

Limited window for donation of convalescent plasma with high live-virus neutralizing antibodies for COVID-19 immunotherapy

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One Sentence Summary

Evaluation of SARS-CoV-2 anti-spike protein IgM, IgG, and live-virus neutralizing titer profiles reveals that the optimal window for donating convalescent plasma for use in immunotherapy is within the first 60 days of symptom onset.

ABSTRACT

The optimal timeframe for donating convalescent plasma to be used for COVID-19 immunotherapy is unknown. To address this important knowledge deficit, we determined *in vitro* live-virus neutralizing capacity and persistence of IgM and IgG antibody responses against the receptor-binding domain and S1 ectodomain of the SARS-CoV-2 spike glycoprotein in 540 convalescent plasma samples obtained from 175 COVID-19 plasma donors for up to 142 days post-symptom onset. Robust IgM, IgG, and viral neutralization responses to SARS-CoV-2 persist, in the aggregate, for at least 100 days post-symptom onset. However, a notable acceleration in decline in virus neutralization titers ≥ 160 , a value suitable for convalescent plasma therapy, was observed starting 60 days after first symptom onset. Together, these findings better define the optimal window for donating convalescent plasma useful for immunotherapy of COVID-19 patients and reveal important predictors of an ideal plasma donor, including age and COVID-19 disease severity score.

[MAIN TEXT]

The kinetics and longevity of the antibody response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are poorly understood. This knowledge is essential for determining if individuals have been infected, elucidating host and virus factors that influence the magnitude and persistence of serological responses, assessing whether an individual is sufficiently protected from re-infection, and evaluating the effectiveness of vaccination strategies to contain the pandemic. Additionally, understanding antibody kinetics and persistence is essential to determine correlates of live-virus neutralization (VN) titers required for qualifying donors of convalescent plasma for use in immunotherapy.

Antibodies directed to the SARS-CoV-2 surface spike glycoprotein (S) ectodomain (S/ECD) and receptor-binding domain (S/RBD) neutralize SARS-CoV-2 *in vitro*, and their titers can serve as effective surrogates for virus neutralization (VN)¹⁻³. These titers have also been used to identify suitable convalescent plasma donors for COVID-19 immunotherapy^{1,3}. However, there is considerable uncertainty about the robustness and persistence of the serological responses to SARS-CoV-2. Some reports suggest variable duration and resilience of serum IgG or IgM antibodies to S or other viral proteins²⁻⁴, whereas others report that serological and neutralizing responses begin to wane and approach undetectable levels within weeks after infection³⁻⁶.

To better understand the kinetics of the serological response to SARS-CoV-2, we determined the temporal profiles of IgM, IgG, and VN responses in a cohort of 175 convalescent plasma donors, including 105 who had donated multiple times. Plasma samples ($n=540$) were collected up to 142 days after the onset of the donors' first symptoms [days post-symptom onset (DPO); Tables 1,S1]. We used a Fab fragment-based assay to assess total antibody titers against S/ECD and S/RBD, an isotype-specific assay to measure anti-S/RBD IgM and IgG titers, and a live-virus assay to determine SARS-CoV-2 VN titers¹.

We discovered robust IgM, IgG, and VN responses in the majority of individuals, with moderate to strong correlation regardless of assay type (Figure 1a,b). Only 4 of 175 [2.3%; 95% confidence interval (CI): 0.9-5.7%] individuals had undetectable levels of IgG, IgM, or total antibody to S/RBD or S/ECD at initial sampling, whereas a significantly higher fraction (29 of 114; 25.4%; 95% CI: 18.3-34.1%) had undetectable VN titers (z -score=6; $P<0.01$). Thus, ~75% of RT-PCR-confirmed symptomatic individuals were serologically positive for anti-spike protein antibody, and their convalescent plasma had demonstrable ability to neutralize SARS-CoV-2 in VN assays.

We next determined the patterns of distribution of IgM and IgG background-corrected optical density (OD) values and titers over time (Figure 1c-f). Titers peaked at approximately 30 DPO and persisted through 140 DPO (Figure 1c-f), with the IgG titer consistently higher than the IgM titer. The titer ratios began to diverge after 60 DPO (Figure 1d,f), but remained strongly correlated over the first 140 DPO (Pearson's $r=0.71$; 95% CI: 0.67–0.75). The observed persistence of IgG responses in many convalescent individuals through 140 DPO is encouraging from the perspective of antibody durability to SARS-CoV-2. The data are consistent with the expected serological responses to rapidly replicating RNA viruses, including SARS-Cov-1⁷. In contrast, the persistence of IgM well beyond the acute phase was unexpected and differs from reports suggesting a rapid decline in IgM by 4-6 weeks^{7,8}.

To further study the trajectory of antibody persistence, we performed survival analyses on IgM and IgG titers on all 540 samples obtained from 175 individual donors (Figure 2). Consistent with the temporal distribution of titers, survival analyses showed that the proportion of S/RBD IgG Fc seropositive convalescent individuals remained high through 140 DPO (Figure 2a,b).

It is clear that antibodies directed against SARS-CoV-2 S/ECD and S/RBD neutralize the virus *in vitro*. Consistent with this, several vaccines targeting the S glycoprotein have shown promise in animal infection models and human clinical trials⁹⁻¹³. We and others have recently

reported that anti-S/RBD and S/ECD IgG titers are excellent surrogates for *in vitro* VN and help identify plasma donors for therapeutic uses^{1,14}. Specifically, we have shown that anti-S/RBD or anti-S/ECD antibody titers of ≥ 1350 are strong proxies for a VN titer ≥ 160 , the FDA-recommended value for use in COVID-19 convalescent plasma therapy¹, and transfusion of anti-S/RBD IgG ≥ 1350 titer plasma within 72 hours (h) of hospitalization significantly improves survival and health outcomes^{15,16}.

Our large and well-characterized convalescent plasma library with longitudinally donated samples enabled detailed assessment of VN response persistence. We found that the proportion of individuals with a VN titer ≥ 160 remained above 80% through the first 60 DPO but declined sharply to less than 20% between DPO 61 and 120 (Figure 2c). These results suggest that the time period in which donated convalescent plasma is likely to have a high VN titer and optimal therapeutic potential is within the first 60 DPO. This has important implications for convalescent plasma donation and passive immunotherapy programs, some of which have already transfused more than 60,000 individuals in the United States as of August 13, 2020 (<https://www.uscovidplasma.org>).

Facile methods to identify suitable convalescent plasma donors are needed as the gold standard live-virus VN assays used herein are labor intensive, cumbersome, take several days to perform, and require specialized expertise and access to a high containment (Biosafety Level 3) laboratory and regulatory clearances. ELISAs are easier to implement than VN assays, especially in resource-limited countries and environments. We previously reported that an S/RBD ≥ 1350 titer may serve as a good marker for identifying plasma donors with VN ≥ 160 ¹ (Supplementary Table S2). Here we confirm a high positive likelihood ratio (LR+; 13.43) for a VN ≥ 160 when S/RBD titers are ≥ 1350 early (1-30 DPO) post onset of symptoms (Supplementary Table S2). However, extended longitudinal analyses through 140 DPO show that S/ECD and S/RBD ≥ 1350 persist longer than VN ≥ 160 , with significantly different survival curves ($P < 0.001$) for 1-140 DPO and overall LRs+ of 1.34 for S/ECD and 1.61 for S/RBD

(Figure 2c,d; Supplementary Table S2). Thus, an S/RBD ≥ 1350 titer is a promising marker for identifying suitable plasma donors early, but not late, after first symptom onset. In contrast, S/RBD IgG ≥ 1350 appears to be a reliable predictor of VN ≥ 160 , and S/RBD IgG ≥ 1350 survival is statistically indistinguishable from that of VN ≥ 160 (Figure 2e), with an overall LR+ of 3.18 and a negative likelihood ratio (LR-) of 0.26 (Supplementary Table S2).

We next investigated the survival and predictive values of S/RBD IgM ≥ 450 as compared to VN ≥ 160 (Figure 2f, Supplementary Table S2). An S/RBD IgM titer ≥ 450 was selected because the magnitude of IgM response was approximately three-fold lower than that of IgG (Figure 1f). The results showed that S/RBD IgM ≥ 450 had a similar survival profile to VN ≥ 160 but waned significantly faster ($P < 0.01$; Figure 2f). While S/RBD IgM ≥ 450 had an overall LR+ of 3.72, it also had a LR- of 0.69, which would likely result in an unacceptable number of suitable donors with VN ≥ 160 being excluded. Together, these results indicate that S/RBD IgG ≥ 1350 , but not IgM ≥ 450 or S/RBD or S/ECD total antibody ≥ 1350 , serves as a good marker to identify suitable plasma donors for COVID-19 immunotherapy.

To determine the kinetics and persistence of IgM, IgG, and VN responses, we next performed longitudinal analyses of the initial and final observed titers in 105 subjects with multiple plasma donations [median 4 donations, interquartile range (IQR): 2-6; median interval between initial and final donation of 42 days (range 6-101; IQR: 26-68), Extended Data Figure 1]. The data confirm the robustness of IgG and IgM levels through the 140 DPO observation period. All individuals with a detectable starting titer remained, on average, between one or two dilutions above or below the initial titer (Extended Data Figure 1). Of particular note, only 5 of 60 individuals (8.3%, 95% CI: 2.8-18.4%) with an initial VN titer of ≥ 5.3 (1:40) showed a subsequent increase in titer, emphasizing the importance of recruiting and screening convalescent plasma donors quickly, as VN titers are unlikely to rise from initial levels.

We next assessed whether particular donor characteristics predicted a more robust serological and neutralization response. The results show that individuals 30 years of age or

younger had significantly lower VN, IgG and IgM antibody titers than those in the older age groups (Figure 3a). Individuals between 20-30 years of age also had significantly faster decline in IgG ($P < 0.05$) and IgM ($P < 0.05$) than did those > 60 years of age (Figure 3b-d, Extended Figure 2a). Consistent with recent evidence that disease severity correlates with the magnitude and duration of serological response^{1,17,18}, we found that individuals with disease severity scores of 4 or 5 on a 5-point disease severity scale had significantly higher IgM and IgG antibody titers than those with lower severity scores (Figure 3e). In addition, survival analyses of IgG and IgM antibody titers revealed that individuals with mild/moderate symptoms scores of 1, 2, or 3 had significantly different survival curves for IgM ($P < 0.001$) and VN ($P < 0.05$) than did those with higher disease severity scores (3f-h, Extended Figure 2b). Notably, all individuals with high severity scores had detectable IgM at their last measurement point, as did all individuals who were >60 years of age. This may be indicative of potential confounding or interaction between age and disease severity affecting the magnitude and persistence of serological response. The rate of loss of IgM seropositivity to S/RBD was significantly higher for the youngest (20-30 years) compared to the oldest (>60 years) age groups (log-rank test, $P < 0.01$), and this effect remained significant when individuals with high severity scores were excluded. Age and severity score were only weakly correlated (Spearman rank correlation=0.08; $P = .07$), but formal analysis of confounding or interactions between age and severity was precluded due to data frailty and requires further study. Regardless, these findings suggest that convalescent individuals <30 years of age and those with lower disease severity scores are less likely to represent suitable donors of convalescent plasma for immunotherapy for COVID-19 patients than individuals in >30 age group with a history of more severe disease. Finally, the results show that individuals with dyspnea had significantly higher VN, IgG and IgM and antibody titers than those who did not (Figure 3i), and IgM seropositivity declined significantly faster in individuals with dyspnea (log-rank test, $P < 0.0001$) (Figure 3j-l).

In conclusion, these data refine our understanding of the kinetics, magnitude, and durability of human serologic responses to SARS-CoV-2 spike protein, the primary vaccine candidate being studied worldwide. This integrative analysis of serological and VN profiles identifies an optimal donation window of up to 60 DPO for high-titer anti-spike protein convalescent plasma as immunotherapy for COVID-19 patients. Our analysis found that additional characteristics of an ideal potential donor include a recovered patient >30 years old with a high COVID-19 disease severity score. In the aggregate, these data permit a more focused strategy for identifying suitable donors for COVID-19 convalescent plasma and passive immunotherapy programs.

Online Methods

Data Availability. All data generated or analyzed during this study are included in this published article (and its supplementary information files) or will be made available by the authors on reasonable request.

Cohort and sample description

Plasma samples (n=540) from 175 COVID-19 convalescent patients collected at Houston Methodist Hospital in Houston, Texas were included in the study. Patients were confirmed to be positive for SARS-CoV-2 by RT-PCR. The severity of infection in these patients was scored on a scale of 1-5, (median 2, IQR: 1-2). Clinical improvement relative to DPO 0 was defined as a 1 point improvement in ordinal scale [1, discharged (alive); 2, not hospitalized, experiencing dyspnea not requiring supplemental oxygen but requiring ongoing medical care (for COVID-19 or otherwise); 3, hospitalized, requiring low-flow supplemental oxygen; 4, hospitalized, on non-invasive ventilation or high-flow oxygen devices; 5, hospitalized and on invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO)].

Per FDA guidelines (<https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma#Patient%20Eligibility>), all subjects were asymptomatic for at least 14 days at the time of plasma collection. Of the 175 subjects, 105 eligible individuals underwent plasmapheresis and donated plasma at least twice (range 2-12 times). All donors were confirmed negative for SARS-CoV-2 by RT-PCR and provided written consent before plasmapheresis. The study cohort consisted of 88 females (50.3%) and 87 males (49.7%), ranging in age between 20-78 years (median 46, IQR: 36-54). Samples were collected from 17-142 DPO (median 68 days, IQR: 48-93). Plasma from donors was collected with the transfusion apheresis system (Trima Accel® Terumo BCT) and standard blood banking protocols were followed. An aliquot of collected plasma was tested for antibodies by ELISA and/or VN assays. Cohort characteristics are described in Table 1 and Supplementary Table 1.

Study approvals

Informed consent was obtained from either the patient or an authorized representative of the patient when applicable for collection of plasma samples. All procedures were approved by the Institutional Review Board of Houston Methodist Hospital (IRB# PRO00025121). Serological analyses were performed at the Pennsylvania State University under BSL-2 (ELISA assays) and BSL-3 (VNs) conditions, following the Pennsylvania State University Institutional Biosafety Committee (IBC) approved protocols.

Quantitative estimation of antibodies against SARS-CoV-2

SARS-CoV-2 antibodies in plasma samples were detected and quantified against purified recombinant SARS-CoV-2 spike ectodomain (S/ECD) or receptor-binding domain (S/RBD) proteins using in-house indirect Fab antibody-based or isotype-specific (IgM and IgG) ELISA assays. The protocols were performed as previously described^{1,19} and deposited in protocols.io

([dx.doi.org/10.17504/protocols.io.bivgke3w](https://doi.org/10.17504/protocols.io.bivgke3w)). Two isotypes of CR3022, a human monoclonal antibody reactive to spike regions of SARS-CoV-1 and SARS-CoV-2, were used as positive controls in the assays (IgG1: Ab01680-10.0; IgM: Ab01680-15.0, Absolute Antibody, USA). The cutoff for the assays was determined as an optical density (absorbance at 450 nm) higher than three or six standard deviations above the mean of the tested pre-COVID-19 serum samples ($n=100$). Sample titers were estimated as reciprocals of the highest dilution resulting in an OD greater than the cutoff. The class specificity of the IgM ELISA was tested by treating the plasma samples ($n=10$) with 1,4-Dithiothreitol (DTT, 10708984001, Millipore Sigma, USA) as previously described²⁰. Briefly, samples were allowed to react with 0.005 M DTT in PBS at $36\pm 2^{\circ}\text{C}$ for 30 min and then tested with isotype-specific ELISAs for titer estimation (Extended data Figure 3).

Virus neutralization assay

The VN titers of the plasma samples were quantified on a cell-based assay using SARS-CoV-2 strain USA-WA1/2020 (NR-52281-BEI Resources, USA) based on procedures described previously^{1,21}. Briefly, Vero E6 cells (CRL-1586, ATCC, USA) were grown as monolayers in 96-well microtiter plates. Heat-inactivated plasma samples were diluted two-fold in triplicate and incubated with 100 tissue culture infective dose 50 (TCID₅₀) of the virus at 5% CO₂ at $36\pm 2^{\circ}\text{C}$ for 60 min. This plasma-virus mixture was added to cell monolayers and incubated further for 72 h at 5% CO₂ at $36\pm 2^{\circ}\text{C}$. Plates were treated with crystal violet formaldehyde stain for 1 h and visually inspected for cytopathic effect (CPE) or protection. The reciprocal of the highest dilution of the plasma where at least two of the three wells were protected (no CPE) was determined as the VN titer of the sample.

Statistical analyses

Tests for normality were performed using the Kolmogorov-Smirnov test and a P value of <0.05 was considered statistically significant. Data dispersion was indexed by standard errors of mean

or quartile and IQR. The agreement between the various assays was determined using Pearson correlation coefficient with log₂-transformed titers. The non-parametric regression method LOESS was used for scatterplot smoothing to visualize antibody trajectories. The geom_smooth (method="loess") function in R was used with default span of 0.75. The proportion of the sample population remaining seropositive over the 100-day period was determined using a log-rank test and Kaplan-Meier survival curves were plotted with "survival" and "survminer" packages in R Studio²²⁻²⁴. Statistical differences in antibody titers and survival curves of patient characteristics—including severity score, age, and presence of dyspnea—were analyzed using one-way ANOVAs (Tukey's multiple comparison tests) and a log-rank test, respectively. Individual level interval-censored data were used to fit semi-parametric accelerated failure time models using the icenReg R package. DTComPair R package (<https://cran.r-project.org/web/packages/DTComPair/DTComPair.pdf>) was used to compare the sensitivity, specificity, and positive and negative predictive values for detection of S/RBD, S/ECD, and S/RBD IgG titers ≥1350, as well as S/RBD IgM titer ≥450 using VN titer ≥160 as the gold standard. Positive and negative predictive values were compared with the generalized score statistics, whereas the sensitivity and specificity were compared using an exact binomial test. All analyses were completed using R (versions 3.6.1 or 3.6.3) within R Studio (version 1.2.5019) or Graphpad PRISM 8 (version 8.4.3).

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Author Contributions

Project concept (AG, SS, ES, SVK, JMM, VK); acquired data (AG, SS, ES, MSN, RHN, DG, IMB, IP, RK, SEL, AMM, RR, PAC, BC, JC, TNE, XY, PZ, CL, RJO, DWB, JG); analyzed data (AG, SS, MSN, CH, MJF, SVK, JMM, VK); wrote manuscript (VK, JMM, AG, SS, SVK); prepared figures (AG, SS, MSN, CH, VK). All authors reviewed the manuscript and gave final approval for publication.

Competing Interests

ES is the local principal investigator for a clinical trial sponsored by Regeneron assessing an investigational therapy for COVID-19.

References

1. Salazar, E., *et al.* Relationship between Anti-Spike Protein Antibody Titers and SARS-CoV-2 In Vitro Virus Neutralization in Convalescent Plasma. *bioRxiv* (2020).
2. Iyer, A.S., *et al.* Dynamics and significance of the antibody response to SARS-CoV-2 infection. *medRxiv*, 2020.2007.2018.20155374 (2020).
3. Wajnberg, A., *et al.* SARS-CoV-2 infection induces robust, neutralizing antibody responses that are stable for at least three months. *medRxiv*, 2020.2007.2014.20151126 (2020).
4. Ibarondo, F.J., *et al.* Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild Covid-19. *New England Journal of Medicine* (2020).
5. Long, Q.-X., *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nature Medicine* **26**, 1200-1204 (2020).
6. Seow, J., *et al.* Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection. *medRxiv*, 2020.2007.2009.20148429 (2020).
7. Sethuraman, N., Jeremiah, S.S. & Ryo, A. Interpreting Diagnostic Tests for SARS-CoV-2. *Jama* (2020).
8. Liu, X., *et al.* Patterns of IgG and IgM antibody response in COVID-19 patients. *Emerg Microbes Infect* **9**, 1269-1274 (2020).
9. Zhu, F.C., *et al.* Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. *Lancet* **395**, 1845-1854 (2020).
10. Jackson, L.A., *et al.* An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. *N Engl J Med* (2020).
11. Bar-Zeev, N. & Moss, W.J. Encouraging results from phase 1/2 COVID-19 vaccine trials. *Lancet* **396**, 448-449 (2020).
12. Folegatti, P.M., *et al.* Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* **396**, 467-478 (2020).
13. Zhu, F.C., *et al.* Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* **396**, 479-488 (2020).
14. Tan, C.W., *et al.* A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nat Biotechnol* (2020).
15. Salazar, E., *et al.* Treatment of COVID-19 Patients with Convalescent Plasma Reveals a Signal of Significantly Decreased Mortality. *Am J Pathol* (2020).
16. Joyner, M.J., *et al.* Effect of Convalescent Plasma on Mortality among Hospitalized Patients with COVID-19: Initial Three-Month Experience. *medRxiv*, 2020.2008.2012.20169359 (2020).
17. Lee, N., *et al.* Anti-SARS-CoV IgG response in relation to disease severity of severe acute respiratory syndrome. *J Clin Virol* **35**, 179-184 (2006).
18. Lynch, K.L., *et al.* Magnitude and kinetics of anti-SARS-CoV-2 antibody responses and their relationship to disease severity. *Clin Infect Dis* (2020).
19. Stadlbauer, D., *et al.* SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol* **57**, e100 (2020).

- 377 20. Okuno, T. & Kondelis, N. Evaluation of dithiothreitol (DTT) for inactivation of IgM
378 antibodies. *J Clin Pathol* **31**, 1152-1155 (1978).
- 379 21. Sui, J., *et al.* Potent neutralization of severe acute respiratory syndrome (SARS)
380 coronavirus by a human mAb to S1 protein that blocks receptor association. *Proc Natl*
381 *Acad Sci U S A* **101**, 2536-2541 (2004).
- 382 22. R Development Core Team. R: a language and environment for statistical computing. (R
383 Foundation for Statistical Computing, Vienna, Austria, 2014).
- 384 23. Therneau, T.M. A Package for Survival Analysis in R (Springer, New York, 2020).
- 385 24. Kassambara and Kosinski. “Survminer: Drawing Survival Curves using “ggplot2”.”
386 (2018).
- 387

Figure Legends

Figure 1. Distribution, correlation, and trajectories of antibody titers against SARS-CoV-2.

(a) Violin plots showing distribution of virus neutralization titers ($n=305$); total antibody ($n=538$), and specific isotype antibody IgG and IgM ($n=540$) titers to SARS-CoV-2 spike-ectodomain (S/ECD) and spike-receptor binding domain (S/RBD) in convalescent plasma samples (Log_2 transformed values). The means of the distribution among the titers were significantly different, except between S/ECD and S/RBD [One-way ANOVA, Tukey's multiple comparison (mixed-effects model), $P<0.05$]. The dashed line at Log_2 titer represents VN titer of 1:160. (b) Pairwise comparison of the assays show a moderate to strong correlation between the total and isotype specific IgG and IgM antibody estimates with virus neutralization assays. (c) & (d) Optical density (OD) (at 450nm) for the indirect ELISAs indicating total or isotype specific IgG and IgM antibody levels; (e) & (f) Titers of the total or isotype specific IgG and IgM antibodies. The IgG and IgM titers appear to peak around 30 days post onset (DPO) of symptoms. High IgG titers persist until 140 DPO, while IgM titers trend lower but persist until 140 DPO. (g) Neutralizing antibody titers persist until 140 DPO. A locally estimated scatterplot smoothing (LOESS) regression curve is fitted to the data.

Figure 2. Survival analysis of IgG and IgM antibody titers to SARS-CoV-2 spike-receptor binding domain (S/RBD) in 540 samples and virus neutralizing antibody (VN) titers in 305 samples collected from convalescent individuals ($n=175$) during the first 140 days post onset of symptoms (DPO). (a) Proportion of S/RBD IgG seropositive convalescent individuals remains high through 140 DPO, while IgM seropositivity remains high through the first 60 DPO and then steadily declines over the next 60 days (Log rank test; **** $P<0.0001$). The proportion of individuals with VN responses also begins to decline 60 DPO, with ~50% of individuals remaining seropositive with VN test through 140 DPO (Log rank test; *** $P<0.001$). (b) Violin plots showing a significant decline in VN and IgM titers with time (Ordinary one-way ANOVA,

Tukey's multiple comparison test; $*P<0.05$; $**P<0.01$); the IgG titers remain stable until after 120 DPO. Comparison of proportion of individuals seropositive with S/RBD, S/ECD, and S/RBD IgG titers ≥ 1350 as well as with S/RBD IgM titer ≥ 450 to the proportion of individuals possessing VN titers ≥ 160 through 140 DPO are depicted in c, d, e, and f respectively ($***P<0.001$; $****P<0.0001$).

Figure 3. Distribution of antibody titers against SARS-CoV-2 based on age, severity scores, and presence of dyspnea. These data represent samples collected from convalescent individuals ($n=175$) during the first 140 days post symptom onset (DPO). (a) Individuals <31 years of age have significantly lower IgG, IgM, and viral neutralizing antibody (VN) titers than those >40 years of age in this cohort (Ordinary one-way ANOVA, Tukey's multiple comparison test; $**P<0.01$; $***P<0.001$; $****P<0.0001$). Survival analysis of (b) IgG, (c) IgM, and (d) VN antibody titers during the first 140 DPO in convalescent individuals within the age groups of 20-30 ($n=95$ samples) and >60 ($n=45$ samples) (Log-rank test, $*P<0.05$ for IgG and IgM, $P>0.05$ for VN antibodies). (e) Individuals with a severity score of 1 have significantly lower IgM and IgG titers than those above a score of 3 (Ordinary one-way ANOVA, Tukey's multiple comparison test; $**P<0.01$; $***P<0.001$; $****P<0.0001$). Survival analysis of (f) IgG, (g) IgM, and (h) VN antibody titers in relation to severity scores grouped as mild (1/2/3) and severe (4/5) in convalescent individuals during the first 140 DPO (Log-rank test, $P>0.05$ for IgG, $****P<0.0001$ for IgM, $*P<0.05$ for VN antibodies). (i) Individuals with dyspnea had significantly higher VN, IgM, and IgG titers (Ordinary one-way ANOVA, Tukey's multiple comparison test; $**P<0.01$; $****P<0.0001$). Survival analysis of (j) IgG, (k) IgM, and (l) VN antibody titers in relation to occurrence of dyspnea in convalescent individuals during the first 140 DPO (Log-rank test, $P>0.05$ for IgG, $****P<0.0001$ for IgM, $P>0.05$ for VN).

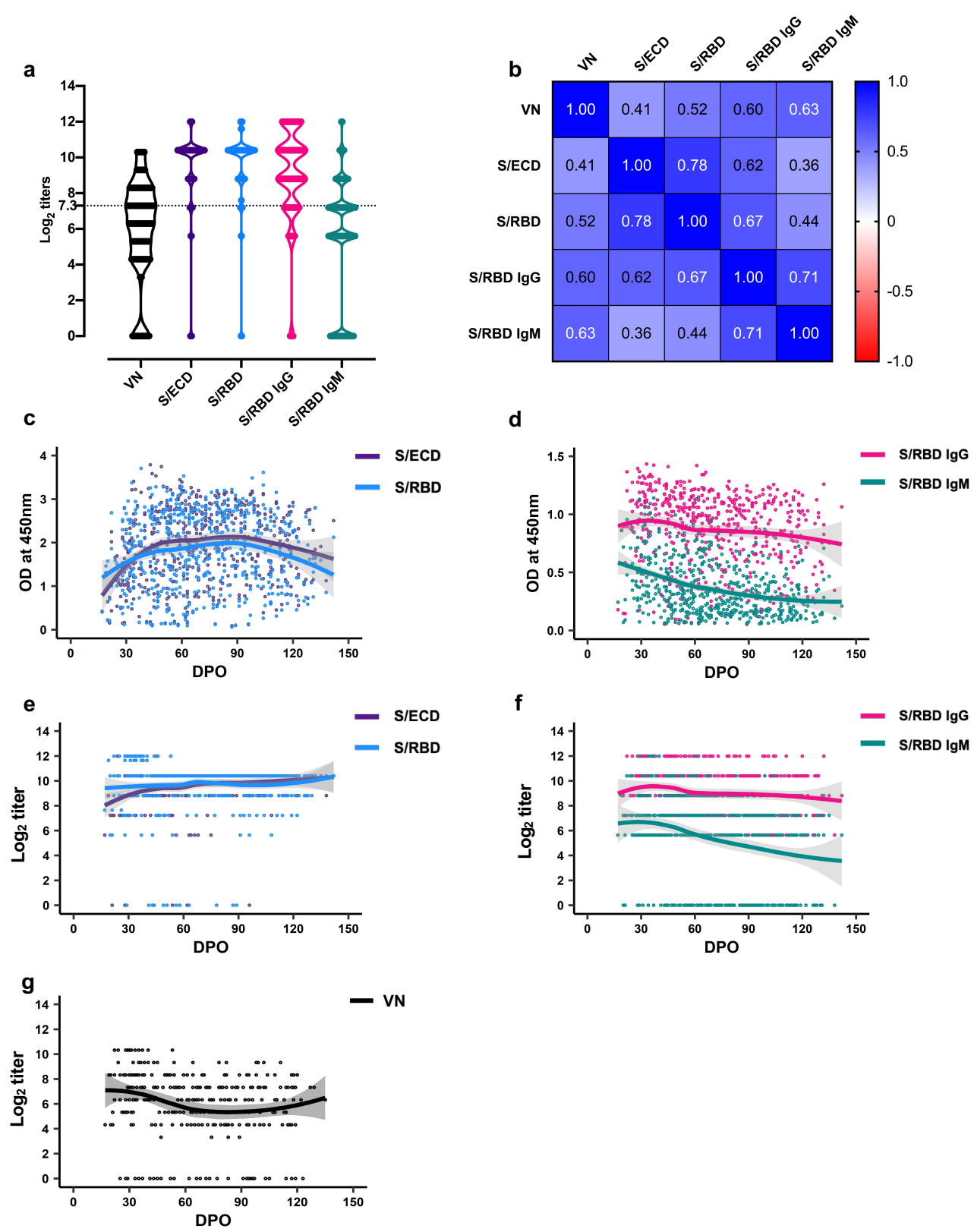
Extended Data Figure 1. Trajectories (first and last donation only) of (a) SARS-CoV-2 spike-receptor binding domain (S/RBD) IgM, (b) S/RBD IgG, and (c) virus neutralizing (VN) antibody titers against SARS-CoV-2 in subjects who donated plasma more than once. Initial (Log_2) S/RBD IgM and IgG titers ≥ 5.3 remain stable or vary by one or two dilutions below or above the initial titer. A majority of individuals (33 out of 39) with initial (Log_2) VN titers ≥ 7.3 begin to drop beyond ~60 DPO.

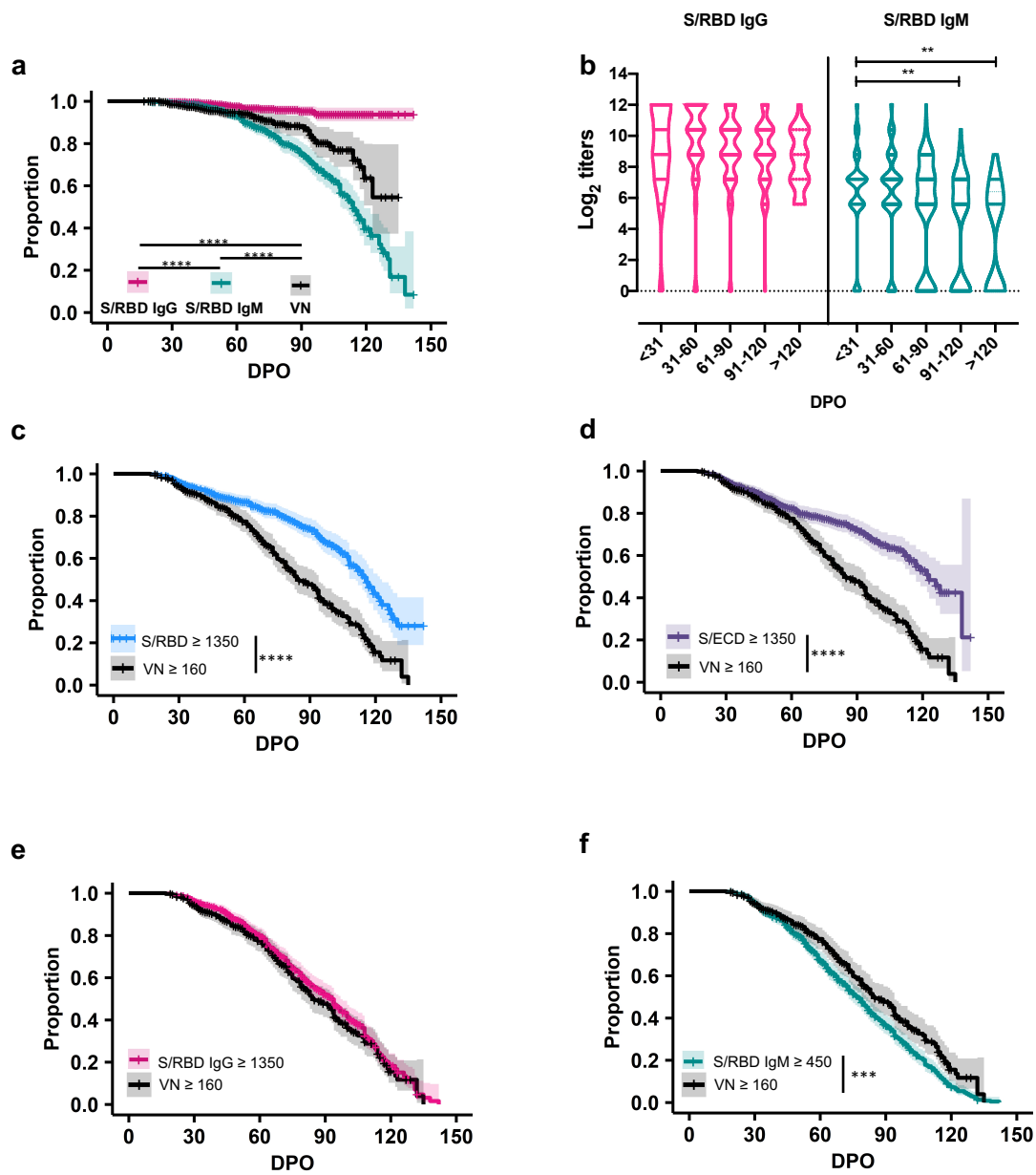
Extended Data Figure 2. Survival analysis of SARS-CoV-2 spike ectodomain (S/ECD), SARS-CoV-2 spike-receptor binding domain (S/RBD), S/RBD IgM, S/RBD IgG, and neutralizing (VN) antibody titers in 175 convalescent individuals during the first 140 days post onset (DPO) of symptoms stratified by (a) age and (b) severity (Log-rank test, $*P < 0.05$, $P < 0.01$).** Significant differences were observed in the titers of ELISAs between the age groups: 20-30 versus 31-40 (S/ECD $**P < 0.01$, S/RBD IgG $*P < 0.05$); 20-30 versus 41-50 (S/ECD $*P < 0.05$, S/RBD IgG $*P < 0.05$, S/RBD IgM $*P < 0.05$, VN $*P < 0.05$); 20-30 versus 51-60 (S/ECD $**P < 0.01$, S/RBD $*P < 0.05$, S/RBD IgG $*P < 0.05$, S/RBD IgM $**P < 0.01$, VN $*P < 0.05$); 20-30 versus > 60 (S/RBD IgM $*P < 0.05$); and 31-40 versus 51-60 (S/RBD IgM $*P < 0.05$). Significant differences were observed in the S/RBD IgM titers of the donors with the severity scores 1 versus 3 ($*P < 0.05$); 1 versus 4,5 ($**P < 0.01$); and 2 versus 4,5 ($**P < 0.01$).

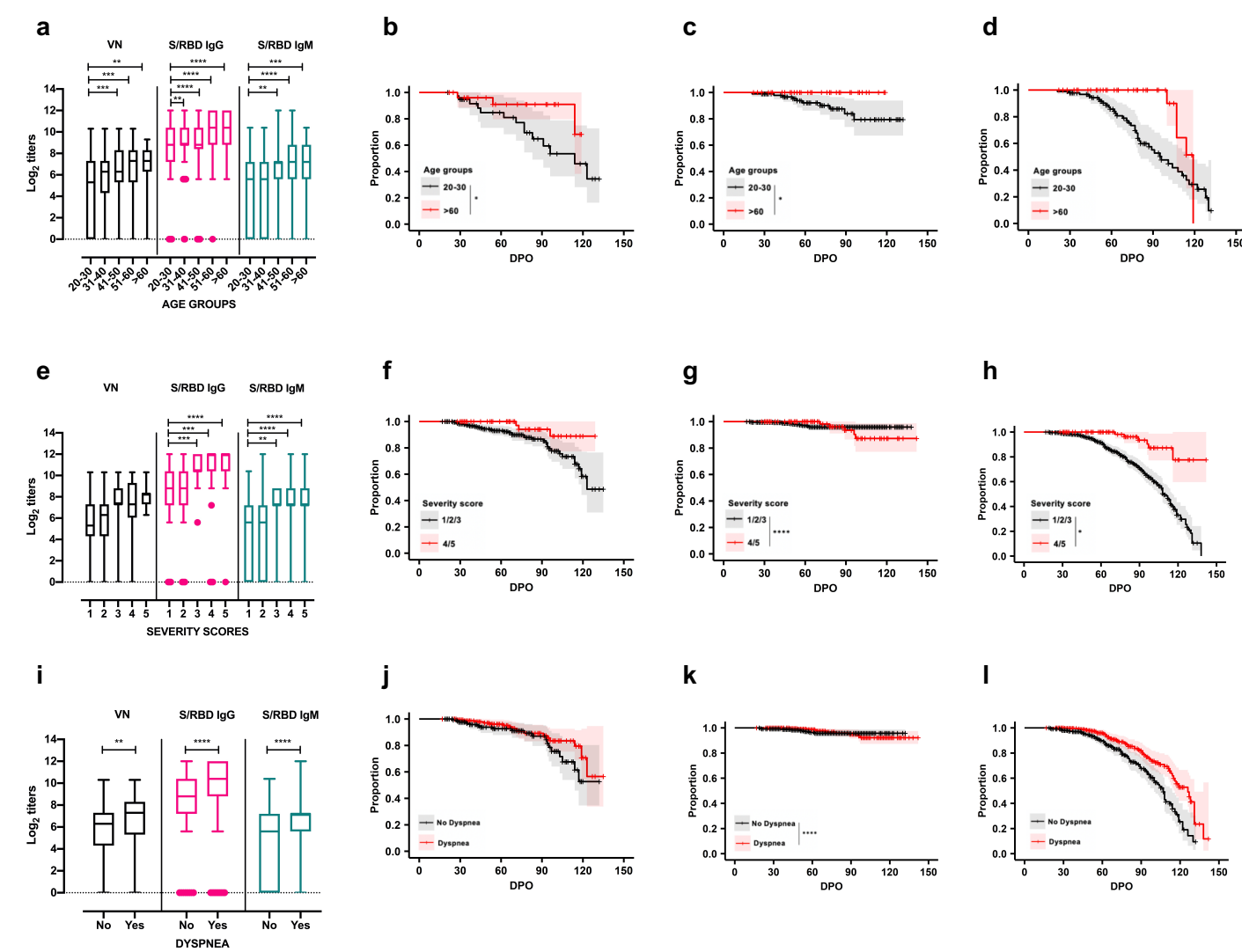
Extended Data Figure 3. Class specificity test for SARS-CoV-2 spike-receptor binding domain (S/RBD) isotype specific indirect ELISAs. 1,4-Dithiothreitol (DTT) treatment of convalescent plasma abrogates S/RBD IgM antibody titers but not IgG titers (n=10) (paired t test, $****P < 0.0001$).

Extended Data Figure 4. Forest plot depicting the positive and negative predictive values for detection of SARS-CoV-2 spike-receptor binding domain (S/RBD), SARS-CoV-2 spike

465 **ectodomain (S/ECD), and S/RBD IgG titers ≥ 1350 using virus neutralization (VN) titer ≥ 160**
466 **as the standard.** Likelihood ratios (LR) for each assay are shown on the right panel. P values
467 were generated using the generalized score statistic for pairwise comparisons. For positive
468 predictive values (PPV) S/ECD ≥ 1350 versus S/RBD ≥ 1350 $**P < 0.01$; S/RBD ≥ 1350 versus
469 S/RBD IgG ≥ 1350 $***P < 0.001$; S/RBD IgG ≥ 1350 versus S/RBD IgM ≥ 450 $P > 0.05$; S/RBD IgM
470 ≥ 450 versus S/RBD ≥ 1350 $***P < 0.001$. For negative predictive values (NPV) S/ECD ≥ 1350
471 versus S/RBD ≥ 1350 $**P < 0.01$; S/RBD ≥ 1350 versus S/RBD IgG ≥ 1350 $P > 0.05$; S/RBD IgG
472 ≥ 1350 versus S/RBD IgM ≥ 450 $****P < 0.0001$; S/RBD IgM ≥ 450 versus S/RBD 1350
473 $****P < 0.0001$.

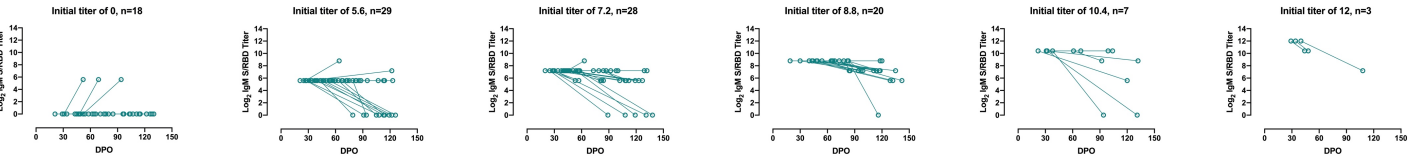




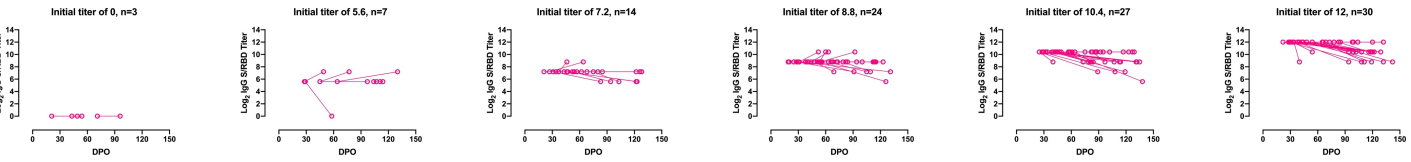


Extended data Figure-1

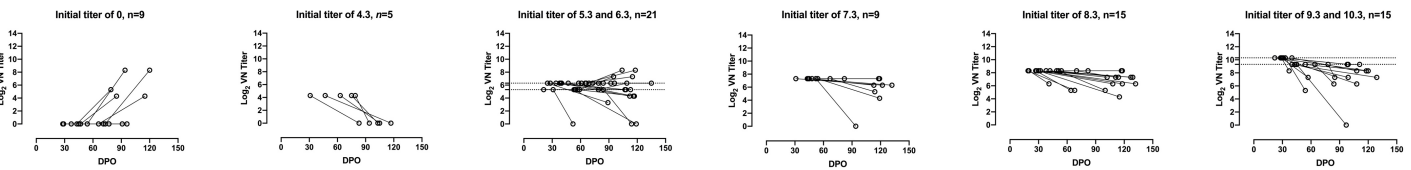
a: S/RBD IgM



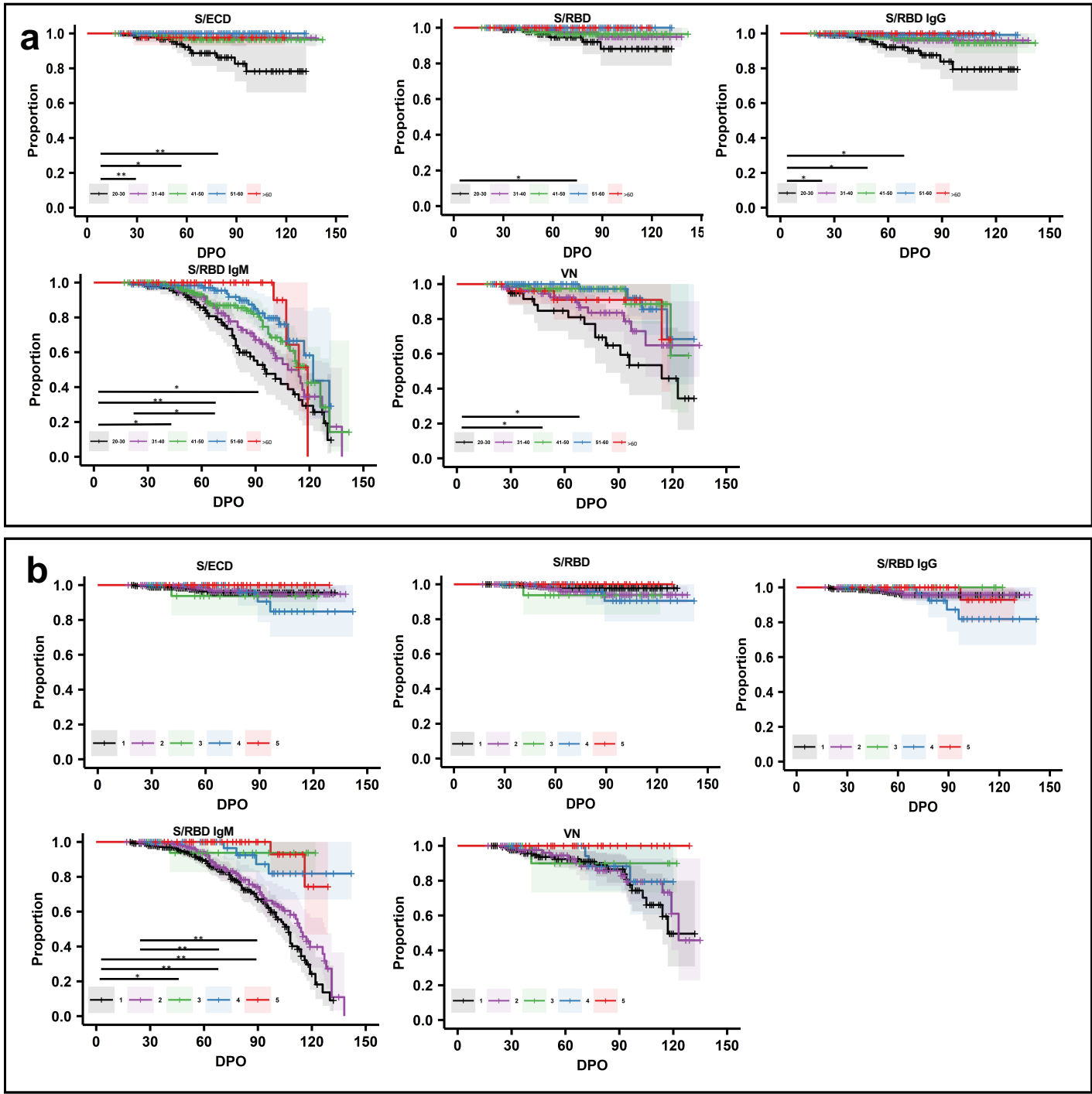
b: S/RBD IgG



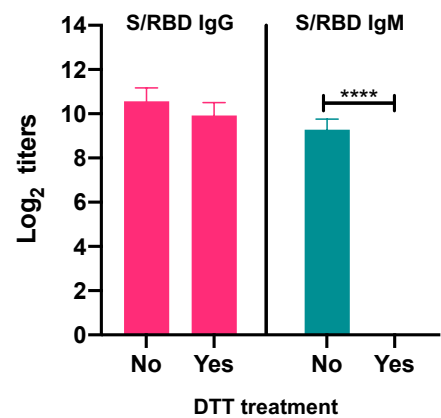
c: VN



Extended Data Figure 2



Extended data Figure-3



Extended data Figure- 4

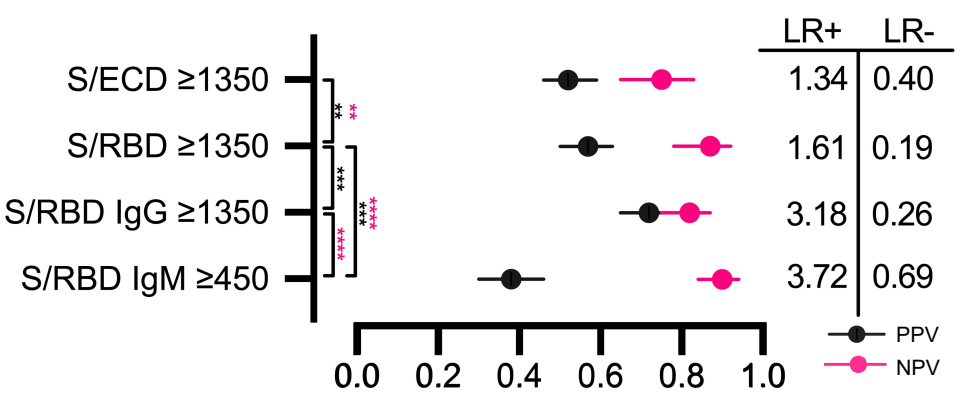


Table 1: Demographics and characteristics of the plasma donor cohort.

Patient characteristics	Samples n (%)	Individuals n (%)
Sex		
Female	213 (39.4)	88 (50.3)
Male	327 (60.6)	87 (49.7)
Age		
20-30	95 (17.6)	26 (14.9)
31-40	117 (21.7)	39 (22.3)
41-50	166 (30.7)	51 (29.1)
51-60	117 (21.7)	40 (22.9)
> 60	45 (8.3)	19 (10.9)
Average (95% CI)	43.8 (42.7 - 44.9)	44.9 (43.0 - 46.8)
Median (IQR)	44 (33 - 53)	46 (36 - 54)
Range	20 - 78	20 - 78
Severity		
1	244 (45.2)	76 (43.4)
2	182 (33.7)	63 (36.0)
3	23 (4.3)	10 (5.7)
4	44 (8.1)	15 (8.6)
5	47 (8.7)	11 (6.3)
Median (IQR)	2 (1 - 2)	2 (1 - 2)
Range	1 - 5	1 - 5
Dyspnea		
No	250 (46.3)	79 (45.1)
Yes	290 (53.7)	96 (54.9)
DPO		
< 31	39 (7.2)	35 (20.0)
31-60	181 (33.5)	89 (50.9)
61-90	173 (32.0)	44 (25.1)
91-120	122 (22.6)	7 (4.0)
> 120	25 (4.6)	-
Average (95% CI)	70.8 (68.4 - 73.3)	49.5 (46.5 - 52.5)
Median (IQR)	68 (48 - 93)	46 (32 - 63)
Range	17 - 142	17 - 108
Hospitalization		
No	428 (79.3)	141 (80.6)
Yes	112 (20.7)	34 (19.4)
Total	540	175

Supplementary Table 1: Convalescent plasma donor demographics and sample characteristics.

Subject	Age	Sex	Hospitalization	Severity	Dyspnea	DPO	VN titer	S/ECD titer	S/RBD titer	S/RBD IgG titer	S/RBD IgM titer
0001	44	M	NO	1	NO	20	320	150	1350	450	150
0001						24	640	-	-	450	150
0001						27	320	150	450	1350	150
0001						31	320	150	3200	1350	450
0001						34	320	150	3200	450	150
0001						38	160	450	450	450	150
0001						41	80	450	450	450	150
0002	54	M	NO	1	NO	28	40	50	150	150	50
0003	36	M	NO	1	NO	25	80	450	1350	1350	150
0003						28	80	150	450	450	0
0003						33	80	450	3200	1350	50
0003						35	0	450	450	1350	50
0003						42	20	150	450	1350	50
0003						47	10	1350	1350	450	50
0003						61	20	1350	1350	450	50
0003						68	0	450	1350	150	0
0003						75	40	1350	450	450	0
0003						82	20	1350	450	150	0
0003						89	10	1350	1350	150	0
0004	54	F	NO	2	YES	32	320	1350	1350	1350	1350
0004						36	640	1350	1350	4050	1350
0004						68	0	1350	1350	1350	150
0004						75	80	1350	1350	1350	450
0004						103	160	1350	1350	1350	150
0004						118	80	1350	1350	1350	150
0004						131	-	1350	1350	450	0
0005	58	M	NO	2	YES	91	-	150	150	150	0
0007	36	M	NO	1	NO	88	-	1350	1350	1350	50
0007						123	-	1350	1350	1350	50
0009	38	F	NO	2	YES	30	80	450	450	450	50
0011	67	F	NO	1	NO	28	0	0	50	50	50
0011						49	-	450	150	150	50
0012	46	F	NO	1	NO	30	320	150	450	150	150
0013	43	F	NO	1	NO	28	320	1350	3200	4050	450
0016	47	F	NO	1	NO	32	640	4050	4050	4050	1350
0020	41	F	NO	2	YES	17	20	50	200	50	50
0022	22	M	NO	1	NO	47	-	450	150	150	50
0022						57	-	450	1350	150	50
0028	23	M	NO	1	NO	31	20	150	150	150	50
0028						46	-	450	150	150	50

0028						63	-	0	450	150	150
0028						77	-	150	150	150	50
0028						83	0	450	450	50	50
0029	66	F	NO	1	NO	22	80	150	450	4050	150
0032	65	M	NO	2	YES	25	320	450	4050	4050	1350
0035	50	M	NO	2	YES	28	320	1350	3200	4050	150
0035						38	640	1350	1350	4050	150
0035						52	-	1350	1350	4050	150
0035						59	160	1350	1350	1350	50
0035						82	640	1350	1350	1350	50
0035						108	80	1350	1350	450	0
0040	52	M	NO	2	YES	29	1280	-	-	4050	4050
0040						35	320	4050	4050	4050	4050
0040						37	320	1350	4050	4050	4050
0040						44	-	4050	4050	4050	1350
0045	23	F	NO	1	NO	33	1280	4050	1350	4050	50
0045						47	-	1350	1350	4050	150
0045						54	40	1350	1350	1350	50
0049	57	F	NO	1	NO	27	320	150	450	150	50
0049						64	40	1350	1350	450	450
0050	41	M	NO	2	YES	30	320	1350	1350	450	150
0050						33	0	150	150	1350	50
0050						44	20	1350	450	450	150
0050						58	20	1350	1350	150	50
0050						66	40	1350	450	150	50
0050						72	20	1350	1350	150	50
0050						94	20	1350	1350	150	50
0050						102	20	1350	1350	150	50
0050						108	-	1350	450	450	50
0050						115	20	1350	450	150	50
0050						131	-	1350	1350	150	0
0051	50	F	NO	1	NO	30	160	150	450	150	150
0052	27	F	YES	3	YES	31	160	1350	1350	4050	50
0052						59	-	1350	1350	1350	150
0052						87	160	1350	1350	1350	150
0052						122	80	1350	1350	1350	150
0053	29	M	NO	2	YES	28	0	450	450	450	50
0053	30	M	NO	2	YES	56	-	450	450	450	0
0053						63	-	450	450	150	0
0053						77	0	1350	1350	150	0
0053						91	0	150	50	150	0
0055	61	M	YES	3	YES	33	320	1350	3200	1350	150
0057	44	F	NO	2	YES	34	160	450	450	450	150

0058	36	M	NO	2	YES	92	-	1350	1350	1350	50
0062	24	F	NO	1	NO	32	320	1350	1350	1350	1350
0062						35	1280	1350	1350	1350	1350
0062						46	-	1350	1350	1350	1350
0062						53	-	1350	1350	1350	1350
0062						62	-	1350	1350	1350	450
0062						69	320	1350	1350	1350	450
0062						101	80	1350	1350	450	450
0062						118	160	1350	1350	450	450
0062						132	80	1350	1350	450	450
0065	50	M	NO	1	NO	34	-	1350	1350	150	0
0065						54	-	1350	1350	450	150
0065						126	-	450	450	150	0
0069	49	F	NO	1	NO	28	80	450	450	1350	50
0069						32	80	450	450	1350	50
0069						63	-	50	450	450	0
0069						108	40	1350	450	150	0
0070	37	F	NO	2	YES	38	160	450	1350	4050	50
0072	23	F	NO	1	NO	29	0	0	0	50	0
0072						37	0	50	50	0	0
0072						44	-	0	0	0	0
0072						51	-	0	0	0	0
0072						58	-	0	0	0	0
0073	39	F	NO	2	YES	47	0	150	150	150	0
0073						55	-	450	450	150	0
0073						85	20	1350	450	150	0
0077	59	F	NO	1	NO	71	-	1350	1350	1350	150
0081	64	F	NO	2	YES	52	-	450	1350	1350	150
0088	29	F	NO	1	NO	21	20	0	50	0	0
0088						54	-	0	50	0	0
0089	42	F	NO	2	YES	38	160	450	450	4050	150
0090	33	M	NO	1	NO	37	1280	150	150	150	50
0090						46	-	1350	1350	450	50
0095	61	F	NO	1	NO	54	160	1350	1350	4050	150
0095						76	-	1350	1350	1350	50
0095						83	-	1350	1350	1350	50
0095						119	20	1350	1350	450	0
0096	44	F	NO	1	NO	59	-	1350	1350	1350	50
0099	54	F	NO	1	NO	20	20	50	50	0	0
0109	33	F	NO	1	NO	67	160	1350	1350	450	50
0109						73		1350	1350	450	50
0109						80	160	1350	1350	450	0
0109						93	0	450	150	450	0

0109						100	-	450	450	450	0
0109						108	-	1350	450	450	0
0109						114	40	1350	450	450	0
0112	47	F	NO	1	NO	32	40	450	450	450	150
0113	52	F	NO	1	NO	29	40	1350	150	150	0
0115	70	M	YES	5	NO	54	320	1350	1350	4050	450
0115						62	-	1350	1350	4050	150
0115						102	640	1350	1350	1350	150
0115						118	320	1350	1350	1350	150
0116	27	M	NO	1	NO	32	20	450	450	450	50
0117	27	F	NO	2	YES	30	320	1350	1350	1350	150
0117						44	-	1350	1350	1350	150
0117						71	-	1350	1350	1350	150
0117						79	160	1350	1350	1350	150
0117						85	-	1350	1350	1350	150
0117						129	160	1350	1350	1350	150
0118	50	F	NO	1	NO	34	320	1350	450	4050	150
0118						40	-	1350	1350	450	150
0119	35	F	NO	1	NO	25	0	450	450	1350	50
0119						34	-	1350	1350	450	50
0119						40	-	1350	450	450	50
0120	41	F	NO	1	NO	19	320	450	3200	450	450
0120						68	40	1350	1350	450	450
0121	51	F	NO	1	NO	21	40	150	200	150	50
0121						54	40	450	450	150	50
0132	61	M	YES	5	YES	78	640	1350	1350	4050	450
0132						85	160	1350	1350	4050	150
0133	51	M	NO	2	YES	25	-	150	450	450	150
0133						53	-	1350	1350	450	50
0135	47	F	NO	2	YES	32	320	4050	4050	4050	150
0137	53	F	NO	1	NO	38	80	450	3200	450	50
0137						59	-	450	1350	450	50
0137						66	-	1350	1350	450	50
0137						73	80	1350	1350	450	50
0140	54	M	NO	1	NO	98	-	1350	1350	450	50
0143	49	F	NO	2	YES	37	160	1350	3200	4050	150
0144	48	M	YES	5	YES	40	640	1350	3200	4050	450
0144						45	640	4050	1350	4050	1350
0144						50	320	450	1350	4050	450
0144						53	1280	4050	4050	1350	150
0144						64	320	1350	1350	1350	150
0144						73	80	1350	1350	1350	150
0144						80	160	1350	1350	1350	50

0144						94	80	1350	1350	1350	150
0144						115	160	1350	1350	1350	50
0144						121	-	1350	450	1350	50
0144						129	160	1350	1350	1350	50
0156	59	M	NO	1	NO	22	1280	4050	1350	4050	1350
0156						29	1280	1350	1350	4050	450
0156						43	80	1350	1350	4050	450
0156						50	-	1350	1350	4050	450
0156						65	-	1350	1350	4050	150
0156						71	160	1350	1350	1350	150
0156						85	-	1350	1350	1350	50
0156						92	-	1350	1350	1350	50
0156						99	160	1350	1350	1350	50
0156						106	-	1350	1350	1350	50
0156						113	-	1350	1350	1350	50
0156						120	320	1350	1350	1350	50
0158	33	M	NO	2	YES	26	40	150	200	450	50
0159	23	F	NO	2	YES	46	-	4050	4050	4050	450
0162	51	F	YES	3	YES	34	160	150	450	450	50
0177	55	M	NO	1	NO	44	160	4050	4050	4050	450
0177						132	80	1350	1350	450	50
0215	38	M	NO	2	YES	53	-	1350	1350	450	50
0215						60	-	1350	1350	1350	50
0229	32	M	NO	2	YES	40	80	450	1350	1350	450
0229						61	320	1350	1350	1350	450
0229						68	-	1350	1350	450	150
0229						76	-	1350	1350	450	150
0229						100	160	1350	1350	450	150
0229						110	-	1350	1350	450	150
0229						117	160	1350	1350	450	50
0229						135	80	1350	1350	450	150
0234	40	M	NO	2	YES	27	40	150	150	150	150
0245	51	M	YES	5	YES	38	320	1350	4050	4050	150
0245						52	-	1350	1350	4050	450
0245						59	-	1350	1350	4050	150
0245						81	320	1350	1350	1350	150
0245						102	160	1350	1350	1350	50
0249	56	M	NO	1	NO	22	320	4050	4050	4050	150
0255	40	M	NO	2	YES	31	40	450	450	450	0
0255						45	-	1350	1350	1350	50
0255						52	0	1350	1350	1350	50
0260	44	M	NO	2	YES	24	1280	4050	1350	1350	1350
0262	36	F	YES	4	YES	31	1280	4050	4050	4050	1350

0262						49	-	1350	1350	4050	1350
0262						99	640	1350	1350	4050	1350
0263	20	M	NO	1	NO	43	0	1350	1350	1350	50
0263						52	-	150	1350	450	50
0263						59	-	1350	450	450	0
0263						79	40	1350	1350	450	0
0265	53	F	NO	2	YES	31	320	4050	4050	4050	150
0280	37	M	YES	4	YES	73	0	1350	1350	4050	450
0280						98	320	1350	1350	4050	450
0280						120	320	1350	1350	4050	450
0284	35	F	NO	4	YES	53	-	1350	1350	1350	150
0285	51	F	NO	1	NO	40	-	1350	1350	450	150
0285						56	-	1350	1350	450	150
0287	40	F	NO	2	YES	56	-	50	0	0	0
0301	59	M	NO	1	NO	83	-	1350	1350	1350	450
0301						90	-	1350	1350	1350	450
0301						97	-	1350	1350	450	150
0302	25	M	NO	1	NO	84	80	1350	1350	450	150
0302						96	80	450	150	450	50
0302						112	-	450	1350	450	50
0313	78	M	YES	3	YES	36	160	4050	4050	4050	50
0339	31	M	YES	3	YES	54	640	150	1350	4050	450
0339						62	-	1350	1350	4050	450
0339						68	160	1350	1350	4050	450
0339						82	-	1350	1350	1350	150
0339						89	-	1350	1350	1350	150
0339						96	160	1350	1350	1350	150
0339						110	-	1350	1350	1350	150
0339						118	320	1350	1350	1350	150
0345	62	M	NO	1	NO	54	0	1350	1350	4050	450
0345						94	320	1350	1350	1350	150
0350	53	M	NO	1	NO	45	160	1350	1350	1350	150
0350						59	-	1350	1350	1350	150
0350						82	160	1350	1350	450	50
0354	59	M	YES	4	YES	98	640	1350	1350	4050	150
0354						104	-	1350	1350	4050	150
0354						111	640	1350	1350	4050	450
0354						132	-	1350	1350	4050	150
0363	56	M	NO	1	NO	34	80	450	1350	1350	50
0363						44	-	1350	1350	1350	50
0363						59	-	1350	1350	450	50
0363						64	-	1350	1350	450	50
0363						73	80	1350	1350	450	50

0363						86	-	450	1350	450	0
0363						100	20	1350	1350	450	50
0363						107	40	1350	450	450	50
0367	58	F	NO	2	YES	89	-	1350	1350	4050	450
0368	37	F	NO	2	YES	29	160	450	1350	4050	50
0369	41	M	NO	1	NO	39	80	150	450	1350	50
0369						46	40	450	450	450	50
0369						49	40	1350	1350	450	50
0369						56	80	1350	1350	450	50
0369						63	20	1350	1350	450	0
0369						69	20	1350	450	450	50
0369						76	20	1350	450	150	50
0369						83	10	1350	1350	150	50
0369						97	20	450	450	450	0
0369						104	-	450	150	150	0
0369						112	20	1350	450	150	0
0369	41	M	NO	1	NO	119	-	450	450	150	0
0376	52	M	YES	4	YES	28	1280	1350	4050	4050	150
0376						32	160	4050	4050	4050	150
0376						60	-	1350	1350	1350	150
0376						67	80	1350	1350	1350	50
0376						88	160	1350	1350	1350	50
0376						108	80	1350	1350	450	50
0377	49	M	NO	2	YES	52	160	450	1350	150	0
0377						66	-	50	450	150	0
0377						80	-	450	450	50	0
0377						94	0	450	450	50	50
0385	45	M	NO	2	YES	63	-	0	0	0	0
0398	55	F	YES	4	YES	58	-	1350	1350	1350	150
0398						67	-	1350	1350	1350	150
0398						73	-	1350	1350	450	150
0412	53	M	NO	1	NO	75	20	1350	450	150	0
0412						89	-	1350	1350	150	0
0412						103	0	1350	450	50	0
0419	40	M	NO	2	YES	68	--	1350	1350	1350	150
0422	41	M	NO	1	NO	64	640	1350	1350	1350	450
0422						84	80	1350	450	450	150
0423	39	M	NO	2	YES	57	40	450	1350	1350	150
0423						65	20	1350	1350	450	50
0423						75	20	50	1350	450	50
0423						83	20	1350	1350	450	50
0423						89	20	450	1350	150	50
0423						117	20	450	450	150	0

0423						127	-	1350	450	150	0
0423						131	-	1350	1350	150	0
0423						138	-	450	1350	50	0
0430	44	M	YES	4	YES	35	1280	4050	4050	4050	150
0436	32	F	NO	1	NO	45	640	1350	1350	50	0
0436						55	-	1350	1350	50	0
0436						62	-	450	1350	50	0
0436						69	-	1350	1350	50	0
0436						90	-	1350	1350	50	0
0436						97	0	1350	1350	50	0
0437	49	M	NO	1	NO	55	40	1350	1350	450	50
0437						64	-	1350	1350	450	50
0437						78	-	1350	1350	450	50
0437						113	40	1350	1350	450	50
0448	49	M	NO	2	YES	43	160	1350	1350	4050	150
0448						46	-	1350	1350	4050	150
0448						91	160	1350	1350	1350	150
0448						105	-	1350	1350	4050	150
0448						112	-	1350	1350	1350	50
0448						119	160	1350	1350	1350	50
0462	47	F	NO	2	YES	61	-	450	1350	450	0
0464	31	F	NO	2	YES	48	160	1350	1350	4050	450
0464						55	160	1350	1350	4050	450
0464						62	80	1350	1350	450	450
0464						69	80	1350	1350	1350	450
0464						83	80	1350	1350	1350	150
0464						90	-	1350	1350	1350	450
0464						118	160	1350	1350	1350	450
0479	56	F	NO	1	NO	79	-	1350	1350	4050	450
0488	37	F	NO	1	NO	62	-	150	150	0	0
0515	58	M	YES	4	YES	69	80	1350	1350	4050	1350
0515						83	-	1350	1350	4050	1350
0515						104	320	1350	1350	4050	1350
0524	35	F	YES	3	YES	44	-	1350	1350	4050	450
0525	33	F	NO	2	YES	43	-	450	50	150	50
0525						68	-	50	450	150	50
0526	74	M	NO	2	YES	45	-	1350	1350	1350	150
0530	39	F	NO	1	NO	71	-	1350	1350	450	50
0533	32	F	NO	1	NO	47	-	450	450	150	0
0548	40	M	NO	4	NO	52	-	1350	1350	4050	150
0554	68	F	NO	1	NO	57	-	1350	1350	4050	1350
0576	50	F	YES	3	YES	41	0	0	0	50	0
0579	70	M	NO	2	YES	43	640	1350	1350	4050	450

0579						50	-	1350	1350	1350	150
0579						57	-	1350	1350	4050	450
0579						64	80	1350	1350	1350	150
0579						99	160	1350	1350	1350	150
0580	43	F	YES	3	YES	29	1280	4050	4050	1350	150
0580						35	1280	4050	4050	4050	150
0580						57	160	1350	1350	1350	50
0581	50	M	YES	4	YES	108	-	1350	1350	150	50
0591	51	F	NO	1	NO	44	-	450	1350	450	150
0595	26	F	NO	2	YES	104	-	150	150	50	0
0595						111	-	150	150	50	0
0598	46	M	YES	4	YES	30	1280	4050	4050	4050	450
0598						99	640	1350	1350	4050	150
0599	42	M	YES	3	YES	82	320	1350	1350	1350	450
0599						117	320	1350	1350	1350	150
0605	54	F	NO	1	NO	58	-	1350	1350	1350	150
0610	48	F	NO	2	YES	64	-	1350	1350	150	0
0612	36	F	NO	1	NO	69	-	1350	1350	50	0
0618	58	F	YES	5	YES	65	80	1350	1350	4050	450
0618						118	320	1350	1350	1350	150
0620	59	M	YES	5	YES	40	1280	4050	4050	4050	4050
0620						52	320	1350	1350	4050	1350
0620						59	320	1350	1350	4050	1350
0620						66	160	1350	1350	4050	1350
0620						73	160	1350	1350	4050	450
0620						94	320	1350	1350	450	450
0620						101	--	1350	1350	4050	150
0620						108	320	1350	1350	1350	150
0622	20	F	NO	1	NO	45	0	450	150	50	0
0622						52	-	450	150	50	0
0622						77	0	1350	450	150	0
0631	58	M	NO	1	NO	47	20	150	450	150	50
0631						53	20	150	1350	50	50
0631						61	20	1350	1350	50	0
0631						67	20	1350	150	50	0
0631						74	10	450	1350	50	0
0631						81	20	450	450	50	0
0631						95	0	450	450	50	0
0631						108	-	150	50	50	0
0631						117	0	450	450	50	0
0631						122	-	450	150	50	0
0633	63	M	YES	4	YES	79	-	1350	1350	1350	150
0634	53	M	YES	5	YES	33	1280	4050	4050	4050	450

0636	30	M	NO	2	YES	64	-	1350	1350	1350	450
0664	46	M	NO	1	NO	75	-	1350	1350	1350	50
0664						86	-	1350	1350	1350	50
0694	30	M	NO	2	YES	42	320	1350	1350	1350	150
0694						127	160	1350	1350	1350	50
0695	22	F	NO	2	YES	62	0	0	1350	150	0
0695						72	-	450	1350	150	0
0695						79	-	450	450	150	0
0695						114	0	450	150	150	0
0695						128	-	450	450	150	0
0698	32	M	NO	2	YES	51	320	1350	1350	450	150
0698						59	-	1350	1350	450	150
0698						72	-	1350	450	450	50
0698						100	40	1350	150	450	150
0699	61	M	YES	3	NO	35	640	4050	450	450	450
0701	41	M	NO	1	NO	47	80	1350	1350	450	150
0701						53	40	1350	1350	1350	150
0701						63	40	1350	1350	450	150
0701						70	40	1350	1350	450	150
0701						74	80	1350	1350	150	150
0701						105	20	1350	450	450	50
0701						109	-	1350	1350	50	0
0701						116	20	1350	450	450	50
0701						123	-	1350	1350	450	50
0719	63	M	NO	1	NO	107	-	1350	1350	50	0
0719						114	-	1350	1350	50	0
0720	39	M	NO	2	YES	63	-	50	0	0	0
0731	50	M	YES	4	YES	66	80	1350	1350	4050	450
0731						73	40	1350	1350	4050	150
0731						80	40	1350	1350	1350	50
0731						87	80	1350	1350	450	150
0731						94	40	1350	1350	1350	150
0731						101	-	1350	1350	4050	150
0731						115	160	1350	1350	1350	150
0731						128	-	1350	1350	1350	150
0731						142	-	1350	1350	450	50
0749	45	M	NO	1	NO	48	-	450	1350	450	0
0749						55	-	450	1350	450	0
0749						69	-	450	450	150	50
0750	29	M	NO	2	YES	46	-	450	150	150	150
0750						50	-	450	150	450	50
0750						53	-	150	1350	150	50
0750						123	0	450	450	50	50

0759	48	F	NO	2	YES	45	-	1350	1350	450	150
0762	47	M	NO	2	YES	44	160	450	1350	1350	50
0762						51	40	1350	1350	1350	150
0762						58	80	1350	1350	450	150
0762						64	160	1350	1350	450	150
0762						78	160	1350	1350	1350	50
0762						85	-	1350	1350	150	0
0762						92	40	1350	1350	450	0
0762						113	80	1350	1350	450	0
0786	46	F	NO	2	YES	48	-	450	1350	1350	150
0789	55	F	NO	2	YES	62	-	1350	1350	1350	150
0796	45	M	YES	5	YES	52	-	1350	1350	1350	450
0820	56	F	NO	1	NO	82	-	1350	1350	450	50
0820						113	-	1350	1350	450	50
0834	48	M	NO	2	YES	60	-	1350	1350	4050	450
0835	52	F	YES	5	YES	61	-	1350	1350	4050	4050
0838	74	F	NO	1	NO	49	320	1350	1350	1350	450
0838							160	1350	1350	1350	450
0838							160	1350	1350	450	450
0838							80	1350	1350	1350	450
0838							320	1350	1350	1350	150
0838							-	1350	1350	1350	150
0838							160	1350	1350	450	150
0850	34	F	NO	2	YES	67	-	1350	1350	1350	150
0879	50	M	YES	4	YES	34	160	4050	4050	4050	4050
0879							-	1350	1350	4050	1350
0905	41	F	NO	1	NO	57	-	1350	1350	150	0
0913	34	F	YES	5	YES	83	-	1350	1350	4050	450
0913						90	-	1350	1350	4050	150
0913						101	-	1350	1350	4050	150
0913						116	-	1350	1350	1350	0
0933	67	M	NO	1	NO	65	-	1350	1350	4050	450
0970	29	F	NO	2	YES	56	-	1350	1350	1350	50
0992	45	F	NO	2	YES	43	-	0	0	0	0
0992						49	-	0	0	0	0
1033	33	F	NO	1	NO	61	80	1350	1350	1350	1350
1033						75	-	1350	450	150	0
1033						92	80	1350	1350	1350	450
1052	28	F	NO	2	YES	59	40	1350	1350	1350	50
1052						73	-	150	1350	450	50
1052						95	160	1350	1350	1350	50
1062	44	F	NO	2	YES	53	40	1350	1350	450	50
1062						88	-	1350	1350	150	0

1062						93	20	450	150	150	0
1062						98	-	450	450	150	0
1062						112	-	450	450	150	0
1062						119	0	1350	150	150	0
1062						126	-	1350	1350	50	0
1121	28	F	YES	4	YES	71	0	1350	1350	0	0
1121						78	-	0	0	0	0
1121						89	-	0	0	0	0
1121						96	0	0	50	0	0
1145	39	F	NO	2	YES	66	0	1350	1350	450	0
1145						73	-	1350	1350	450	0
1145						80	-	1350	450	450	0
1145						94	40	1350	1350	450	50
1145						101	-	1350	1350	450	0
1145						115	20	1350	1350	450	0
1215	54	M	NO	1	NO	79	40	1350	1350	450	150
1215						109	80	1350	1350	150	50
1234	49	F	NO	2	YES	38	-		1350	4050	1350
1234						44	-	1350	1350	4050	150
1234						52	-	1350	1350	4050	450
1234						60	-	1350	1350	4050	150
1234						68	-	1350	1350	4050	150
1234						82	-	1350	1350	4050	150
1234						94	-	1350	1350	450	0
1278	23	M	NO	1	NO	64	-	50	1350	50	0
1278						74	-	1350	1350	150	0
1278						81	-	1350	1350	150	0
1278						95	-	1350	450	150	0
1278						116	-	1350	450	150	0
1278						130	-	1350	450	150	0
1288	27	F	NO	2	YES	47	-	1350	1350	1350	150
1288						54	-	1350	1350	1350	150
1288						61	-	1350	1350	1350	150
1288						68	-	1350	450	450	0
1288						81	80	1350	1350	450	50
1344	32	F	NO	1	NO	63	20	1350	450	450	50
1344						105	0	1350	1350	150	0
1401						87	-	0	0	1350	150
1401						102	-	1350	450	1350	50
1432	54	M	NO	1	NO	58	80	1350	1350	1350	150
1432						84	40	1350	450	450	50
1457	23	M	NO	1	NO	80	-	1350	1350	150	0
1457						87	-	450	150	150	0
1457						94	-	1350	1350	450	50

1457						101	-	450	1350	50	0
1457						108	-	1350	450	150	0
1457						122	-	1350	1350	150	0
1462	70	M	NO	1	NO	72	-	1350	1350	450	50
1462						79	80	1350	1350	450	50
1462						86	-	450	450	450	50
1462						93	-	1350	450	450	50
1462						100	20	150	1350	450	0
1462						107	-	1350	450	150	0
1462						114	0	450	1350	450	50
1499	42	M	NO	1	NO	79	20	1350	1350	450	50
1499						94	0	1350	450	450	0
1551	50	M	YES	5	YES	65	-	1350	1350	4050	450
1551						70	320	1350	1350	4050	450
1551						76	-	1350	1350	1350	150
1551						83	-	1350	1350	4050	450
1551						89	320	1350	1350	4050	450
1551						97	-	1350	1350	0	0
1551						104	-	1350	1350	1350	150
1551						111	160	1350	1350	450	150
1678	48	F	NO	2	YES	54	-	1350	1350	450	150
1678						85	-	1350	1350	1350	450
1678						92	-	1350	1350	1350	150
1817	28	F	NO	1	NO	44	-	1350	1350	450	150
1817						51	-	1350	1350	1350	450
1817						63	-	1350	1350	1350	450

DPO Days post onset of symptoms; S/ECD Spike ectodomain; S/RBD Spike receptor-binding domain;
VN Virus neutralization

Supplementary Table 2: Predictive values and likelihood ratios of the ELISA methods as a surrogate for virus neutralizing antibody titer of ≥ 160 .

DPO	Effect	S/ECD ≥ 1350		S/RBD ≥ 1350		S/RBD IgG ≥ 1350		S/RBD IgM ≥ 450	
		Value	95% CI	Value	95% CI	Value	95% CI	Value	95% CI
Overall 0-142	PPV	0.52	0.46 to 0.59	0.57	0.50 to 0.63	0.72	0.65 to 0.79	0.38	0.30 to 0.46
	NPV	0.75	0.65 to 0.83	0.87	0.78 to 0.92	0.82	0.75 to 0.87	0.90	0.84 to 0.94
	LR+	1.34		1.61		3.18		3.72	
	LR-	0.40		0.19		0.26		0.69	
1-30	PPV	0.92	0.65 to 1.00	0.94	0.72 to 0.99	0.78	0.55 to 0.91	1.00	0.68 to 1.00
	NPV	0.67	0.47 to 0.82	0.80	0.58 to 0.92	0.65	0.43 to 0.82	0.56	0.39 to 0.73
	LR+	9.85		13.43		2.83		-	
	LR-	0.45		0.22		0.44		0.62	
31-60	PPV	0.79	0.67 to 0.88	0.69	0.57 to 0.78	0.73	0.61 to 0.82	0.90	0.74 to 0.96
	NPV	0.56	0.41 to 0.70	0.72	0.52 to 0.83	0.72	0.55 to 0.84	0.55	0.43 to 0.66
	LR+	1.80		1.56		1.92		6.23	
	LR-	0.38		0.28		0.28		0.59	
61-90	PPV	0.32	0.23 to 0.43	0.35	0.25 to 0.47	0.58	0.41 to 0.72	0.50	0.31 to 0.69
	NPV	1.00	0.65 to 1.00	1.00	0.74 to 1.00	0.90	0.79 to 0.96	0.78	0.66 to 0.86
	LR+	1.13		1.30		3.20		2.40	
	LR-	0.00		0.00		0.27		0.69	
91-120	PPV	0.52	0.40 to 0.64	0.61	0.48 to 0.73	0.87	0.71 to 0.95	0.80	0.49 to 0.96
	NPV	1.00	0.77 to 1.00	1.00	0.85 to 1.00	0.87	0.74 to 0.94	0.62	0.50 to 0.73
	LR+	1.43		2.05		8.80		5.21	
	LR-	0.00		0.00		0.20		0.79	
>120	PPV	0.43	0.16 to 0.75	0.43	0.16 to 0.75	0.75	0.30 to 0.99	0.00	0.00 to 0.95
	NPV	1.00	0.05 to 1.00	1.00	0.05 to 1.00	1.00	0.51 to 1.00	0.57	0.25 to 0.84
	LR+	1.25		1.25		5.00		0.00	
	LR-	0.00		0.00		0.00		1.25	

DPO Days post onset of symptoms; S/ECD Spike ectodomain; S/RBD Spike receptor-binding domain; PPV Positive predictive value; NPV Negative predictive value; LR+ Positive likelihood ratio; LR- Negative likelihood ratio