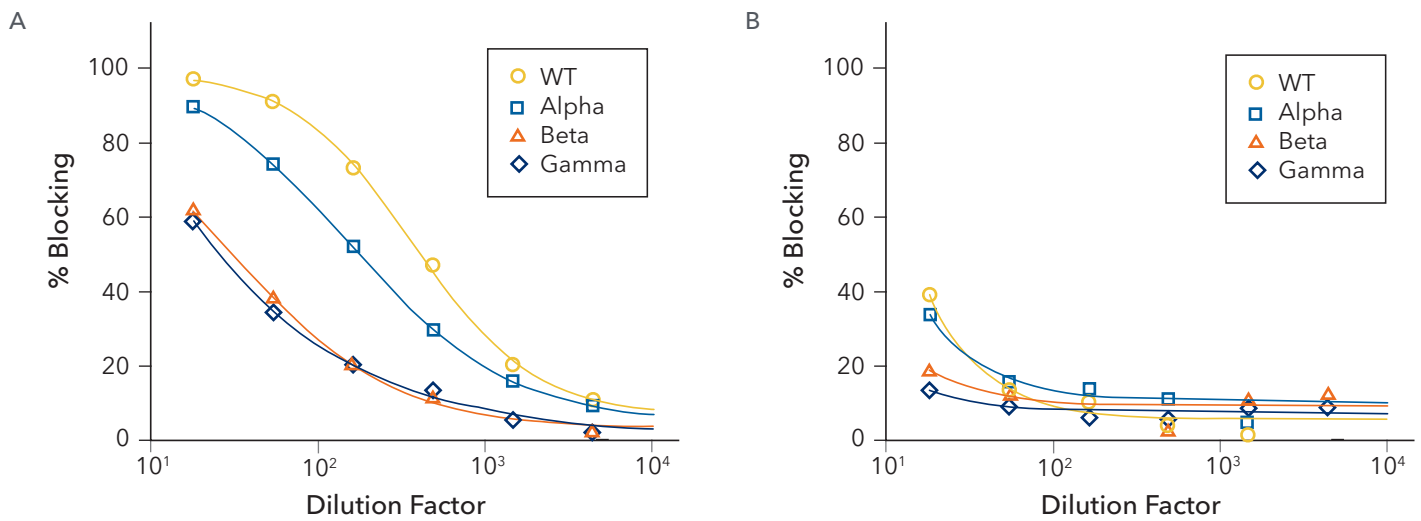


FIGURE 2. Assay plots with sera of two COVID-19 patients with the highest (A.) and lowest (B.) antibody levels. Samples COVID-53 and COVID-31 in TABLE 2.

| SAMPLE ID | AU/ML | RBD: 50% INHIBITION VS FOLD-SERUM DILUTION |       |      |       | RBD: 50% INHIBITION VS AU/ML |       |      |       |
|-----------|-------|--|-------|------|-------|------------------------------|-------|------|-------|
|           |       | WT   | Alpha | Beta | Gamma | WT                           | Alpha | Beta | Gamma |
| COVID-31  | 8.449 | ~8   | ~6    | *    | *     | ~1.06                        | ~1.4  | *    | *     |
| COVID-25  | 14.07 | ~4   | *     | *    | *     | ~3.5                         | *     | *    | *     |
| COVID-29  | 11.39 | 30   | 10    | 8    | 8     | 0.38                         | 1.14  | 1.4  | 1.4   |
| COVID-80  | 23.58 | ~10  | ~8    | *    | *     | ~2.36                        | ~2.95 | *    | *     |
| COVID-14  | 25.72 | 120  | 40    | 40   | 40    | 0.22                         | 0.65  | 0.65 | 0.65  |
| COVID-50  | 31.97 | 160  | 70    | 50   | 50    | 0.2                          | 0.46  | 0.64 | 0.64  |
| COVID-49  | 34.13 | 20   | 14    | ~4   | ~4    | 1.7                          | 2.43  | ~8.5 | ~8.5  |
| COVID-94  | 68.49 | 70   | 30    | ~4   | ~4    | 0.97                         | 2.26  | ~17  | ~17   |
| COVID-44  | 81.22 | 210  | 150   | 20   | 20    | 0.39                         | 0.54  | 4.05 | 4.05  |
| COVID-53  | 90.96 | 420  | 190   | 20   | 20    | 0.21                         | 0.48  | 4.55 | 4.55  |

TABLE 2. Serum dilution and antibodies concentration required to give 50% inhibition of RBDs/ACE2 association. The antibody concentration was measured by serological assay. Asterisk (\*) indicates samples that did not achieve 50% inhibition in assay.

## MATERIALS AND METHODS

RBDs with a C-terminal His-tag of the original Wuhan SARS-CoV-2 and variants were expressed in mammalian cell lines and purified to over 95% purity. ACE-2 was fused to the Fc region of human IgG1 bearing an Avi-tag at the C-terminus. TABLE 1 contains a complete listing of recombinant protein reagents and sources. Sequence alignment of the RBDs of variants in this study are in FIGURE 1.

96-well microtiter plates were pre-coated with mouse anti-His antibody, and His-tagged RBD proteins were captured onto the plate. Capturing of the His-tagged RBDs by the anti-His antibody provides uniform orientation of the RBDs on the plate. After pre-incubating immobilized SARS-CoV-2-RBD His-Tag protein with serially diluted serum or plasma samples, Biotinylated Recombinant Human ACE-2 Fc Chimera Avi-Tag is added to the plate. The binding between immobilized Recombinant SARS-CoV-2-RBD His-Tag and Biotinylated Recombinant Human ACE-2 Fc Chimera Avi-Tag is determined by Streptavidin-HRP followed by color reaction.

Sera of infected donors was collected in March 2020 (Italy). Plasma of vaccinated donors with the Moderna vaccine was from Precision for Medicine. Blood draws were prior to vaccination, three weeks after the first dose and four weeks after the second dose, respectively.

Concentration of IgGs specific for the wild type spike protein were determined with COVID-SeroKliar Kantaro SARS-CoV-2 IgG Ab Kit, Cat # COV219-100, a serological assay which has received Emergency Use Authorization by the US FDA.

|            |         |       |         |                   | 50% INHIBITION (FOLD-SERUM DILUTION) |      |       |      |       |       |       |
|------------|---------|-------|---------|-------------------|--------------------------------------|------|-------|------|-------|-------|-------|
| LOT #      | VAX #   | CI    | AU/ML   | DAYS POST 1ST VAX | DAYS POST 2ND VAX                    | WT   | ALPHA | BETA | GAMMA | KAPPA | DELTA |
| AB21275059 | Pre-Vax | 0.722 | 8.2     |                   |                                      | *    | *     | *    | *     | *     | *     |
| AB21275059 | 1st Vax | 5.712 | 1509.4  | 26                |                                      | 2140 | 1090  | 908  | 1140  | 1380  | 1890  |
| AB21275059 | 2nd Vax | 5.612 | 1210.5  | 41                | 14                                   | 2310 | 1080  | 800  | 933   | 1160  | 1670  |
| AB21275060 | Pre-Vax | 0.046 | 0.105   |                   |                                      | *    | *     | *    | *     | *     | *     |
| AB21275060 | 1st Vax | 0.420 | 6.096   | 15                |                                      | *    | *     | *    | *     | *     | *     |
| AB21275060 | 2nd Vax | 5.346 | 151.574 | 43                | 14                                   | 304  | 130   | 90   | 110   | 160   | 200   |
| AB21275061 | Pre-Vax | 0.054 | 0.266   |                   |                                      | *    | *     | *    | *     | *     | *     |
| AB21275061 | 1st Vax | 3.918 | 56.315  | 30                |                                      | *    | *     | *    | *     | *     | *     |
| AB21275061 | 2nd Vax | 5.655 | 361.5   | 43                | 14                                   | 472  | 260   | 120  | 129   | 245   | 369   |
| AB21275062 | Pre-Vax | 0.189 | 1.033   |                   |                                      | *    | *     | *    | *     | *     | *     |
| AB21275062 | 1st Vax | 1.635 | 14.891  | 26                |                                      | *    | *     | *    | *     | *     | *     |
| AB21275062 | 2nd Vax | 5.514 | 376.7   | 43                | 14                                   | 781  | 347   | 354  | 256   | 414   | 434   |
| AB21275063 | Pre-Vax | 0.154 | 0.511   |                   |                                      | *    | *     | *    | *     | *     | *     |
| AB21275063 | 1st Vax | 5.529 | 342.2   | 26                |                                      | 445  | 178   | 130  | 130   | 158   | 193   |
| AB21275063 | 2nd Vax | 5.695 | 1818.45 | 41                | 13                                   | 3550 | 1470  | 1150 | 1060  | 1500  | 1400  |
| AB21275066 | Pre-Vax | 0.069 | 0.34    |                   |                                      | *    | *     | *    | *     | *     |       |
| AB21275066 | 1st Vax | 5.571 | 370.85  | 26                |                                      | 471  | 221   | 153  | 170   | 220   |       |
| AB21275066 | 2nd Vax | 5.866 | 2283.25 | 41                | 14                                   | 4060 | 2040  | 1490 | 1200  | 2180  |       |
| AB21275069 | Pre-Vax | 0.041 | 0.501   |                   |                                      | *    | *     | *    | *     | *     | *     |
| AB21275069 | 1st Vax | 2.777 | 41.861  | 26                |                                      | *    | *     | *    | *     | *     | *     |
| AB21275069 | 2nd Vax | 5.323 | 455.4   | 42                | 14                                   | 816  | 582   | 201  | 282   | 306   | 511   |
| AB21275071 | Pre-Vax | 1.307 | 28      |                   |                                      | *    | *     | *    | *     | *     | *     |
| AB21275071 | 1st Vax | 5.502 | 3340.15 | 26                |                                      | 4230 | 2260  | 1720 | 1720  | 1680  | 1750  |
| AB21275071 | 2nd Vax | 5.705 | 3323.25 | 40                | 12                                   | 9030 | 5600  | 4510 | 4340  | 3960  | 3690  |
| AB21275073 | Pre-Vax | 2.25  | 29.331  |                   |                                      | 25   | 30    | *    | *     | *     | *     |
| AB21275073 | 1st Vax | 3.339 | 68.044  | 26                |                                      | 40   | *     | *    | *     | *     | 30    |
| AB21275073 | 2nd Vax | 5.366 | 568.1   | 42                | 14                                   | 858  | 385   | 248  | 244   | 413   | 414   |
| AB21275075 | Pre-Vax | 0.748 | 0.761   |                   |                                      | *    | *     | *    | *     | *     | *     |
| AB21275075 | 1st Vax | 1.214 | 7.035   | 26                |                                      | *    | *     | *    | *     | *     | *     |
| AB21275075 | 2nd Vax | 5.125 | 268     | 45                | 17                                   | 267  | 120   | 180  | 157   | 194   | 180   |

TABLE 3. Serum samples of vaccinated individuals with Moderna's vaccine were obtained from Precision Medicine. The concentration of IgGs specific for the spike protein of the original Wuhan virus is in the AU/ml column. Confidence index (CI) higher than 0.7 suggest statistically significant values of antibody levels (COVID-SeroKlar Kantaro SARS-CoV-2 IgG Ab Kit). Asterisk (\*) indicates samples with less than 50% blocking.

|            |         | 50% INHIBITION (AU/ML) |       |       |       |       |       |
|------------|---------|------------------------|-------|-------|-------|-------|-------|
| LOT #      | VAX #   | WT                     | ALPHA | BETA  | GAMMA | KAPPA | DELTA |
| AB21275059 | Pre-Vax | *                      | *     | *     | *     | *     | *     |
| AB21275059 | 1st Vax | 0.705                  | 1.385 | 1.662 | 1.324 | 1.094 | 0.799 |
| AB21275059 | 2nd Vax | 0.524                  | 1.121 | 1.513 | 1.297 | 1.044 | 0.725 |
| AB21275060 | Pre-Vax | *                      | *     | *     | *     | *     | *     |
| AB21275060 | 1st Vax | *                      | *     | *     | *     | *     | *     |
| AB21275060 | 2nd Vax | 0.499                  | 1.166 | 1.684 | 1.378 | 0.947 | 0.758 |
| AB21275061 | Pre-Vax | *                      | *     | *     | *     | *     | *     |
| AB21275061 | 1st Vax | *                      | *     | *     | *     | *     | *     |
| AB21275061 | 2nd Vax | 0.766                  | 1.390 | 3.013 | 2.802 | 1.476 | 0.980 |
| AB21275062 | Pre-Vax | *                      | *     | *     | *     | *     | *     |
| AB21275062 | 1st Vax | *                      | *     | *     | *     | *     | *     |
| AB21275062 | 2nd Vax | 0.482                  | 1.086 | 1.064 | 1.471 | 0.910 | 0.868 |
| AB21275063 | Pre-Vax | *                      | *     | *     | *     | *     | *     |
| AB21275063 | 1st Vax | 0.769                  | 1.922 | 2.632 | 2.632 | 2.166 | 1.773 |
| AB21275063 | 2nd Vax | 0.512                  | 1.237 | 1.581 | 1.716 | 1.212 | 1.299 |
| AB21275066 | Pre-Vax | *                      | *     | *     | *     | *     |       |
| AB21275066 | 1st Vax | 0.787                  | 1.678 | 2.424 | 2.181 | 1.686 |       |
| AB21275066 | 2nd Vax | 0.562                  | 1.119 | 1.532 | 1.903 | 1.047 |       |
| AB21275069 | Pre-Vax | *                      | *     | *     | *     | *     | *     |
| AB21275069 | 1st Vax | *                      | *     | *     | *     | *     | *     |
| AB21275069 | 2nd Vax | 0.558                  | 0.782 | 2.266 | 1.615 | 1.488 | 0.891 |
| AB21275071 | Pre-Vax | *                      | *     | *     | *     | *     | *     |
| AB21275071 | 1st Vax | 0.790                  | 1.478 | 1.942 | 1.942 | 1.988 | 1.909 |
| AB21275071 | 2nd Vax | 0.368                  | 0.593 | 0.737 | 0.766 | 0.839 | 0.901 |
| AB21275073 | Pre-Vax | 1.173                  | 0.978 | *     | *     | *     | *     |
| AB21275073 | 1st Vax | 1.701                  | *     | *     | *     | *     | 2.268 |
| AB21275073 | 2nd Vax | 0.662                  | 1.476 | 2.291 | 2.328 | 1.376 | 1.372 |
| AB21275075 | Pre-Vax | *                      | *     | *     | *     | *     | *     |
| AB21275075 | 1st Vax | *                      | *     | *     | *     | *     | *     |
| AB21275075 | 2nd Vax | 1.004                  | 2.233 | 1.489 | 1.707 | 1.381 | 1.489 |

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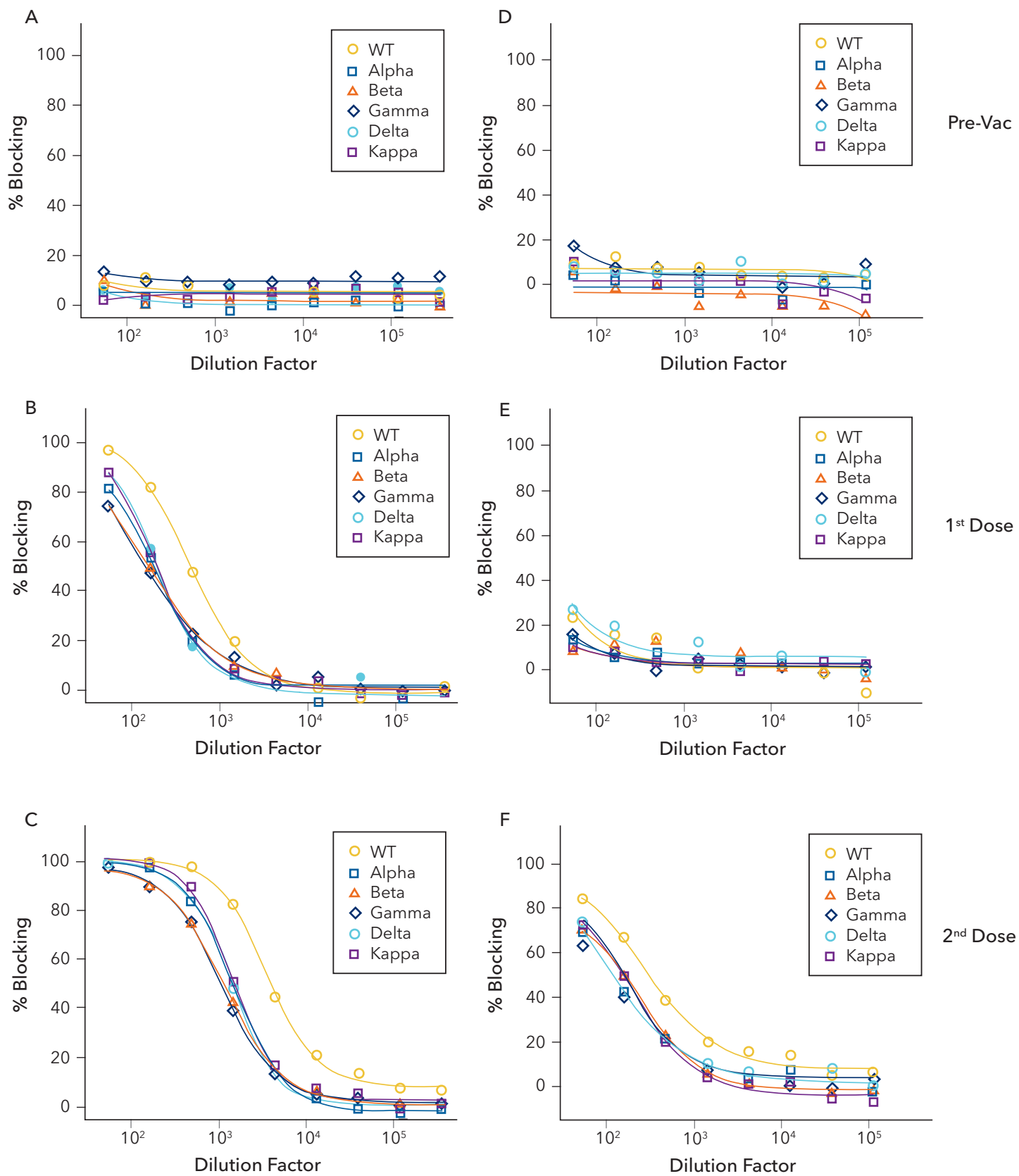


Figure 3. Assay plots of two donors who had different antibody levels after the first and second doses.

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