

Loss of Neutralizing Antibody Response to mRNA Vaccination against SARS-CoV-2 Variants: Differing Kinetics and Strong Boosting by Breakthrough Infection

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31 **Abstract:**

32 The waning efficacy of SARS-CoV-2 vaccines combined with the continued emergence of
33 variants resistant to vaccine-induced immunity has reignited debate over the need for booster
34 vaccines. To address this, we examined the neutralizing antibody (nAb) response against four
35 major SARS-CoV-2 variants—D614G, Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2)—in
36 health care workers (HCWs) at pre-vaccination, post-first and post-second mRNA vaccine dose,
37 and six months post-second mRNA vaccine dose. Neutralizing antibody titers against all variants,
38 especially the Delta variant, declined dramatically from four weeks to six months post-second
39 mRNA vaccine dose. Notably, SARS-CoV-2 infection enhanced vaccine durability, and mRNA-
40 1273 vaccinated HCWs also exhibited ~2-fold higher nAb titers than BNT162b2 vaccinated HCWs.
41 Together these results demonstrate possible waning of protection from infection against SARS-
42 CoV-2 Delta variant based on decreased nAb titers, dependent on COVID-19 status and the
43 mRNA vaccine received.

44

45 **Introduction:**

46 Since its emergence in late 2019, the COVID-19 pandemic has led to over 252 million
47 confirmed cases and over 5 million deaths as of November 14, 2021 (1). In response, several
48 vaccines have been developed against SARS-CoV-2, the causative agent of COVID-19, including
49 two novel mRNA vaccines, Moderna mRNA-1273 and Pfizer/BioNTech BNT162b2. These highly
50 effective vaccines have helped to stem COVID-19 hospitalizations and deaths. However, the rapid
51 evolution of SARS-CoV-2, combined with waning vaccine efficacy, remain a threat to public health.

52 Following its introduction into the human population, several SARS-CoV-2 variants of
53 concern (VOCs) have emerged. Very soon after zoonotic transmission, SARS-CoV-2 acquired a
54 predominant D614G mutation in its spike (S) protein. This mutation leads to enhanced
55 transmissibility, likely due to increased stability of the S protein, increased viral titers in the
56 nasopharynx, and increased infectivity (2). As a result, nearly all currently circulating SARS-CoV-
57 2 strains bear the D614G mutation (3). However, as greater proportions of the world population
58 acquired immunity against SARS-CoV-2, through infection or vaccination, new VOCs emerged
59 that had reduced susceptibility to antibody-mediated immune responses and continued to become
60 more transmissible (4, 5). One VOC, Alpha (B.1.1.7), is characterized by N-terminal domain (NTD)
61 deletions and a key N501Y mutation in its receptor-binding domain (RBD). Alpha exhibited
62 enhanced transmissibility and rapidly spread from Europe to other parts of the world (6). Another
63 VOC to emerge at about the same time was Beta (B.1.351), which is characterized by other NTD
64 mutations and deletions, as well as key RBD mutations, including K417N, E484K, and N501Y.
65 While the Beta variant did not disseminate as widely as Alpha, it harbored strong resistance to
66 vaccine-induced immunity (7). Finally, Delta (B.1.617.2) is responsible for the most recent wave
67 of the COVID-19 pandemic and is characterized by new NTD alterations, together with key RBD
68 mutations (L452R and T478K). Delta has led to an alarming number of vaccine breakthrough
69 infections worldwide and has prompted debate about the need for vaccine booster doses.

70 The extent to which the rise in breakthrough infections is caused by increased resistance
71 to vaccine-induced immunity in these variants and/or to waning durability of immunity and efficacy
72 of vaccines in preventing infection remains unclear. Reports from India, where the population was
73 still pursuing mass vaccination efforts, show minor differences in breakthrough infection rates
74 between Alpha and Delta. Specifically, BNT162b2 efficacy against symptomatic infection was
75 reported to drop from 93.4% against Alpha to 87.9% against Delta (8). However, reports from the
76 U.S. indicate that vaccine efficacy of BNT162b2 against Delta infection declined from 93% one
77 month after vaccination to 53% at four months (9), consistent with an overall waning of vaccine
78 efficacy over time (10). A critical goal of this study is to better understand how the durability of
79 vaccine efficacy contributes to rates of breakthrough infections, especially in the context of
80 evolving SARS-CoV-2 variants. Such insights will improve strategies for allocation of booster
81 doses, recommendations for immunocompromised patients, and could guide any reformulation
82 of future SARS-CoV-2 booster doses.

83 To address these issues, we examined neutralizing antibody (nAb) levels in 48 vaccinated
84 health care workers (HCWs) against the major SARS-CoV-2 variants using serum collected pre-
85 vaccination, one month after the first dose of BNT162b2 or mRNA-1273, and one and six months
86 after the second dose of vaccine. Indeed, prior studies have shown that neutralizing antibody
87 levels are a major correlate for protection from SARS-CoV-2 infection (11).

88

89 **Results:**

90 We produced lentiviral pseudotypes expressing a *Gaussia* luciferase reporter gene and
91 bearing SARS-CoV-2 spike derived from D614G, Alpha, Beta, or Delta (**Fig. 1A**). Pseudotyped
92 virus infectivity was then determined by infection of HEK293T-ACE2 cells. *Gaussia* luciferase
93 secreted into the media of infected cells was assayed to determine the infectivity of produced
94 lentiviral pseudotypes. We did not find significant differences in pseudotyped lentivirus infectivity

95 for the four variants (all containing D614G) tested (**Fig. 1B**), despite some reports of drastically
96 increased transmission and spread for some VOCs, especially the Delta variant (12).

97 We used our previously reported (13, 14) highly-sensitive SARS-CoV-2 pseudotyped
98 lentivirus-based virus neutralization assay to assess nAb titers in HCW samples collected under
99 approved IRB protocols (2020H0228 and 2020H0527). The 48 HCW samples included 22 mRNA-
100 1273 and 26 BNT162b2 vaccinated individuals, with a median age of 37 years (IQR = 31.75-
101 43.25). Samples were collected from HCWs with median time points of 222 days (IQR = 215-
102 225.75) pre-first vaccine dose (Pre), 21 days (IQR = 19.25-23) post-first vaccine dose (Post 1st),
103 26 days (IQR = 22.5-28) post-second vaccine dose (Post 2nd), and 194 days (IQR = 190-197.75)
104 post-second vaccine dose (Six Months). According to the titer of pseudotyped viruses, we
105 adjusted the volumes of each so that equivalent infectious viruses were used in neutralization
106 assays. HCW serum samples underwent 4-fold serial dilutions followed by the addition of
107 pseudotyped virus for one hr neutralization, with final dilutions of 1:80, 1:320, 1:1280, 1:5120,
108 1:20480, and no serum control. HEK293T-ACE2 cells were then infected with neutralized virus
109 and *Gaussia* luciferase activity was assayed 48 hrs and 72 hrs after infection. Neutralizing titer
110 50% (NT₅₀) values were determined by least-squares fit, non-linear regression in GraphPad Prism
111 5.

112 We compared the strength of the nAb titers over time against all four variants tested.
113 Following the first dose of mRNA vaccine, a strong nAb response was induced among HCWs
114 compared to pre-vaccination across all variants ($p < 0.001$), which efficiently blocked virus entry;
115 this was despite the huge variation in nAb titers of these individuals including against D614G
116 (mean = 1140, 95% CI = 317-1963, range = 100-15954) (**Fig. 1C**). However, across all variants,
117 between 14.6% (7/48) and 45.8% (22/48) of HCWs exhibited NT₅₀ values below detection limit
118 (NT₅₀ < 100) following the first dose of vaccine (**Fig. 1C-F**). These initial nAb titers fell to 0.0%
119 (0/48) to 4.2% (2/48) for all variants following a second vaccine dose, with a 2-3-fold increase in
120 mean nAb titers compared to the first dose ($p < 0.001$) (**Fig. 1C-F**). Notably, four HCWs with

121 higher nAb titers after the first vaccine dose did not show an increase, but a plateau or slight
122 decline in nAb titers following the second dose (**Fig. 1C-F**). These four individuals included one
123 that was anti-SARS-CoV-2-N positive at pre-vaccination, and three that were anti-N positive post-
124 first vaccine dose—indicating infection either prior to or shortly after their first vaccine dose. We
125 found that, following two vaccine doses, the Alpha, Beta, and Delta VOCs exhibited a 1.3- (p <
126 0.001), 3.2- (p < 0.001), and 2.2-fold (p < 0.001) lower NT₅₀ values compared to D614G,
127 respectively (**Fig. 1C-F**). Critically, six months post-vaccination, there was a 3.5-10.7-fold
128 reduction in nAb levels against all variants examined, with 37.5% (18/48) to 56.3% (27/48) of
129 HCWs exhibiting NT₅₀ levels below the limit of detection (**Fig. 1C-F**). The mean NT₅₀ values for
130 Alpha, Beta, and Delta variants at six months were 1.3-, 1.7-, and 3.6-fold lower than that of
131 D614G, respectively, although the differences in these low nAb titer groups were not statistically
132 significant (**Fig. 1C-F**).

133 We also examined the correlation between time post-second dose and log₁₀ transformed
134 NT₅₀ values. We found a statistically significant association between these values for all four
135 variants (**Fig. 1G-J**). This corresponded to an approximately 10-fold decline in NT₅₀ for D614G,
136 Alpha, and Delta ($R^2 = 0.0452-0.594$, p < 0.0001) every ~22 weeks compared with Beta ($R^2 =$
137 0.286, p < 0.001) every ~37 weeks (**Fig. 1G-J**).

138 Prior COVID-19 status is a critical parameter for the nAb response to vaccination (15). Of
139 the 48 HCWs examined, one was anti-SARS-CoV-2 N positive by ELISA pre-vaccination, four
140 were anti-N positive at their post-first vaccine dose sample, three at their post-second vaccine
141 dose sample, and four at their six-month vaccine sample—indicating that these 12 subjects were
142 infected by SARS-CoV-2 at different phases of vaccination (**Fig. 2A**). At the time of pre-
143 vaccination sample collection D614G was the major circulating SARS-CoV-2 variant, while at the
144 time off post-first dose and post-second dose D614G and Alpha were circulating, and at the six
145 month time point Delta was the dominant strain. Notably, not all patients remained anti-N positive,
146 but were still considered to have been infected for the purpose of analysis. Following the first

147 vaccine dose, anti-N positive HCWs exhibited 11.7-fold higher mean NT_{50} ($p < 0.001$) against all
148 four viruses compared to the anti-N negative HCWs (**Fig. 2B**). This difference diminished to 2.3-
149 fold following a second vaccine dose ($p < 0.001$) (**Fig. 2B**). However, at six months post-
150 vaccination, anti-N positive HCWs exhibited 6.1-fold higher NT_{50} values than anti-N negative
151 HCWs for all variants ($p = 0.042$) (**Fig. 2B**). Interestingly, we found that the differences in NT_{50}
152 between anti-N positive and negative HCWs were greater and more statistically significant for
153 D614G and Alpha compared with the Beta and Delta variants, likely due to the strong
154 neutralization resistance of the latter VOCs (**Fig. 2C**). Notably, for anti-N negative HCWs,
155 between 41.7% (15/36) and 66.7% (24/36) of subjects exhibited NT_{50} against all four variants that
156 were below detection limit at six months, in sharp contrast to anti-N positive individuals, who were
157 between 8.3% (1/12) and 25.0% (3/12) (**Fig. 2C**).

158 We further examined the difference in nAb durability between Moderna mRNA-1273 and
159 Pfizer/BioNTech BNT162b2 vaccinated HCWs. Across all variants over the full-time course, we
160 observed that mRNA-1273 elicited an overall 2.2-fold higher nAb response than the BNT162b2
161 ($p < 0.001$) (**Fig. 2D**). In particular, following two vaccine doses, mRNA-1273 vaccinated HCWs
162 exhibited 2.1-, 2.3-, 2.4-, and 1.3-fold higher nAb response compared to BNT162b2-vaccinated
163 HCWs for D614G, Alpha, Beta, and Delta variants, respectively (**Fig. 2E**). The slightly higher NT_{50}
164 values of mRNA-1273 vaccinated HCWs persisted through their six-month collection (**Fig. 2E**),
165 with 18.2% (4/22) to 36.4% (8/22) of mRNA-1273-vaccinated HCWs falling below detection limit
166 for the four variants compared to 53.8% (14/26) to 73.1% (19/26) for BNT162b2 (**Fig. 2E**).

167 We examined additional factors that may contribute to strength and duration of the nAb
168 response to vaccination, including age and sex. We observed no significant correlation for age
169 and NT_{50} against D614G at any time point (**Fig. 3A-C**), potentially influenced by our relatively
170 younger pool of study subjects. However, male HCWs exhibited significantly higher NT_{50} titers
171 compared to females (**Fig. 3D**).

172

173 **Discussion:**

174 In summary, we report a dramatic decline of SARS-CoV-2 nAb at six months post-mRNA
175 vaccination and examined several key factors accounting for these kinetics. Most critically, we
176 observed a drastic drop in nAb titers from 3-4 weeks to six months post-second vaccine dose,
177 with more than 50% of HCWs exhibiting NT_{50} values below detection limit against Delta at the
178 latter time. This number increased to almost 70% for the anti-N negative HCWs, which was in
179 sharp contrast to that of anti-N positive HCWs, with 25% below the background. Thus, additional
180 antigen exposures are necessary to improve the durability of the SARS-CoV-2 nAb response,
181 consistent with data from administration of mRNA vaccine booster doses (16). Together, these
182 results support a rationale for the need for boosters and alternative vaccination strategies to
183 achieve long-term protection from infection with SARS-CoV-2.

184 Additionally, we observed that individuals vaccinated with BNT162b2 exhibited lower nAb
185 titers than individuals vaccinated with mRNA-1273. However, the trend for declining nAb titers
186 was consistent for both vaccines. Thus, both mRNA vaccines require booster doses to maintain
187 protective nAb levels, although the waning of nAb responses likely occurs over a relatively longer
188 period of time for mRNA-1273. Further examination of the durability of cellular immunity following
189 mRNA vaccination is needed, as this more persistent immunity may limit the rates of
190 hospitalization and death, which remain low for mRNA vaccinated individuals (17).

191 In this study, we found that all three VOCs consistently had reduced NT_{50} values compared
192 to D614G at all time points, with Beta showing the most pronounced nAb resistance, followed by
193 Delta. These results are consistent with preliminary reports from ours and other groups (14, 18,
194 19). However, we found that the Delta variant exhibited comparable or even higher resistance to
195 nAbs than Beta for samples collected at six months post vaccination. The more modest drop in
196 NT_{50} values at six months for the Beta variant was unclear, but likely the result of this variant's
197 pre-existing strong resistance to neutralization following the second dose of vaccination. Further,
198 the more dramatic decline in nAb titers against Delta could be attributed to a lower frequency and

199 durability of neutralizing antibody-producing plasma cells. As reported by others, the rampant
200 spread of Delta in vaccinated and unvaccinated populations is likely related to other factors such
201 as its high replication kinetics and transmissibility (20) coupled with its comparable neutralization
202 resistance.

203

204 **Materials and Methods:**

205 *Health Care Worker Cohort:*

206 De-identified vaccinated health care worker (HCW)'s serum samples were collected under
207 approved IRB protocols (2020H0228 and 2020H0527). These 48 HCWs ranged in age from 22-
208 61 years (median = 37; IQR = 31.75-43.25) and included 26 male and 22 female HCWs. HCWs
209 were vaccinated with either Moderna mRNA-1273 (n = 22) or Pfizer/BioNTech BNT162b2 (n =
210 26). Sera were collected from HCWs at 4 time points, with median time points being 222 days
211 (IQR = 215-225.75) pre-first vaccine dose (Pre), 21 days (IQR = 19.25-23) post-first vaccine dose
212 (Post 1st), 26 days (IQR = 22.5-28) post-second vaccine dose (Post 2nd), and 194 days (IQR =
213 190=197.75) post-second vaccine dose (6 Months). HCWs received their second vaccine dose
214 between January and February of 2021.

215 HCW COVID-19 status was determined by anti-N ELISA (described below). Of the 48
216 HCWs examined, one was anti-SARS-CoV-2 N positive by ELISA pre-vaccination, four became
217 anti-N positive for their post-first vaccine dose sample, three for their post-second vaccine dose
218 sample, and four for their six-month vaccine sample—indicating that these 12 subjects were
219 infected by SARS-CoV-2 at the different phases of vaccination.

220

221 *Constructs for Pseudotyping Virus Production:*

222 Production of lentiviral pseudotyped virus was performed using a previously reported
223 protocol using pNL4-3-HIV-1-inGluc vector (13, 14, 21-23). This vector is a pNL4-3-HIV-1 ΔEnv
224 construct and contains a *Gaussia* luciferase reporter gene with a CMV promoter both oriented in

225 an anti-sense orientation relative to the HIV-1 genome. This *Gaussia* luciferase reporter gene
226 then contains a sense orientation intron, which prevents expression of *Gaussia* luciferase in the
227 virus producing cells. However, after the intron is spliced from full length virus genomes and upon
228 integration into target cells, target cells can produce *Gaussia* luciferase, which is secreted in
229 mammalian cell culture (24). Constructs encoding N- and C-terminal flag-tagged SARS-CoV-2
230 spike (S) for each variant — D614G, Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2) —
231 were synthesized and cloned into pcDNA3.1 vector using KpnI/BamHI restriction enzyme cloning
232 by GenScript BioTech (Piscataway, NJ).

233

234 *Cell Lines and Maintenance:*

235 HEK293T cells (CRL-11268, CVCL_1926, ATCC, Manassas, VA) and HEK293T-ACE2
236 cells (NR-52511, BEI Resources, ATCC, Manassas, VA) were maintained in Dulbecco's Modified
237 Eagles Medium (Gibco, 11965-092, ThermoFisher Scientific, Waltham, MA) supplemented with
238 10% (v/v) fetal bovine serum (F1051, Sigma-Aldrich, St. Louis, MO) and 1% (v/v)
239 penicillin/streptomycin (SV30010, HyClone Laboratories Inc., Logan, UT). Cells were maintained
240 in at 37°C and 5% CO₂.

241

242 *Pseudotyped Virus Production and Titering:*

243 Pseudotyped lentivirus was produced by co-transfection of HEK293T cells with pNL4-3-
244 HIV-1-inGluc and pcDNA3.1 vector expressing the spike of interest (D614G, B.1.1.7, B.1.351, or
245 B.1.617.2) in a 2:1 ratio using polyethylenimine (PEI) transfection. Virus was collected 24 hrs, 48
246 hrs, and 72 hrs after transfection, then was pooled and stored at -80°C.

247 To determine relative titers of harvested virus, the pseudotyped virus for each of the
248 SARS-CoV-2 variants were used to infect HEK293T-ACE2 cells. Then, 48 hrs and 72 hrs after
249 infection, *Gaussia* luciferase activity in the media of infected cells was determined. 20 µL of cell
250 culture media and 20 µL of *Gaussia* luciferase substrate (0.1M Tris (T6066, MilliporeSigma,

251 Burlington, MA) pH 7.4, 0.3M sodium ascorbate (S1349, Spectrum Chemical Mfg. Corp., New
252 Brunswick, NJ), 10 μ M coelenterazine (CZ2.5, GoldBio, St. Louis, MO)) were combined in a white
253 polystyrene 96-well plate. Luminescence was immediately measured by a BioTek Cytation5 plate-
254 reader.

255

256 *Virus Neutralization Assays:*

257 Virus neutralization assays were performed as previously reported (13, 14, 23). In a 96-
258 well format, HCW serum was 4-fold serial diluted and 100 μ L of pseudotyped virus was added
259 (final dilutions of 1:80, 1:320, 1:1280, 1:5120, 1:20480, and no serum). Note that, to ensure
260 comparable results between SARS-CoV-2 variants, equivalent amounts of infectious virus were
261 used based on the pre-determined virus titers. The virus was incubated with HCW serum for 1 hr
262 at 37°C, followed by infection of HEK293T-ACE2 cells seeded on a 96-well polystyrene tissue
263 culture plate. *Gaussia* luciferase activity in cell culture media was then assayed 48 hrs and 72 hrs
264 after infection as described above. Neutralizing titer 50% (NT₅₀) for each serum sample was
265 determined by non-linear regression with least squares fit in GraphPad Prism 5 (GraphPad
266 Software, San Diego, California).

267

268 *Anti-N ELISA:*

269 Anti-N ELISA was performed as previously reported (13). ELISA was performed by using
270 the EDI Novel Coronavirus COVID-19 N protein IgG ELISA Kit (KT-1032, EDI, San Diego, CA)
271 following manufacturer's protocol. Briefly, 100 μ L of a 1:100 dilution of HCW serum was added to
272 microplates coated with SARS-CoV-2 nucleocapsid (N) antigen and incubated for 30 min. Plates
273 were then washed and treated with 100 μ L of HRP labeled anti-human-IgG antibody (31220, EDI,
274 San Diego, CA) for 30 min. Then plates were washed and 100 μ L of ELISA HRP substrate (10020,
275 EDI, San Diego, CA) was added and incubated for 20 min before 100 μ L of stop solution (10030,

276 EDI, San Diego, CA) was added. Absorbance at 450 nm was read by spectrophotometric plate
277 reader using Gen 5 software.

278

279 **Statistical Analyses:**

280 Statistical analysis was done with GraphPad Prism 5. Comparisons between multiple
281 groups were done using one-way ANOVA with Bonferroni's multiple testing correction (Figs. 1A,
282 2B) or one-way repeated measures ANOVA with Bonferroni's multiple testing correction (Figs.
283 1C-F). For comparisons between two "treatments" across multiple groups, a two-way ANOVA
284 with Bonferroni's multiple testing correction was used (Figs. 2C and 2E). For comparisons
285 between two groups, an unpaired, two-tailed student's t-test with Welch's correction was used
286 (Figs. 2D, S1D). For correlative analyses between two continuous variables, a linear regression
287 model with least squares fit was used with \log_{10} transformed NT₅₀ values to better approximate
288 linearity (Figs. 1G-J, S1A-C).

289

290 **Competing Interests:**

291 The authors declare no competing interest.

292

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303 **Author Contributions:**

304 J.P.E. conducted neutralization assays, analyzed data, and drafted the manuscript. C.Z. aided in
305 neutralization assays, review of manuscript, and provided valuable discussion and insight. C.C.
306 contributed to recruitment of HCWs and sample collection. R.J.G. contributed to study design,
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308 discussion and insight. G.L. provided anti-N ELISA data. S.-L.L. contributed to study design,
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387 **Figure 1: The durability of vaccine-induced immunity wanes over time, with a virtual loss**
388 **at six months for the Delta variant.** *Gaussia* luciferase reporter gene containing lentivirus
389 pseudotypes were produced bearing the spike (S) protein from SARS-CoV-2 variants. **(A)**
390 Schematic representations of the SARS-CoV-2 variant spikes tested are shown which contain the
391 indicated mutations. These include D614G, Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2).
392 The schematics highlight the location of the S1 and S2 subunits as well as the N-terminal domain
393 (NTD), receptor binding domain (RBD), fusion peptide (FP), and transmembrane region (TM). **(B)**
394 Lentivirus pseudotypes were used to infect HEK293T-ACE2 cells and 48hrs after infection media
395 was harvested from infected cells and assayed for *Gaussia* luciferase activity to determine the
396 relative infectivity of variant pseudotyped virus. **(C-F)** Lentivirus pseudotyped with SARS-CoV-2
397 S from D614G **(C)**, Alpha **(D)**, Beta **(E)**, and Delta **(F)** were incubated for 1 hr to neutralize with
398 serial dilutions (1:80, 1:320, 1:1280, 1:5120, 1:20480, and no serum) of health care worker (HCW)
399 serum collected pre vaccination, post vaccination with a first dose of Pfizer/BioNTech BNT162b2
400 or Moderna mRNA-1273, post vaccination with a second dose of mRNA vaccine, and six months
401 post vaccination with a second dose of mRNA vaccine. Neutralized virus was then used to infect
402 HEK293T-ACE2 cells and *Gaussia* luciferase activity was assayed 48 hrs and 72 hrs after
403 infection. Neutralization titers 50% (NT₅₀) were determined by least-squares fit non-linear
404 regression. Mean NT₅₀ are shown at the top of the plots, and NT₅₀ values below 100 were
405 considered background. **(G-J)** Log₁₀ transformed (to better approximate linearity) NT₅₀ values
406 against D614G **(G)**, Alpha **(H)**, Beta **(I)**, and Delta **(J)** variants were plotted against days post-
407 second vaccine dose of sample collection. The equation of the fitted curve, the goodness of fit
408 (R²), and p-value for the curve are displayed on each plot. The dotted lines correspond to the
409 background level (NT₅₀ < 100). Statistical significance was determined by one-way ANOVA with

410 Bonferroni's correction (**B**), one-way repeated measures ANOVA with Bonferroni's correction (**C**-
411 **F**), or by least-squares fit linear regression (**G-J**). In call cases, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;
412 ns: not significant.

413

414 **Figure 2: The durability of the nAb response is dependent on prior COVID-19 status, mRNA**
415 **vaccine type but not age.** (**A**) Anti-N ELISA results are presented for HCWs who became anti-
416 N positive ($OD_{450} > 0.4$ at any time point; $n = 12$) and HCWs who never became anti-N positive
417 ($OD_{450} < 0.4$ for all time points; $n = 36$). (**B, C**) HCWs were divided by prior COVID-19 status as
418 determined by anti-SARS-CoV-2 N ELISA. HCWs with anti-N above the cut-off value of 0.4 for
419 any time point ($n = 12$) were considered as COVID-19 positive during the study period. NT_{50}
420 values against all four variants combined (**B**) or separated (**C**) for anti-N positive HCWs are
421 compared to anti-N negative HCWs for samples collected post first mRNA vaccine dose, post
422 second mRNA vaccine dose, and six months post second mRNA vaccine dose, respectively. (**D,**
423 **E**) HCWs were divided by types of mRNA vaccine received, either Moderna mRNA-1273 ($n = 22$)
424 or Pfizer/BioNTech BNT162b2 ($n = 26$), and all variants at post-first vaccine dose, post-second
425 vaccine dose, and six months post-second vaccine doses were plotted together (**D**) or grouped
426 by variant and time point (**E**). Mean NT_{50} values are indicated at the top of plots (**B, D**) Statistical
427 significance was determined by one-way ANOVA with Bonferroni's correction (**B**) two-way,
428 repeated measures ANOVA with Bonferroni's correction (**C, E**) or unpaired two-tailed t-test with
429 Welch's correction (**D**). In call cases, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant.

430

431 **Figure 3: Impact of age and sex on response to mRNA vaccination.** (**A-C**) \log_{10} transformed
432 NT_{50} titers against D614G-SARS-CoV-2 S pseudotyped lentivirus are plotted against age (in
433 years) at time of second vaccine dose for HCW samples collected post-first vaccine dose (**A**),
434 post-second vaccine dose (**B**), and 6 months post-second vaccine dose (**C**). (**D**) NT_{50} values
435 against all variants at all time points were compared for male and female HCWs, with mean NT_{50}

436 values displayed at the top of the plot. Statistical significance was determined by linear regression
437 with least squares residual fit (**A-C**) or by unpaired, two-tailed student's t-test with Welch's
438 correction (**D**). P-values are indicated as 'ns' (not significant) for $p > 0.05$ or * $p < 0.05$, ** $p < 0.01$,
439 *** $p < 0.001$.
440

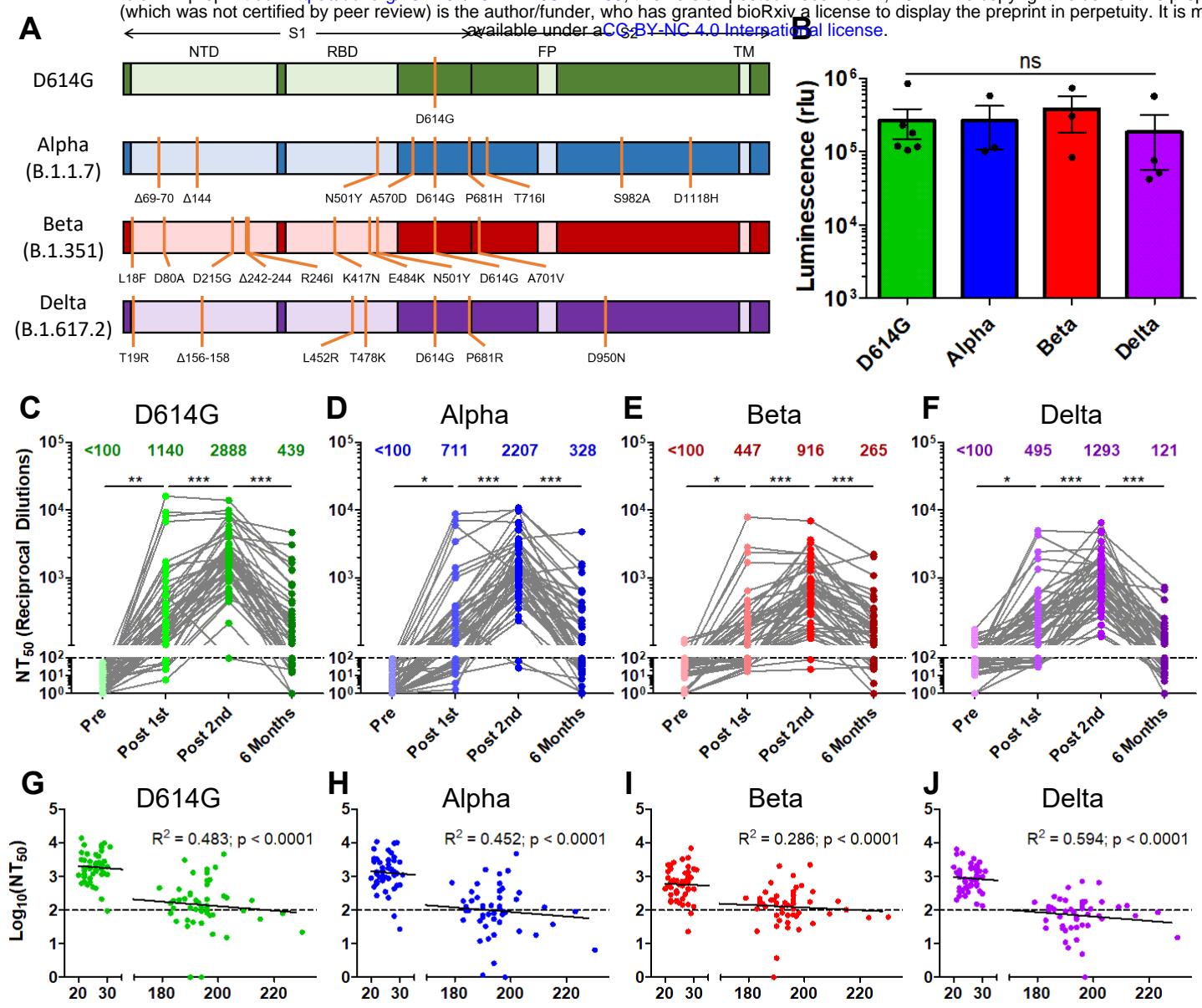


Figure 1

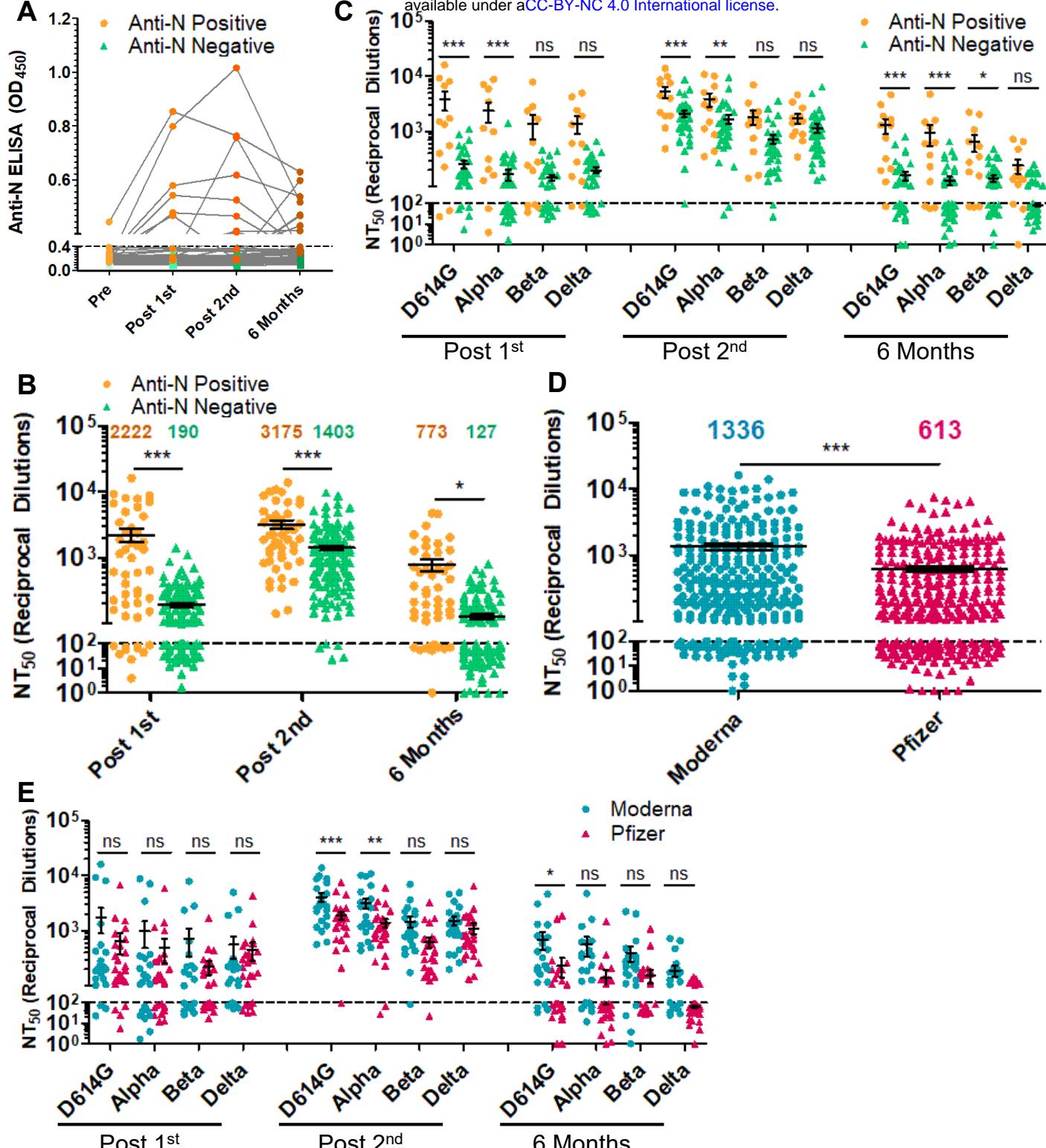


Figure 2

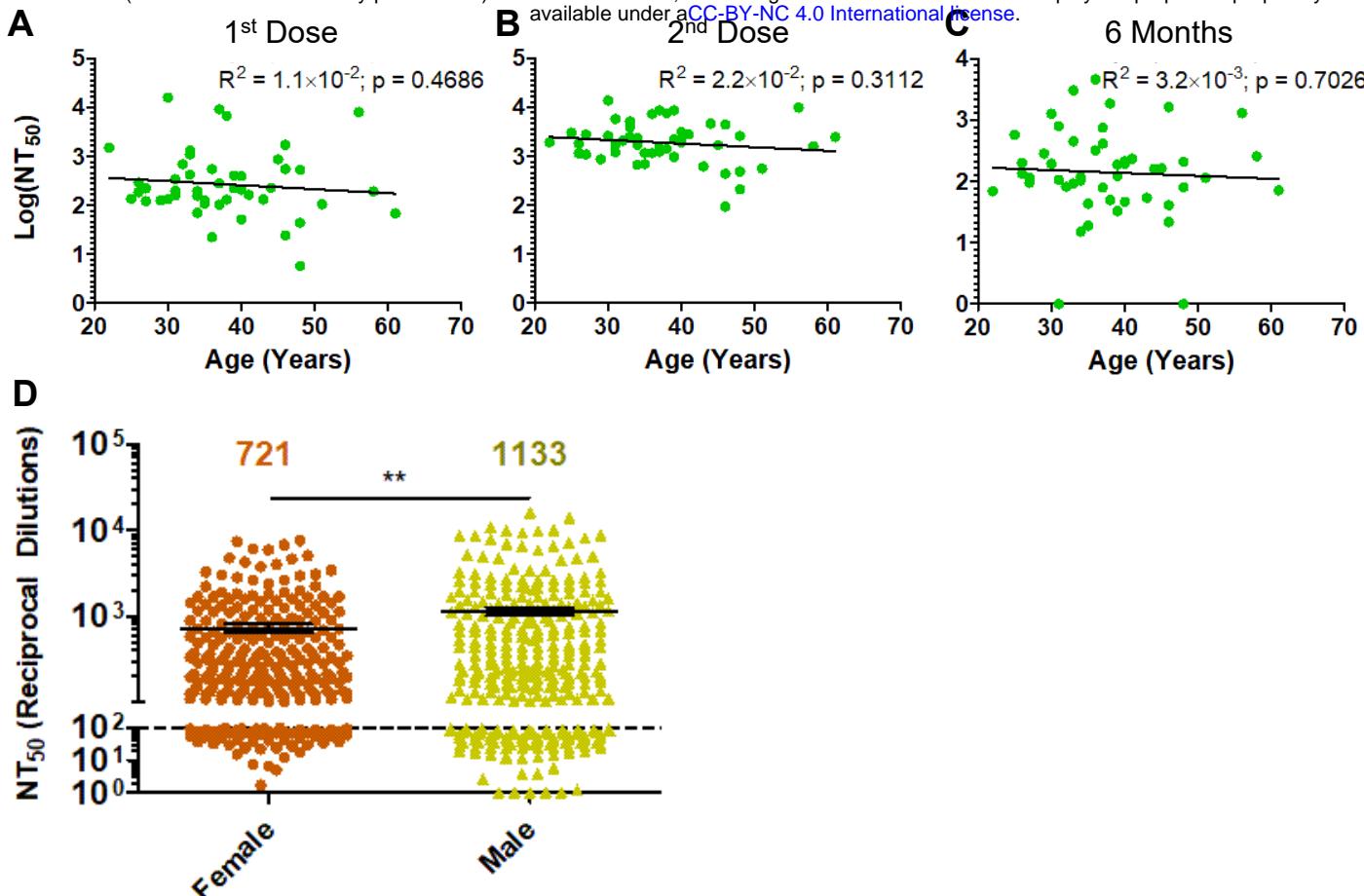


Figure 3