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Measurement of binding antibodies to SARS-CoV-2 variants elicited by natural infection and COVID-19 vaccines in a sub-Saharan African population

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Abstract

Data on immune responses to COVID-19 vaccination in West and Central Africa remain limited, particularly with respect to antibody (Ab) binding across SARS-CoV-2 variants and for different types of vaccine platforms. We assessed anti-spike (anti-S) antibody binding to nine variants using the Meso Scale Discovery 10 plex assay (MSD) in 96 participants from the InVITE cohort, residing in the Democratic Republic of Congo, Guinea, Liberia, and Mali. Pre-vaccination anti-S seropositivity was determined with the Quanterix assay, and 48 participants each were selected from seronegative (no prior infection) and seropositive (prior infection) groups. Participants received either an mRNA, adenovirus-vectored, or inactivated virus vaccine. Anti-S binding levels were measured at baseline visit(V1) and the visit two months following completion of the primary vaccination series (V2). Regression models were applied to examine the effects of variant group, prior infection status, and vaccine platform on antibody binding responses.

At V1, seropositive participants had significantly higher antibody binding than seronegative individuals (fold change 15.65, 95% CI: 10.13–24.18). Binding to ancestral variants (Wuhan, Alpha, Beta, Delta) exceeded that to Omicron (BA.1, BA.2, BA.2.12.1, BA.2.75, BA.5) variants (fold change 3.7, 95% CI: 3.53–3.86). At V2, antibody levels rose substantially across both groups, with the greatest fold increases in seronegative individuals. mRNA vaccines elicited the strongest responses, particularly in the seronegative group (fold change vs. adenovirus vaccines 6.93 for ancestral and 7.57 for Omicron). Post-vaccination, differences between seronegative and seropositive groups were no longer significant.

Vaccination markedly enhanced antibody binding across variants, with mRNA vaccines generating the highest responses. Prior infection conferred an advantage at baseline, but vaccination equalized responses across serostatus groups. These findings highlight the strong immunogenicity of mRNA vaccines and underscore the value of completing the full vaccination series in previously uninfected individuals in West and Central Africa.

ClinicalTrials.gov, NCT05096091, date of registration: October 26, 2021, Clinical trial registry: https://clinicaltrials.gov/study/NCT05096091?term=NCT05096091&rank=1#study-overview

1. Introduction

One of the main response actions during the COVID-19 pandemic was the rapid development and distribution of vaccines encompassing three major vaccine platforms: mRNA, adenovirus vector, and inactivated virus. The primary mechanism of all SARS-CoV-2 vaccine platforms is to generate a protective antibody (Ab) response against the Spike (S) protein[1]. However, the Ab response varies across different vaccine platforms[2,3].

The first licensed vaccines were all based on the ancestral (Wuhan) S protein. Acquisition of mutations in SARS-CoV-2, and especially in S protein, led to emergence of variant strains of the SARS-CoV-2 virus, including Alpha, Beta, Delta, and Omicron[4]. These mutations in the S protein reduce the binding capacity

of Abs elicited by vaccination with ancestral S protein, with a negative impact on vaccine effectiveness[4,5].

While studies have focused on the levels of antibodies produced by different vaccine platforms and on the influence of Spike variants on Ab binding within single regions or sites, few studies have looked at the influence of vaccine platform, and previous SARS-CoV-2 infection on Ab levels and binding across variants. Studying binding levels of SARS-CoV-2 variants in West and Central Africa is particularly important given the region's unique demographic, epidemiologic, and immunologic context, which may influence vaccine effectiveness and cross-protection against emerging strains. This study aimed to understand the relative levels of Ab binding to SARS-CoV-2 Spike variants following vaccination with mRNA, viral vector, or inactivated virus vaccines. It also examined how these binding levels differ based on infection prior to primary vaccination in participants from West and Central Africa.

2. Methods

2.1. Study design and population

Samples used in this study came from the InVITE study, which is an observational, multi-country study of immune response to COVID-19 vaccines[5]. Blood samples were collected within 24 hours of primary COVID-19 vaccination (V1) and again 2 months after completing the vaccination regimen (V2). Previous infection status at V1 (seropositive or seronegative) was determined by anti-Spike IgG levels above or below the Quanterix quantitative Ab assay[6] with a cutoff of 770ng/ml at V1 as recommended by the manufacturer.

For this study, we randomly selected a subset of samples from participants receiving their primary COVID-19 vaccines in the Democratic Republic of the Congo (DRC), Guinea, Liberia, and Mali. Paired samples from both V1 (time of vaccination) and V2 (2 months post completion of vaccination regimen) were included in this study. We selected samples from 16 participants for each of the following pairs of conditions: SARS-CoV-2 seropositive or seronegative at V1 from each of the three different vaccine platforms (resulting in 6 groups of participants. The vaccine platforms included mRNA (Pfizer/BioNTech and Moderna), adenovirus (AstraZeneca/Oxford and Gam-COVID-Vac), and inactivated virus (Sinopharm, Bharat Biotech, and Sinovac).

2.2. Serological assays

The Meso Scale Discovery 10 plex assay (MSD) that measures binding IgG to S antigen from 10 SARS-CoV-2 strains (catalogue # K15651U) was used in this analysis as previously described[7]. The assay measures binding of IgG to the S protein of the 4 ancestral stains; Wuhan, Alpha, Beta, Delta, and 5 Omicron strains; BA.1, BA.2, BA.2.12.1, BA.2.75, and BA.5.

2.3. Statistical Methods

The study was designed to include 16 participants in each of the six serostatus/platform groups. Power calculations showed that a t-test with a two-sided significance level of 0.05 would have 80% power to detect a standardized between-group difference in antibody binding of 1 (calculated as the between-group difference divided by the standard deviation) for each pairwise comparison. For example, a comparison of variant binding between the seropositive mRNA platform versus seropositive adenovirus platform.

MSD-10 plex binding titers were log10 transformed prior to analysis, with values below the limit of detection (LOD) imputed with LOD/2 (Table S1). Log10 binding levels were analyzed at V1, V2, difference between V2 and V1 using linear mixed models with random effects for participant. Geometric mean (GM) fold changes are presented from the models and referred to as fold change. Variants were grouped as: Ancestral: (Wuhan, Alpha, Beta, Delta) and Omicron (BA.1, BA.2, BA.2.12.1, BA.2.75, BA.5).

Models analyzing binding at V1 included an interaction term for variant group and baseline serostatus, allowing binding to vary across variant-serostatus combinations. Analyses of binding at V2 and for the fold change from V2 to V1 included interaction terms between baseline serostatus and vaccine platform (mRNA, inactivated virus, adenovirus), serostatus and variant group (Ancestral, Omicron), and platform and variant group. These interactions allowed binding response to differ across variant-serostatus-platform combinations. All analyses were conducted using R version 4.3.3 (2024-02-29). No adjustments for multiplicity were made, and p-values < 0.05 were deemed statistically significant.

2.4. Ethical considerations

Written informed consent was obtained from each participant and ethical approval was provided by each institution's ethical review board prior to the main study protocol implementation: DRC School of Public Health of Kinshasa Ethical Committee, ESP/CEA43C/2021–27 July 2021. Liberia: The National Research Ethics Board Ref: NREB-006-21 August 5, 2021, Mali: University of Science, Bamako Techniques and Technology, #2021/213/USTTB—Sept 2, 2021. Guinea: Comité d'éthique recherche en santé (CNERS), #: 137/CNERS/21 –Sept 8, 2021. The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice.

3. Results

3.1. General characteristics

Participants included in the study were enrolled from four countries: DRC (N = 35), Guinea (N = 16), Liberia (N = 21), and Mali (N = 24) (Table 1). Enrollment took place between September 2021 and May 2022; a period following the dominance of the Delta variant and included the rising prevalence of the Omicron Ba.1 variant globally. Table 1 shows the median anti-S Ab values measured using the Quanterix assay at two time points: V1 and V2. Initially, the seronegative group values were all below the seropositivity cut point of 770ng/ml as intended by the study design, whereas the seropositive group had

a median value of 8366ng/ml. Following vaccination at V2, anti-S Ab values for both serostatus groups increased to comparable levels.

Table 1
Participant country representation and anti-S Ab Quanterix levels (ng/ml) at V1 and V2.

Label	Seronegative	Seropositive
n	48	48
Country (%)		
DRC	19 (39.6)	16 (33.3)
Guinea	6 (12.5)	10 (20.8)
Liberia	9 (18.8)	12 (25.0)
Mali	14 (29.2)	10 (20.8)
V1 enrollment range	September 08, 2021- May 20, 2022	September 09, 2021-May 30, 2022
Anti-S (Quanterix)-V1 (median [IQR])	291.83 [104.01, 400.68]	8366.22 [3228.83, 13788.46]
Anti-S (Quanterix)-V2 (median [IQR])	27,806.21 [8486.78, 90,286.16]	25,375.05 [11,687.10, 67,281.35]

3.2 Comparison of variant and serostatus groups prior to vaccination (V1)

We first compared binding levels between variant groups and between previous infection status groups prior to participants receiving vaccination. Figure 1 shows the mean log10 MSD binding levels and corresponding 95% confidence intervals (CI) for each Spike variant analyzed, categorized by serostatus at V1 (pre-vaccination). The regression model, included terms for variant group, baseline serostatus, and an interaction between variant group and serostatus. No significant interaction was observed between variant and serostatus, suggesting that variant effects were consistent across serostatus groups, and serostatus effects were consistent across variants. Our analysis showed that binding was significantly greater in seropositive compared to seronegative participants, with a fold change of 15.65 (95% CI: 10.13 to 24.18). Furthermore, significantly stronger binding was observed for ancestral variants compared to Omicron variants, with a fold change of 3.7 (95% CI: 3.53 to 3.86) (Fig. 1 and Table S2).

3.3. Comparison of vaccine platform, variant and serostatus group after vaccination (V2)

Figure 2 shows the mean log10 MSD binding levels and 95% confidence intervals for participants, categorized by vaccine platform and serostatus, for each Spike variant at V2, which occurred 2 months after the completion of the vaccine regimen. Regression analyses were conducted with variants grouped by ancestral and Omicron categories.

Figure 3 and Table S3 present results from the regression analysis, incorporating terms for vaccine platform, serostatus group, variant group and 2-way interactions between these three groups. The interaction terms enable the effect of the vaccine platform on binding levels to vary by serostatus group and variant, or similarly, the effect of binding for each serostatus group to be different for each variant.

When comparing mRNA vaccines to adenovirus vaccination stronger binding was observed in the Ancestral negative group with a fold change of 6.93 (95% CI, 3.08–15.60) and in the Omicron negative group (fold change 7.57; 95% CI 3.37–17.03). The fold changes were smaller for the seropositive groups, with fold changes of 2.00 (95% CI 0.89–4.50) for the Ancestral group and 2.18 (95% CI 0.97–4.91) for the Omicron group. The fold change between mRNA vaccines and inactivated vaccination was larger than between mRNA and adenovirus vaccination across all groups: Ancestral seronegative 14.49 (95% CI 6.44–32.62), Omicron seronegative 14.33 (95% CI 6.37–32.24), Ancestral seropositive 4.96 (95% CI 2.20–11.16), Omicron seropositive 4.91 (95% CI 2.18–11.04). The estimated fold changes between adenovirus and inactivated vaccination ranged from 1.89 to 2.48 with only the 95% CI for the Ancestral seropositive group excluding 1 (CI, 1.10–5.58). (Fig. 3A).

When comparing fold changes between seropositive and seronegative groups all 95% CIs include 1 indicating no significant difference (Fig. 3B). Binding levels for ancestral variants consistently exceed those for Omicron variants with fold changes ranging from 3.55 to 3.92 (Fig. 3C).

3.4. Fold Change in binding levels between pre and post vaccine visits

Figure 4A shows the fold changes in MSD binding between V2 and V1 along with 95% CI. Figure 4B and Table S4 present the estimated fold changes and CIs. The largest fold changes were observed in the seronegative group across all vaccine platforms and variant groups, with fold changes ranging between 17–152. For the group receiving an inactivated virus vaccine, the 95% CI includes 1, indicating there is no significant increase in binding after vaccination for either variant. The fold changes in binding for V2 to V1 for the seropositive groups receiving mRNA or adenovirus vaccines are lower than that of the seronegative group (8.13, 7.92 respectively) but still show significant increases.

4. Discussion

This study evaluated the Ab binding levels against various Spike protein variants at pre-vaccination and post-vaccination timepoints in participants from West and Central Africa. These participants were seronegative (previously uninfected) or seropositive (previously infected) before receiving the first dose of the primary vaccination regimen, which used one of the three vaccine platforms. By assessing Ab binding to various Spike proteins in both seronegative and seropositive participants for different vaccine platforms, we explored cross-reactivity across variants due to infection, vaccination platform, and the combination of infection and vaccination.

Unexpectedly, we observed that the group that was uninfected (seronegative) prior to vaccination exhibited greater binding activity for the ancestral variants (Wuhan, Alpha, Beta, and Delta) compared to the Omicron variants (BA.1, BA.2, BA.2.12.1, BA.2.75, BA.5). The higher binding may be attributed to cross-reactivity with seasonal coronaviruses[10,11]. Interestingly, following infection (seropositive) yet prior to vaccination there was consistently greater binding across both the ancestral and Omicron variants compared to seronegatives. This may suggest cross-reactivity across all variants stemming from the infecting variant, which was likely an ancestral strain.

Other studies have demonstrated that hybrid immunity in previously infected individuals can lead to higher Ab levels compared to levels in vaccinated individuals without prior infection. It is believed that the first vaccine dose stimulates humoral immunity acquired during infection, resulting in elevated Ab levels compared to vaccine-only individuals[14]. Interestingly, we observed that administrating two doses after an infection did not increase Ab levels beyond those of people who received two doses alone. Notably, in seronegative individuals, Ab levels rise to match those of seropositive participants after a second vaccine dose, underscoring the importance of offering the full vaccination schedule to ensure maximum immune response in naïve individuals. Furthermore, this finding raises the question of the appropriate number of doses required for previously infected (seropositive) individuals as we observed no increased Ab binding quantity compared to seronegative participants. Future research using a functional assay could determine whether there is an enhancement in quality of the humoral immunity response when previously infected individuals are vaccinated.

Although all vaccines were based on the Wuhan strain, recipients of mRNA vaccines exhibited significantly higher levels of Ab binding across all variants when compared to other available vaccine platforms (adenovirus vectors and inactivated virus). This aligns with observations from pivotal trials conducted in Western countries, which demonstrated over 94% vaccine efficacy for mRNA vaccines[12,13] and 67–70% for the adenovirus vector vaccines[14,15]. Interestingly, the difference between vaccines was more pronounced in the uninfected group than for the previously infected group. Binding for the adenovirus and Inactivated virus vaccine groups were similar in the previously uninfected group for both variants. However, there was slightly higher binding for adenovirus vaccines compared to inactivated virus vaccines for the ancestral variant in the previously uninfected group.

Finally, the estimated effect of infection on binding is reflected in the fold change between the seropositive and seronegative group prior to infection which was 15.65 [10.13–24.18]. The impact of the vaccines alone is evident in the seronegative group after vaccination with fold changes of 148.88 for inactivated and adeno virus vector and 152.08 for the mRNA vaccine across both variant groups. Thus, the fold increase in binding following vaccination was much greater than the fold-increase following infection, although this difference may be partly due to the waning of infection induced antibodies prior to enrollment.

Despite employing rigorous methodology, our study has some limitations. A previous SARS-CoV-2 infection might have been misclassified in some cases due to the rapid decline of anti-SARS-CoV-2

antibodies. In addition, the antibody binding profile does not necessarily indicate protection, as the neutralizing function must be evaluated to confirm protective effects. Finally, our study was unable to control for time between infection and vaccination, which may confound the analysis.

Overall, this analysis contributes to the understanding of Ab binding patterns to variants in seropositive and seronegative individuals who have received different vaccine platforms, particularly in understudied geographical areas.

Declarations

Competing interests

All authors declare no financial or non-financial competing interests.

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Author Contribution

E.L., A.O, O.T., K.S., D.F., and S.H. wrote the main manuscript text. A.O. prepared the figures. D.C., M.H., J.B., N.C., M.M., S.F., M.Si., N.B. collected the data for the manuscript. R.D., T.R., I.R., R.R. interpreted data, conceptualized paper and reviewed versions. K.K., M.Sm., reviewed the paper. L.S. and S.N. performed assays, conceptualized and reviewed the paper. All authors reviewed the manuscript.

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Data Availability

The complete de-identified patient data set that supports the findings of this study will be made available to others upon reasonable request. Data access will be subject to review in accordance with the governing policies, criteria, conditions, and exceptions of the global InVITE study. Requests can be directed to the corresponding author.

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Figures

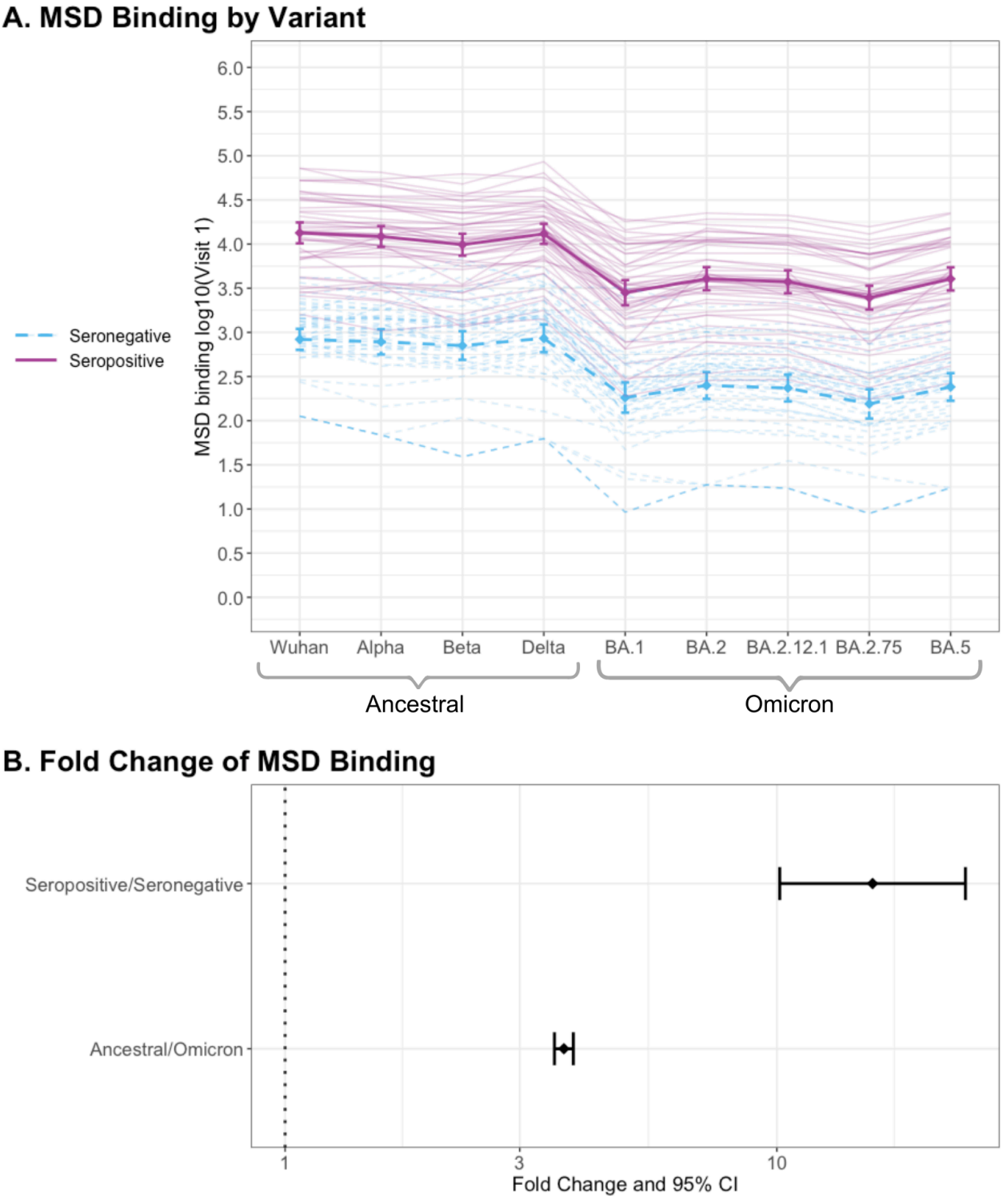


Figure 1

A. Mean log10 antibody (Ab) binding levels with 95% confidence intervals against SARS-CoV-2 variant spikes, measured by MSD 10-plex at baseline (V1). Results are shown for participants who were seronegative (dashed blue lines) or seropositive (solid pink lines) for anti-S at V1. Faint lines represent individual participant values. B. Geometric Mean fold-change and 95% confidence intervals for comparisons of Ab binding: between seropositive and seronegative participants and between Ancestral and Omicron variants at baseline.

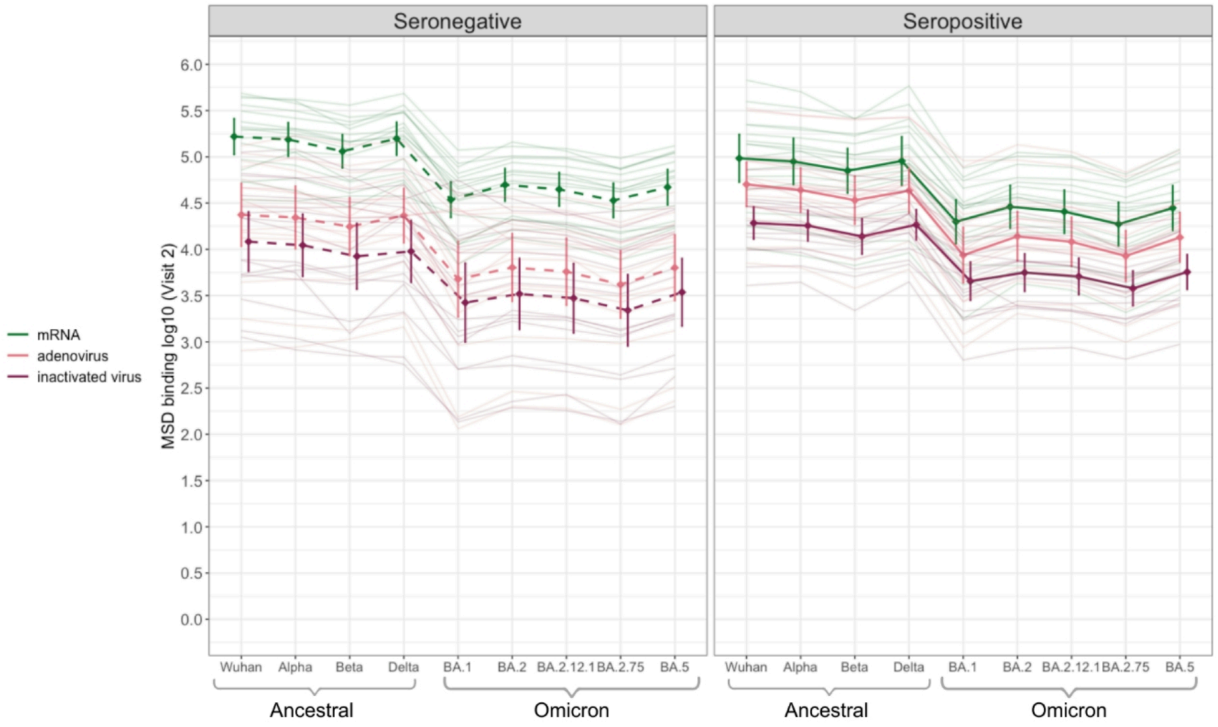


Figure 2

Mean (95% confidence interval) log10 Ab binding levels against SARS-CoV-2 variant spike measured by MSD 10-plex at V2 by baseline serostatus group and vaccine platform. The mRNA platform is represented by green lines, adenovirus is represented by pink lines, and inactivated virus is represented by purple lines. Faint lines represent the individual participant measurements. Dotted lines are used for seronegative and solid lines for seropositive groups.

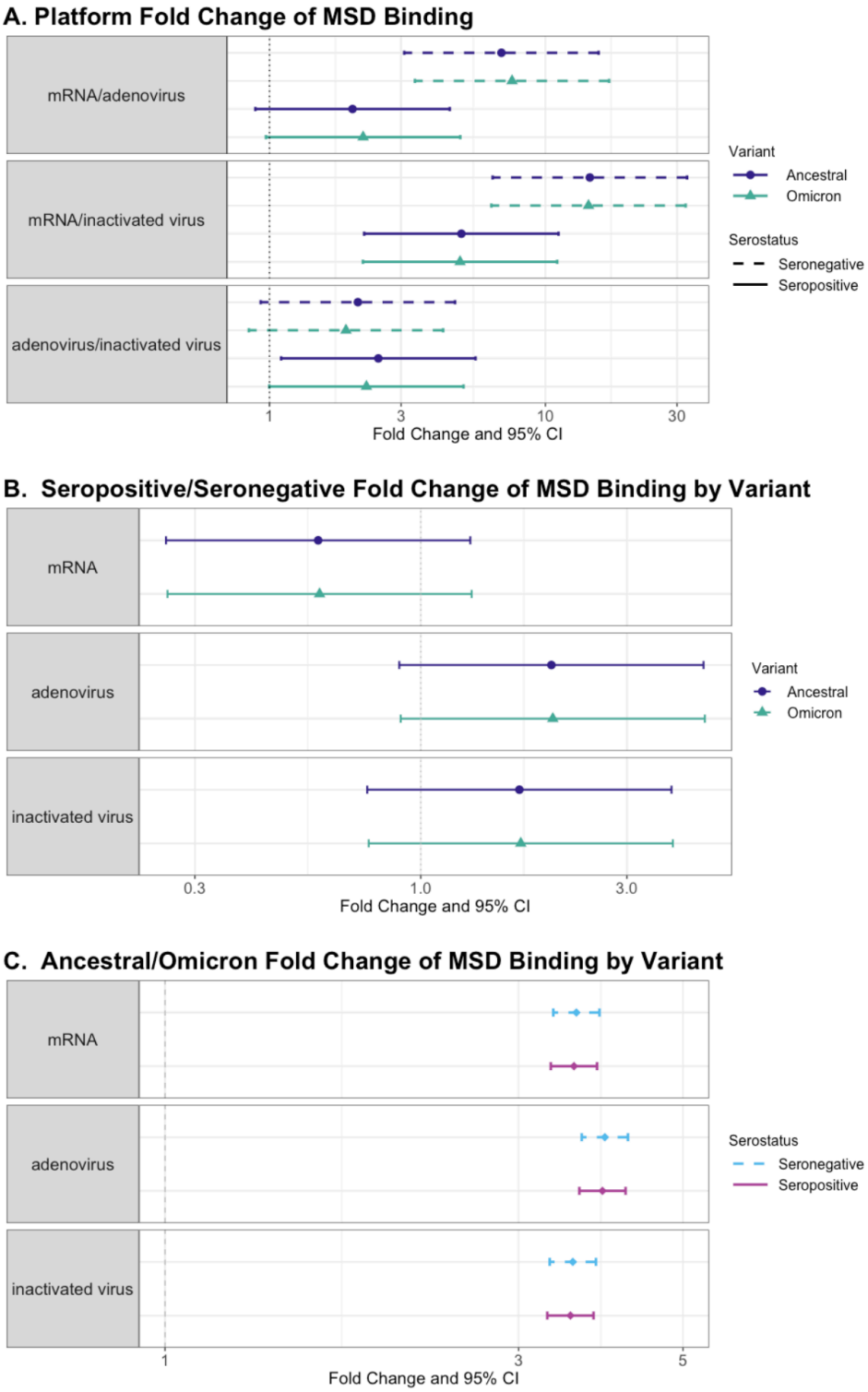


Figure 3

GM Fold changes of Ab binding at V2 for each panel. Ancestral variants are represented by blue lines with circles and Omicron variants by green lines with triangles. Seronegative are represented by dashed lines and seropositive by solid lines. A. GM Fold changes comparing platforms by variant group and serostatus. B. GM fold changes comparing seropositive to seronegative status by vaccine platform

and variant. C. GM fold changes comparing Ancestral to Omicron variants by vaccine platform and serostatus.

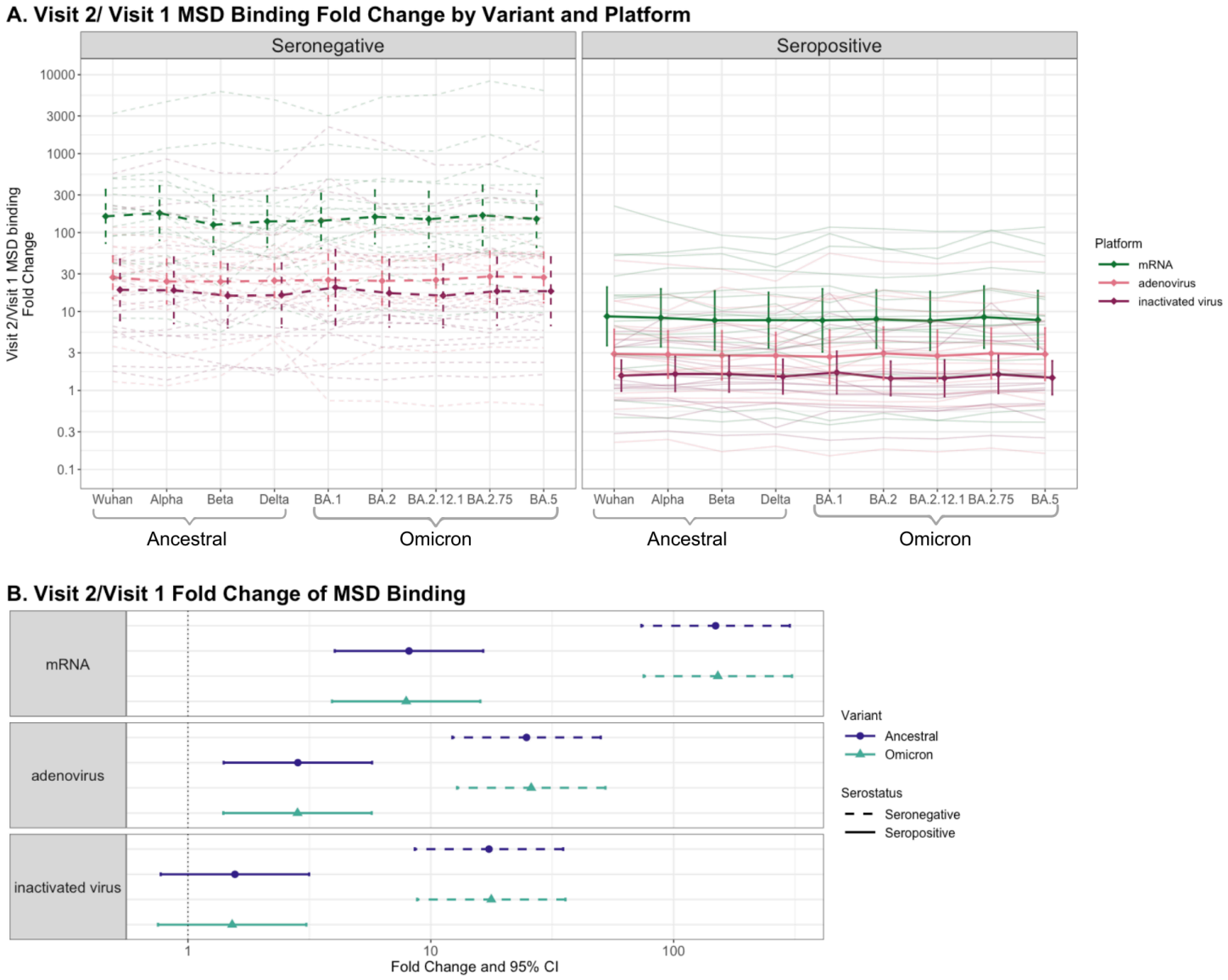


Figure 4

A. GM fold change of Visit 2 to Visit 1 of binding titer against SARS-CoV-2 variant spike measured by MSD 10-plex and 95% confidence intervals by serostatus and platform. The mRNA platform is represented by green; adenovirus is represented by pink, and inactivated virus is represented by purple lines. Faint lines represent the individual participant measurements. Dotted lines represent seronegative and solid lines represent seropositive groups B. GM fold Change of Visit 2 to Visit 1 of binding titer against SARS-CoV-2 variant spike by platform, variant, and serostatus group. Ancestral variants are represented by blue lines with circles and Omicron variants by green lines with triangles

Supplementary Files

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