Durability of Antibody Response and Frequency of SARS-CoV-2 Infection 6 Months after COVID-19 Vaccination in Healthcare Workers

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies decay but persist 6 months postvaccination; lower levels of neutralizing titers persist against Delta than wild-type virus. Of 227 vaccinated healthcare workers tested, only 2 experienced outpatient symptomatic breakthrough infections, despite 59/227 exhibiting serologic evidence of SARS-CoV-2 infection, defined as presence of nucleocapsid protein antibodies.

Neutralizing antibodies (nAbs) and binding antibodies (bAbs) appear to be associated with protection against symptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus disease (COVID-19) (*1*,*2*). Early assessments of the Pfizer-BioNTech (https://www.pfizer.com) BNT162b2 COVID-19 mRNA vaccine observed >95% effectiveness against predominantly Alpha infections (*3*), but the potential effect of waning postvaccine neutralizing titers is an ongoing concern (*4*).

Apparent increases in vaccine-breakthrough infections may result from waning antibody titers, increases in exposure risk, and reduced vaccine effectiveness against Delta and other variants. In mid-2021, Delta became the dominant virus type in the United States (*5*). Delta appears to cause increased hospitalization rates and has increased transmissibility compared with Alpha and other pre-Delta variants (*6*; Bolze et al., unpub. data, https://doi.org/10.1101/2021.06.20.21259195). We report bAb and nAb levels as well as clinically overt and asymptomatic breakthrough infections that occurred among US healthcare workers in the Prospective Assessment of SARS-CoV-2 Seroconversion (PASS) study (*7*), conducted during January–August 2021.

The Study

The PASS study protocol was approved by the Uniformed Services University of the Health Sciences Institutional Review Board (Federalwide Assurance no. 00001628, US Department of Defense Assurance no. P60001) in compliance with all applicable federal regulations governing the protection of human participants. Written consent was obtained from all study participants.

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Table. Demographic characteristics of US healthcare worker participants in the Prospective Assessment of SARS-CoV-2 Seroconversion (PASS) study, January–August 2021*

Characteristic	BNT162b2 vaccinated	Vaccinated with 6-mo follow-up bAb	Vaccinated with 6-mo follow-up nAb titers	Unvaccinated
Total	227 (100)	187 (100)	49 (100)	17 (100)
Sex				
F	159 (70)	131 (70)	33 (67)	13 (76)
M	68 (30)	56 (30)	16 (33)	4 (24)
Ethnicity				
Non-Hispanic	209 (92)	175 (94)	47 (96)	16 (94)
Hispanic	14 (6)	10 (5)	1 (2)	1 (6)
Not reported	4 (2)	2 (1)	1 (2)	0
Race				
White	165 (73)	139 (74)	35 (71)	9 (53)
Black	26 (11.5)	18 (9.5)	4 (8)	6 (35)
Asian	23 (10)	19 (10)	8 (16)	1 (6)
≥2 races	7 (3)	6 (3)	1 (2)	1 (6)
Native Hawaiian or other Pacific Islander	1 (0.5)	1 (0.5)	1 (2)	0
Not reported	5 (2)	4 (2)	0	0
Age, y, mean (range)	41.7 (20–69)	42.8 (21–69)	44.7 (26–69)	32.3 (19–50)

*Values are no. (%) except as indicated. bAb, binding antibodies; nAb, neutralizing antibodies.

For the PASS study, we enrolled and followed generally healthy, adult healthcare workers (HCWs) at Walter Reed National Military Medical Center (Bethesda, MD, USA) who were seronegative for IgG to SARS-CoV-2 spike glycoprotein (spike) and had no history of COVID-19, as previously described (*7*). We collected participants' serum samples monthly and screened them for IgG against SARS-CoV-2 spike and nucleocapsid protein (NP) in multiplex microsphere-based immunoassays, as previously described (Appendix, https://wwwnc.cdc.gov/EID/article/28/4/21-2037-App1.pdf) (E.D. Laing, unpub. data, https://doi.org/10.1101/2021.02.10.21251518). In addition, we asked participants to obtain nasopharyngeal SARS-CoV-2 PCR testing at a designated COVID-19 testing center if they experienced symptoms consistent with SARS-CoV-2 infection.

To quantify spike IgG bAbs in World Health Organization binding antibody units (BAU), we interpolated IgG levels against an internal standard curve calibrated to the Human SARS-CoV-2 Serology Standard (Appendix Figure 1). We assessed serum samples for nAbs against SARS-CoV-2 wild type and Delta as previously described by using a well-characterized SARS-CoV-2 lentiviral-pseudovirus neutralization assay (Appendix) (*8*).

Excluding persons infected before January 31, 2021, the study followed 227 participants fully vaccinated

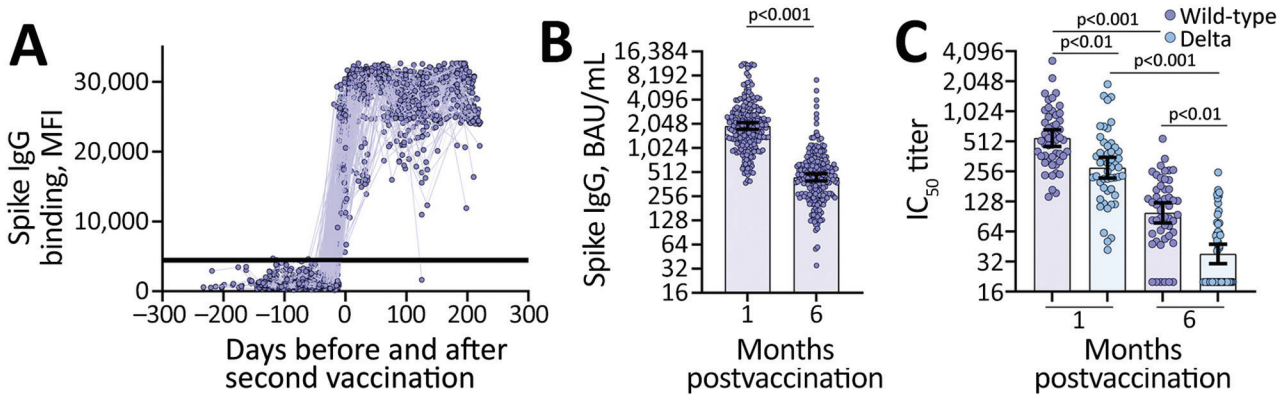

Figure 1. Vaccine-induced binding and neutralizing antibody responses observed among US healthcare worker participants in the Prospective Assessment of SARS-CoV-2 Seroconversion (PASS) study, January–August 2021. A) MFI levels of vaccine-induced spike IgG binding before and after second vaccination in serum samples diluted 1:400 (n = 227 participants). Horizontal line indicates the positive or negative spike IgG threshold. B) Spike IgG binding antibodies (BAU/mL) quantified from serum samples collected 1 month (mean 36.9 days, range 23–81 days) and 6 months (mean 201.1 days, range 151–237 days) postvaccination (n = 187 participants). Wilcoxon matched-pairs signed rank test performed; y-axis is $\log_2$-scale. C) Neutralizing antibody titers against severe acute respiratory syndrome coronavirus 2 wild-type and Delta variant from serum samples collected 1 month (mean 30.8 days, range 28–42 days) and 6 months (mean 200.1 days, range 189–219 days) postvaccination (n = 49 participants). Friedman ANOVA with Dunn's multiple comparisons performed post-hoc; y-axis is $\log_2$-scale. All errors bars represent the geometric mean and 95% CIs. BAU, binding antibody units; IC_{50}, 50% inhibitory concentration; MFI, median fluorescence intensity.

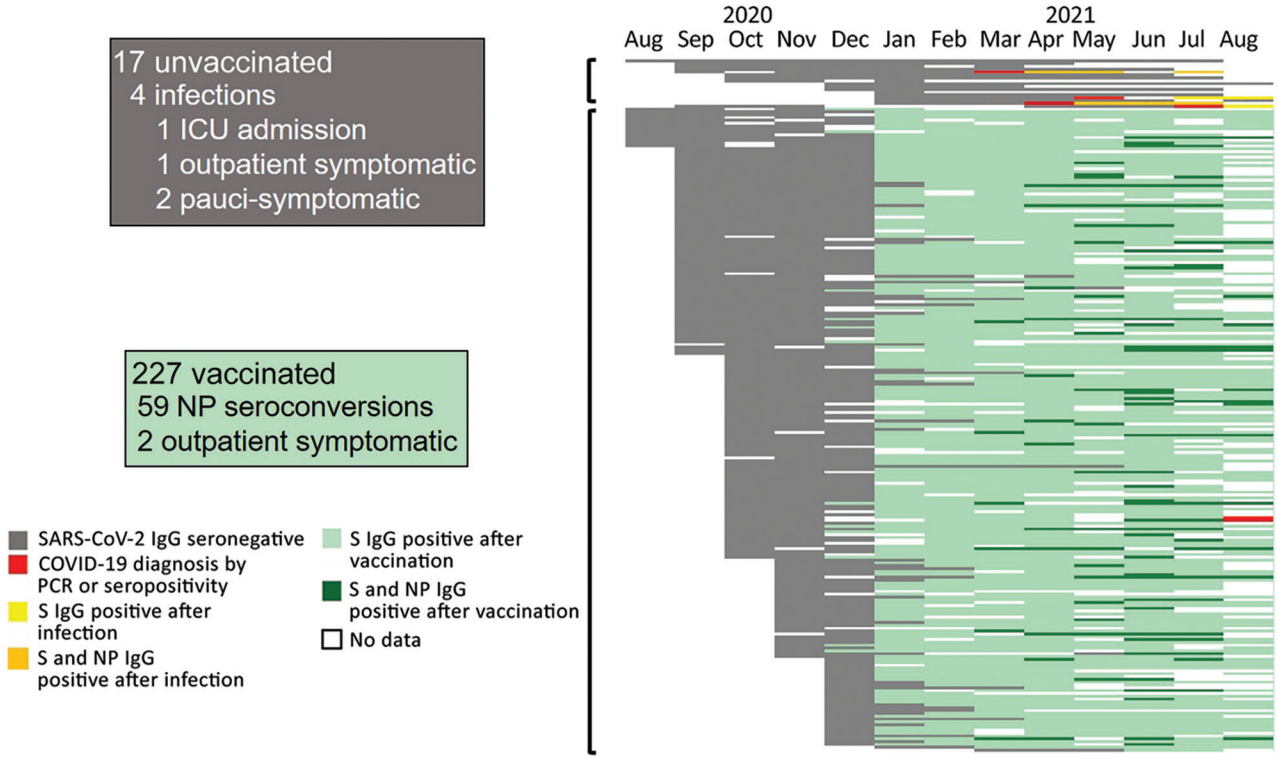

Figure 2. Timeline of antibody responses and SARS-CoV-2 infections among US healthcare worker participants in the Prospective Assessment of SARS-CoV-2 Seroconversion (PASS) study, January–August 2021 (17 unvaccinated and 227 vaccinated participants). Each horizontal bar represents the infection, vaccination, and serologic status obtained monthly in all participants who had not been diagnosed with SARS-CoV-2 by PCR or S protein IgG seroconversion by January 31, 2021. White spaces indicate no data. Gray bars represent negative S protein IgG. Red bars indicate month of SARS-CoV-2 diagnosis by PCR positivity or S protein IgG seroconversion. Yellow bars indicate S protein IgG seroconversion after SARS-CoV-2 diagnosis in unvaccinated persons, and orange bars indicate presence of both S protein and NP antibodies in unvaccinated persons. Light green bars indicate S protein IgG seroconversion after vaccination. Dark green bars indicate detection of NP IgG in addition to S protein antibodies at timepoints postvaccination. S, spike protein; NP, nucleocapsid protein; ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

with BNT162b2 vaccine and 17 unvaccinated participants. Participants were generally healthy, had a mean age of 41.7 (range 20–69) years, and were predominantly women (Table). Vaccinated and unvaccinated participants reported similar in-hospital time; >70% of each group worked in the hospital ≥15 days per month, and had similar rates of direct interaction with COVID-19 positive patients (monthly average of 47% for vaccinated and 45% for unvaccinated participants).

We observed seroconversion in all participants 1 month after the second vaccine dose (Figure 1, panel A). We quantified spike IgG bAbs at 1 and 6 months after full vaccination in the 187 vaccinated participants with serum samples collected at both timepoints. Spike IgG bAbs decreased from a geometric mean of 1,929 BAU/mL (95% CI 1,752–2,124 BAU/mL) at 1 month postvaccination to a geometric mean of 442 BAU/mL (95% CI 399–490 BAU/mL) at 6 months postvaccination (p<0.001) (Figure 1, panel B). Similarly, we observed decay of nAbs between the 1- and 6-month postvaccination timepoints. Peak SARS-CoV-2 wildtype nAbs decreased from a geometric mean titer (GMT) of 551 (95% CI 455–669 GMT) to 98 GMT (95% CI 78–124 GMT) 6 months after full vaccination (Figure 1, panel C). The GMTs of nAbs were significantly higher against wild-type compared with Delta SARS-CoV-2 at both timepoints after vaccination (Figure 1, panel C). In comparison, nAbs against Delta decayed from 279 GMT (95% CI 219–355 GMT) at peak to 38 GMT (95% CI 31–48 GMT) after 6 months. Quantitative IgG bAb (in BAU/mL) correlated with nAb titers ($\rho = 0.70$; p<0.001), demonstrating comparable decay of IgG bAbs and nAbs (Appendix Figure 2).

In addition to spike IgG bAbs, we also monitored for seroconversion of IgG bAbs to NP. Of vaccinated participants, 26.0% (59/227) had NP seroconversion during March–August 2021 (Figure 2). Only 2 had symptomatic, PCR-positive, vaccine-breakthrough infections, both of which were self-limited, outpatient

cases. In the unvaccinated cohort, 4 participants had SARS-CoV-2 infection diagnosed: 2 by PCR while experiencing symptomatic infection (1 outpatient case, 1 requiring intensive care) and 2 diagnosed by spike IgG seroconversion and who reported mild symptoms retrospectively. The frequency of NP seroconversions in the vaccinated population correlated with the frequency of SARS-CoV-2 infections diagnosed in the unvaccinated participants (23.5% [4/17]) (Figure 2), suggesting similar exposure rates.

Conclusions

In this prospective cohort study of generally healthy, adult HCWs, we found that SARS-CoV-2 spike IgG bAbs and nAbs induced by BNT162b2 mRNA COVID-19 vaccination wane but remained detectable through 6 months after vaccination, corroborating results of another study (*9*). Consistent with another report (*10*), we observed significantly lower vaccine-induced nAb titers against Delta compared to wild-type virus. Asymptomatic infections determined by NP seroconversions were relatively frequent, but symptomatic infection was rare, and severe disease was absent.

We observed 1 of 17 unvaccinated persons have onset of severe COVID-19, versus no severe cases among 227 vaccinated participants. Of vaccinated persons, 2 had symptomatic, PCR-proven breakthrough infections, both of which were managed as outpatient cases. We observed that 26% of vaccinated participants developed antibodies against SARS-CoV-2 NP, suggesting that vaccinated persons experienced exposures to SARS-CoV-2 as frequently as the unvaccinated population, yet rarely had onset of overt clinical disease.

The strengths of the study include frequency of serologic assessments and use of variant specific nAb in addition to multiplexed antigen-specific IgG detection. Use of longitudinal serologic assessments (in addition to PCR testing when participants exhibited symptoms) enabled detection of asymptomatic and pauci-symptomatic SARS-CoV-2 exposures. Although our study was powered to show clear differences in antibody titers over time, limitations include the moderate size of the cohort and the small number of unvaccinated participants. Further, seasonal human coronavirus (HCoV) infections may drive cross-reactive IgG responses against SARS-CoV-2 NP. We mitigated the likelihood of HCoV-driven false-positives by using convalescent serum samples from persons with PCR-confirmed HCoV infections to establish the threshold for SARS-CoV-2 NP IgG positivity, which had a specificity of 94% in our multiplex assay (E.D. Laing et al., unpub. data). In a separate study, NP seroconversion reportedly occurred in only 71% of PCR-confirmed vaccine-breakthrough infections (*11*); thus, some instances of asymptomatic vaccine-breakthrough infections may have gone unnoticed.

We observed persistence of nAb titers against SARS-CoV-2 wild-type equal to or greater than the lowest dilution tested in 90% (44/49) of healthy adults 6 months after vaccination with BNT162b2. Neutralizing activity against Delta virus was lower,; only 47% (23/49) of participants maintained nAb titers above the lowest dilution at 6 months post-vaccination. The decrease in nAb does not necessarily mean that persons have lost protection against severe COVID-19, however, given that nAb titers required for protection remain unknown and virus neutralization is only 1 function of antibodies. In addition, memory B cells and T cells have been detected 8–12 months after SARS-CoV-2 infection, demonstrating that adaptive immune memory can be long-lasting (*12*,*13*). Further research is needed to understand the correlates of protection against moderate to severe COVID-19 for known and emerging SARS-CoV-2 variants. Even so, our results suggest that the BNT162b2 vaccine confers protection against severe clinical disease caused by the variants circulating in the United States through August 2021 for ≥6 months in generally healthy adults, even in the face of frequent exposures to the virus and waning antibody titers.

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About the Author

Dr. Laing is an assistant professor at the Uniformed Services University, Bethesda. His primary areas of research are infectious and emerging zoonotic pathogens, with a focus on understanding mechanisms of virus virulence and co-evolutionary adaptations of bat immune responses to virus infections.

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Appendix

Methods

Multiplex Microsphere-based Immunoassay Screening Procedures

Prefusion stabilized spike (S) glycoprotein ectodomain trimers (S-2P) (*1*,*2*), hereafter referred to as spike, of SARS-CoV-2, HCoV-229E, and HCoV-NL63 were purchased from LakePharma, Inc (Hopkinton, MA, USA). HCoV-OC43 and HCoV-HKU1 spike were provided by Dr. Dominic Esposito, National Cancer Institute Frederick National Laboratory (NCI FNL), Protein Expression Laboratory, and have been previously described (*3*). A SARS-CoV-2 NP was sourced from RayBiotech (Peachtree Corners, GA, USA). Multiplexed antigen-based antibody detection has been described previously (E.D. Laing et al., unpub. data, https://doi.org/10.1101/2021.02.10.21251518; E.D. Laing et al., unpub. data, https://doi.org/10.1101/2021.04.27.21256207). Briefly, SARS-CoV-2 spike and NP, and HCoV spike were coupled to magnetic microspheres (Bio-Rad, Hercules, CA, USA). Serum samples were collected from venipuncture in serum separator tubes, processed and stored at −80°C in 500 µL aliquots until use. For weekly screening, neat human serum samples (1.25 µL) were diluted 1:400 in 1X PBS and heat inactivated at 60°C for 30 min after dilutions. Diluted serum samples were incubated with a master mix of SARS-CoV-2, HCoV-OC43, HCoV-HKU1, HCoV-229E, HCoV-NL63 spike, and SARS-CoV-2 NP coupled microspheres. This multiplex microsphere-based immunoassay has a 94% sensitivity and 100% specificity to detect anti-SARS-CoV-2 spike IgG; and a 93% sensitivity and 94% specificity to detect anti-SARS-CoV-2 NP IgG seroconversion between 7–28 days post-symptom onset (E.D. Laing et al., unpub. data, https://doi.org/10.1101/2021.02.10.21251518) (*4*,*5*).

After a 45 minute incubation of diluted serum and antigen-coupled microspheres, with agitation (900 rpm), plates were washed with PBS-Tween20 (0.05%) and 100 µL of biotinylated cross-absorbed anti-human IgG (Thermo Fisher Scientific, Waltham, MA) diluted in 1X PBS-T (1:5000) was added to each well, and plates were incubated for 45 minutes with agitation. Lastly, after washing, streptavidin-phycoerythrin was diluted 1:1000 in PBS-T, and 100 µL were added to each well and plates were incubated for 45 min with agitation (900 rpm). Plates were washed, and microspheres were resuspended with 100 µL PBS-T per well then analyzed on Bio-Plex 200 multiplexing systems (Bio-Rad) and median fluorescence intensity (MFI) values for samples are reported as the PBS adjusted average from duplicate plates. Antibody testing was blind to descriptive data, including demographic data, SARS-CoV-2 PCR status, and clinical phenotype.

Calibration to NCI FNL U.S. Serology Standard and interpolation of Binding Antibody Units (BAU/mL)

An internal reference standard (IR-std), a mixture of nine PASS study serum samples obtained 1 month after PCR-confirmed SARS-CoV-2 infection in 2020, was calibrated against the NCI FNL U.S. serology national standard (U.S.-std) for SARS-CoV-2 spike protein and NP IgG. The IR-std and U.S.-std were diluted 2-fold starting at 1:400 through 1:512,000, and IgG was detected as described above. The concentration of spike-specific IgG in IR-std was determined to be 428 BAU/ml by averaging the results of four separate analyses interpolating IR-std against a standard curve of the U.S.-std with known concentration of 764 BAU/ml. With the established IR-std BAU/mL, PASS participant serum samples were tested at 1:400 and 1:8000 dilutions. All MFI values were adjusted to the PBS-blank control wells, then MFI values were interpolated against the IR-std included on each 96-well microtiter plate. Spike IgG BAU/mL were log10-transformed, checked for normality, and statistical significance was determined by two-tailed Wilcoxon matched-pairs signed rank test.

SARS-CoV-2 S-Pseudovirus Production and Neutralization

A codon-optimized spike gene corresponding to the Wuhan-1 spike with the D614G substitution was used to make the wild-type (WT) pseudovirus. A codon-optimized spike gene used to make the Delta (B.1.617.2) pseudovirus had the following mutations on the WT backbone: T19R, G142D, E156 deletion, F157 deletion, R158G, L452R, T478K, D614G, P681R, and D950N. Neutralization assay were performed as previously described (*6*,*7*). Briefly, 5µg of pCMVΔR8.2, 5µg of pHR'CMVLuc and 0.5µg of S expression plasmids were co-

transfected in 293T cells. Pseudovirus supernatants were collected ≈48 hours post-transfection, filtered through a 0.45 µm low protein binding filter, and used immediately or stored at −80°C. Pseudovirus titers were measured by infecting 293T-ACE2.TMPRSS2 cells, which stably express human angiotensin converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), for 48 hours before measuring luciferase activity (luciferase assay reagent, Promega, Madison, WI). Neutralization titers were calculated using a nonlinear regression curve fit (GraphPad Prism software Inc., La Jolla, CA) using 8-point dilution curves. The mean titer from at least two independent experiments each with intra-assay duplicates was reported as the final titer. Titers below the lowest serum dilution of 1:40 were treated as 20 for statistical analysis. nAb IC_{50} titers were log10-transfomed, checked for normality, and statistical significance was determined by Friedman ANOVA with Dunn's multiple comparisons performed post-hoc.

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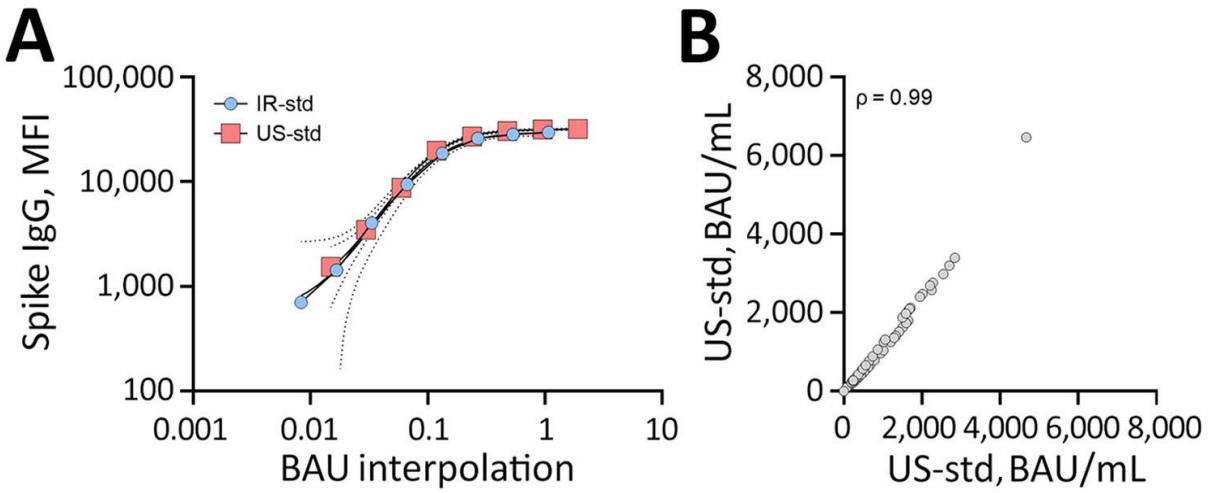
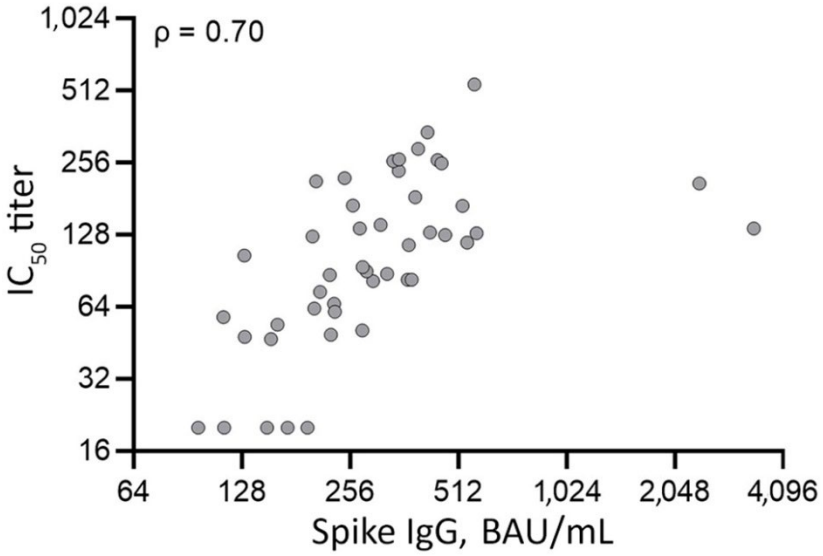


Appendix Figure 1. Comparison of the PASS internal reference standard (IR-std) to the NCI FNL U.S. serology national standard (U.S.-std). A) Comparison of the PASS internal reference standard (IR-std) curve to the NCI FNL U.S. serology national standard (U.S.-std) curve for SARS-CoV-2 spike protein reactive immunoglobulin G (IgG); MFI, median fluorescence intensity; BAU, binding antibody units; curves with dashed lines represent the mean and error bars of independent experiments, axes are log10-scale,representative of four independent experiments. B) Correlation between spike IgG BAU/mL interpolated from the IR-std or the U.S.-std; n = 76 serum samples, Spearman's rho (ρ) = 0.99, two-tailed $p < 0.001$.



Appendix Figure 2. Correlation between spike IgG bAb and nAb titers against SARS-CoV-2 WT 6 months-post vaccination. Six months post-vaccination serum antibodies were evaluated for correlation between spike IgG bAb, and nAb IC_{50} titers against SARS-CoV-2 WT, n = 49. Spearman's rho (ρ) = 0.70, two-tailed $p < 0.001$; axes are log2-scale.