Convalescent plasma anti-SARS-CoV-2 spike protein ectodomain and receptor binding domain IgG correlate with virus neutralization

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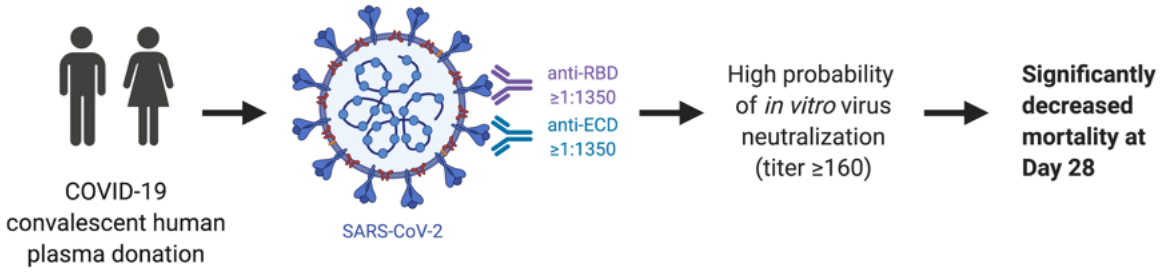
Graphical Abstract



Abstract

The newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) highlights the urgent need for assays that detect protective levels of neutralizing antibodies. We studied the relationship between anti-spike ectodomain (ECD), anti-receptor binding domain (RBD) IgG titers, and SARS-CoV-2 virus neutralization (VN) titers generated by two *in vitro* assays using convalescent plasma samples from 68 COVID-19 patients. We report a strong positive correlation between both plasma anti-RBD and anti-ECD IgG titers and *in vitro* VN titer. The probability of a VN titer ≥160, the FDA-recommended level for convalescent plasma used for COVID-19 treatment, was ≥80% when anti-RBD or anti-ECD titers were ≥1:1350. Of all donors, 37% lacked VN titers ≥160. Dyspnea, hospitalization, and disease severity were significantly associated with higher VN titer. Frequent donation of convalescent plasma did not significantly decrease VN or IgG titers. Analysis of 2,814 asymptomatic adults found 73 individuals with anti-ECD IgG titers of ≥1:50 and strong positive correlation with anti-RBD and VN titers. Fourteen of these individuals had VN titers ≥1:160, all of which had anti-RBD titer ≥1:1350. We conclude that anti-RBD or anti-ECD IgG titers can serve as a surrogate for VN titers to identify suitable plasma donors. Plasma anti-RBD or anti-ECD titers of ≥1:1350 may provide critical information about protection against COVID-19 disease.

Introduction

The recently emerged SARS-CoV-2 novel coronavirus causing COVID-19 disease has spread globally and is now responsible for massive human morbidity and mortality. The pathogen was first documented to cause severe respiratory infections in humans in Wuhan, China, beginning in late December 2019 (1). Soon thereafter, the SARS-CoV-2 virus was characterized as a member of the Betacoronavirus genus and recognized to be related to several bat coronaviruses, SARS, and Middle East Respiratory Syndrome (MERS) coronaviruses. SARS-CoV-2 spread was unusually rapid, and COVID-19 disease has now been reported in virtually all major population centers globally. In the United States, more than 1,500,000 COVID-19 cases have been documented and the virus has caused greater than 100,000 deaths nationwide. Many metropolitan regions have been especially affected, including but not limited to Seattle, New York City, Chicago, Miami, and Detroit (2).

Management of COVID-19 infection has predominantly involved aggressive support care. Various treatment approaches are being studied, including direct viral replication inhibition (3), anti-inflammatory drugs, and passive antibody therapies. Currently, the only available passive antibody therapy for COVID-19 patients is transfusion of convalescent plasma obtained from recovered patients. The therapy is safe, and multiple emerging lines of evidence, including historical precedent, pre-clinical animal studies, small case series, and matched observational studies suggest that convalescent plasma is efficacious in the treatment of COVID-19 (4, 5). Clinical trials assessing efficacy in specific patient populations are underway, and clinical trials assessing the use of hyperimmune IgG may begin soon (6).

The Food and Drug Administration (FDA) has recommended (7) that convalescent plasma with a virus neutralizing (VN) antibody titer of ≥1:160 be used for therapeutic transfusion. While VN assays with live virus are considered the gold standard, these are not widely available, in part because they are labor intensive, cumbersome, and require a Biosafety Level 3 laboratory. Assays with pseudotype virus offer considerable advantages over live virus

assays. These are safe to use in a Biosafety Level 2 environment, and have the potential to assay responses to specific target viral proteins; however, cell culture and maintenance of suitable cell lines is still required. Thus, these assays are not readily integrated into the available donor testing infrastructure. Inasmuch as the VN titers in most donor plasma are not known prior to transfusion, a more facile method to identify suitable convalescent plasma donors is needed. This is an especially pressing matter, as an increasing number of COVID-19 patients are being treated globally with convalescent plasma. For example, under an FDA-approved expanded access protocol, greater than 50,000 transfusions have already occurred in the United States (8).

The trimeric spike (S) protein made by SARS-CoV-2 is a large molecule that is critical to virus dissemination and pathogenesis. S protein is a densely glycosylated molecule present on the surface of the virus. S protein mediates binding of the SARS-CoV-2 virus to the host angiotensin-converting enzyme 2 (ACE2) receptors, thereby acting as the first step in cell entry and infection. Recent work has shown that SARS-CoV-2 and SARS-CoV-1 share the same ACE2 receptor. The molecular mechanism used by S protein to gain entry into host cells is complex and involves a region of the molecule known as the receptor-binding domain (RBD). Engagement of S protein with the host receptor results in considerable changes in molecular conformation. The S protein has a critical function in host-cell entry and thus, is a major target for vaccine research and antibody-mediated VN efforts.

Many lines of evidence from studies of SARS-CoV-1, MERS, and SARS-CoV-2 show that infected hosts make antibodies directed against S protein (9-16). In addition, immunization with S protein can protect laboratory animals against experimental infection with SARS-CoV-1, MERS-CoV, and SARS-CoV-2 (17-21). Similarly, IgG directed against S protein has been reported to have *in vitro* VN activity.

The goal of this study was to test the hypothesis that anti-ECD and/or anti-RBD IgG titer are correlated with VN titer, and thus could be used as a surrogate marker to identify plasma

donors with titers above the FDA threshold value of 1:160. To test this hypothesis, we studied plasma and serum samples from 68 recovered COVID-19 patients with documented disease based on a positive molecular test for SARS-CoV-2. VN titer was determined independently in two laboratories using two different *in vitro* assays. The results show a strong positive correlation between anti-RBD and anti-ECD plasma IgG ELISA titers and the magnitude of *in vitro* VN. Specifically, we report that there is an 80% probability or greater of a VN titer at or above the FDA recommended level of 1:160 for COVID-19 convalescent plasma with anti-RBD or anti-ECD IgG titers of ≥1:1350. The results provide an important quantitative target for therapeutic and prophylactic treatments. We also find that convalescent donors maintain high-titer anti-RBD and anti-ECD IgG with *in vitro* VN activity over many weeks. Frequent plasma donations do not cause a significant decrease in antibody or VN titers. Finally, analysis of anti-ECD and anti-RBD IgG titers in 2,814 asymptomatic individuals in a surveillance cohort identified 14 individuals with VN titers ≥1:160 with all of these having an anti-RBD titer ≥1:1350. Thus, some asymptomatic individuals may have plasma suitable for therapeutic use and may have a degree of relative immunity against SARS-CoV-2.

Results

Plasma Donor Characteristics

Ninety-three samples from 68 unique COVID-19 convalescent plasma donors were assessed (Table 1 and 2). The average age was 45 (range 23 to 78) and 36 donors were female. Most donors had severity scores (as defined in the Methods) consistent with mild to moderate disease, with 44% (30/68) having a symptom severity score of 1; 32% (22/68) having score of 2; 10% (7/68) having a score of 3; 7% (5/68) having a score of 4; and 6% (4/68) having a score of 5. Sixteen donors required hospitalization, with an average length of stay of 4 d (range 2-13 d). Thirteen individuals donated more than once (range 1-7 times) with most (9/13) donating twice only. For all samples assessed, the median interval from symptom onset to donation visit was 32 d (range 17-53 d; IQR 28-36 d), and the median interval from symptom resolution to donation visit was 20 d (range 15-38 d; IQR 17-25 d).

We also studied plasma from 73 asymptomatic individuals identified during an institutional surveillance program involving 2,814 individuals (22). Of these 2,814 individuals, 73 had anti-ECD ELISA titers ≥50. The average age of these 73 asymptomatic subjects was 38 (range 20-69) and 56 of the 73 (77%) were female (Supplemental Table 1).

VN Titers in Convalescent Plasma Donors

VN titers in samples from COVID-19 convalescent plasma donors were assessed with a traditional microneutralization assay evaluating protection from virus infection as determined by crystal violet staining 3 d post-infection. Plasma samples from the majority of donors (43/68; 63%) had a VN titer ≥1:160, the FDA recommended VN antibody titer for convalescent plasma to be used for therapeutic transfusion purposes. In contrast, 25 of 68 donors (37%) had a plasma titer below this recommended cutoff value (Figure 1A and Table 1 and 2).

Correlation between Two VN Assays

VN titers were assessed blinded (that is, without knowledge of the data generated by laboratory one) in a second laboratory with a different microneutralization assay (VN2) that determined the percentage of infected cells 24 h post-infection using a SARS-CoV-2 specific mAb and a fluorescently labeled secondary antibody. The results from the two VN assays were highly correlated ($r=0.66$, $P < 0.001$) (Figure 1B and C).

Association between ELISA IgG Titers and VN Titer

Recognizing the urgent need for assays that could serve as a surrogate for VN, we assessed the association between ELISA anti-ECD and anti-RBD IgG titers and VN titers. The results of all four assays (anti-ECD and anti-RBD ELISAs, VN, and VN2) were strongly correlated (Figure 1C). Anti-RBD IgG had a numerically but not statistically greater correlation than anti-ECD (0.67 versus 0.62) with both microneutralization assays. We found that greater than 80% of donors had a VN titer ≥1:160 in convalescent plasma when their serum anti-RBD or anti-ECD titers were 1:1350 or higher (Figure 2). Among 61 samples from plasma donors with a VN titer ≥1:160, 59 had an anti-RBD assessment, and 41 (70%) had an anti-RBD titer ≥1:1350. Conversely, only 4 of 45 samples with an anti-RBD titer ≥1:1350 had a VN of ≤ 160, indicating a positive predictive value for VN titer ≥1:160 of 91%. Importantly, samples from naïve human plasma specimens obtained before the discovery of SARS-CoV-2 had no detectable titer in any of the four assays (data not shown).

Relationship between Antibody Titers and Donor Characteristics

Inasmuch as approximately one-third of donors lacked convalescent plasma with the FDA recommended VN titer cutoff of ≥1:160, we sought to identify donor characteristics that may be associated with a higher IgG titer. Such characteristics could aid donor recruitment efforts by

identifying which recovered patients may have mounted a strong humoral response. We found that the presence of dyspnea during COVID-19 disease, hospitalization requirement, and more severe disease are all positively and significantly associated with higher IgG titers in all assays (Figure 3). Duration of disease symptoms was not associated with titer. Nor was there an association with time of plasma collection since symptom onset and titer in the donor population. All collections occurred more than 14 d after symptom resolution (as required by the FDA). These results suggest that donors had already plateaued in their IgG titer at the time plasma was obtained, as there was no appreciable trend in titer increase over time (Supplemental Figure 1A and B). There was a trend toward lower titers in younger age group donors and in female donors, but these findings were not consistent across all assays (Supplemental Figure 1C and D).

VN Titers Over Time from the Same Convalescent Plasma Donors

Thirteen individuals donated convalescent plasma more than once (range, 2 to 7 donations). The availability of longitudinal samples from the same plasma donors permitted us to assess the arc of anti-ECD and anti-RBD IgG titers and VN over time within individuals. There was no significant decrease in IgG titers as assessed by the ELISA or VN titer (Supplemental Figure 2), even among donors who donated twice/week for up to seven donations. Thus, we observed stable, high titers both within and between individual donors.

Relationship between Infecting Strain Clade and VN Titer

We had available the virus genome sequences obtained from clinical samples (e.g., nasopharyngeal swab, oropharyngeal swab, or sputum) from 25 plasma donors. Eighty-four percent (21/25) of donors had been infected with strain A2a and the remaining donors had been infected with strain B. Although the number of specimens is small, we tested the hypothesis that

a relationship exists between the VN titer and genetic clade of the infecting SARS-CoV-2 strain. No definitive relationship was evident from analysis of the available data (Table 1).

Asymptomatic Individuals and VN Titers

Having established a relationship between IgG titer and *in vitro* SARS-CoV-2 VN titer, we next determined IgG titers in a sample of 2,814 asymptomatic adults screened under a surveillance protocol. We found that 73 of 2,814 (2.5%) individuals had anti-ECD and anti-RBD IgG ELISA titers of ≥1:50, of which 27 had anti-RBD or anti-ECD IgG titers of ≥1:1350 (Supplemental Table 1). Among the 73 specimens from asymptomatic individuals analyzed for VN titer, the correlation between anti-ECD, anti-RBD, and VN titer remained highly significant with $P<0.001$ in all comparisons (Supplemental Figure 3). In all cases where VN titer was ≥1:160, the anti-RBD titer was ≥1:1350.

Discussion

In the absence of an efficacious vaccine to prevent COVID-19 disease, there is a pressing need for assays that detect neutralizing antibodies against SARS-CoV-2. Here we studied the relationship between anti-RBD and anti-ECD IgG titers present in convalescent plasma obtained from COVID-19 patients and *in vitro* SARS-CoV-2 VN. We discovered a strong positive association between anti-RBD and anti-ECD plasma IgG titers and *in vitro* VN titer.

The data provide important evidence that anti-ECD and anti-RBD IgG titers are a suitable proxy for VN titer. Given the limited availability of VN assays, which are technically complex, require days to set up, run, and interpret, need a Biosafety Level 3 laboratory when performed with live native SARS-CoV-2 virus, and the relative ease with which ELISA assays can be implemented and performed in a high throughput fashion, we believe our data provide a guidepost for proxy assessments of VN titers relevant to the COVID-19 pandemic.

We found that although both anti-ECD and anti-RBD IgG titers correlate well and significantly with *in vitro* VN, anti-RBD IgG titer had a tendency for a stronger correlation than anti-ECD IgG titer. This finding is consistent with a study showing clustering of VN epitopes in the SARS-CoV-1 RBD domain (23). In this study, neutralizing monoclonal antibodies mapped to a region of RBD that has a critical role in attachment to the host ACE2 receptor. Given that the RBD is also the important region for ACE2 receptor binding for SARS-CoV-2 (24, 25), it is not surprising that anti-RBD IgG titers correlate well with VN titers. Importantly, our data from convalescent plasma donors show that anti-RBD or anti-ECD IgG titers of 1:1350 discriminated the presence of an adequate VN titer as recommended by the FDA for COVID-19 convalescent plasma with a probability of ~80%. Using this anti-RBD or anti-ECD IgG titer cutoff, a proportion of donors and plasma units with adequate VN titer would be excluded from use. In an effort to rapidly identify donors and plasma units likely to have efficacy, this exclusion rate is acceptable , while the alternative transfusion of patients with convalescent plasma units with low or no titer of anti-SARS-CoV-2 antibody, is not. Future studies are required to determine if a VN titer of

≥1:160 has therapeutic benefit. Regardless, our findings clearly indicate that an anti-RBD IgG titer cutoff can be established that serves as a suitable proxy for VN titer.

Our findings greatly expand on recent work showing a relationship between anti-S ELISA and microneutralization titer in nine samples using a 48 h post-infection microneutralization assay assessing “whole-well” optical density (13). Suthar et al. have also demonstrated that RBD-specific IgG endpoint titer correlates well with a focus-reduction neutralization assay (12). Li et al. reported a positive correlation between SARS-CoV-2 VN titer and S-RBD–specific IgG titer, with a serum VN titer of 1:80 as approximately equivalent to a titer of 1:1280 for S-RBD-specific IgG (26). Because of differences in the VN assay used, their titers and those we report here are not equivalent (27). Harvala et al. also reported VN and anti-spike ELISA titers were correlated (28), although there were several differences between that study and ours. For example, all donors were male, plasma was collected >28 d after symptom resolution, a different virus strain was used, no repeat donors were studied, and the association with clinical symptoms was not assessed. In addition, they did not study samples obtained during community screening of asymptomatic individuals. Herein, we compared results from two independent neutralization assays run blinded in two independent laboratories. The traditional VN assay (assay one) assessed protection from SARS-CoV-2 virus infection as determined by presence of cytopathic effect three days post-infection. In contrast, assay two (VN2) analyzed the percentage of SARS-CoV-2 virus-infected cells 24 h post-infection as a measure of early virus replication and susceptibility to host-cell infection. These two different approaches to VN assessment, and the robustness of the correlation between the results of the two different assays, add confidence to our conclusion that anti-RBD IgG and anti-ECD IgG titers measured by ELISA serve as a very reliable surrogate of VN.

Of particular note, approximately one-third of convalescent plasma donors in our study did not meet the FDA recommended cutoff of 1:160 for VN titer. This finding is consistent with the 60% that did not meet the target neutralization threshold of 1:100 recently described in the

Harvala study (24). However, the inability to directly compare titers between laboratories highlights an unmet need for the development of international standards to enable comparisons of SARS-CoV-2 serological assays between laboratories (27). An increasing number of COVID-19 patients are being treated globally with convalescent plasma. For example, under an FDA-approved expanded access protocol, >50,000 transfusions have already occurred in the United States alone (8). Inasmuch as convalescent donor plasma likely will continue to play an important role in treatment of COVID-19 patients for the foreseeable future, as efforts are made to manufacture polyclonal hyperimmune immunoglobulin and neutralizing monoclonal antibodies, especially as indications of efficacy are published (29), we felt it necessary to determine if certain donor characteristics may associate with high VN titer. We found that antibody titers were associated with disease severity and hospitalization status. Among all COVID-19 symptoms and donor characteristics assessed, the presence of dyspnea was the best symptom to discriminate the presence of an adequate IgG antibody titer. Although the sample size is small, we found that even for donors who donated plasma twice/week for up to seven donations, there was no significant decrease in titers as assessed by the IgG ELISAs and VN. We believe these data could inform efforts to recruit plasma donors for therapeutic purposes. The finding that increased COVID-19 disease severity is associated with a more robust humoral immune response is consistent with previous studies of SARS and dengue hemorrhagic fever patients (30), but contrasts with a recent report analyzing COVID-19 patients (31). It is possible that differences in antibody testing platforms account for the contrasting observations. The list of emergency use authorized antibody testing platforms is rapidly expanding and test performance, especially as it relates to VN, will be important to understand (32). Regardless, our findings are in agreement with a more recent report finding that strong antivirus antibody responses were associated with male sex, older age, and hospitalization (33).

Analysis of the available genomes for the SARS-CoV-2 strain pairs infecting convalescent donors and recipients found few differences in the inferred amino acid sequences,

and no association between magnitude of humoral immunity, disease severity, or infecting strain genotype. Because our sample size is small, more work is required in this area.

Several important matters remain unanswered with respect to anti-S protein IgG antibodies. First, although many believe, and some experimental animal infection data supports (34), that antibodies directed against S protein confer protection from SARS-CoV-2 infection or reinfection, this remains unproven in humans. Second, although our data and work by others show a strong relationship between anti-S protein IgG titers and *in vitro* VN, it will be important to determine if IgG antibody titer against this protein is a significant correlate of protective immunity in humans. This is an especially important topic given the massive efforts globally on using S protein as a vaccine.

Our study has several limitations. The study was retrospective, only IgG titers were analyzed, and all VN studies were conducted *in vitro*. Plasma from the convalescent donors was used for VN assays, whereas serum samples were used for ELISA assays. As such, the findings may not be entirely applicable to all antibody testing platforms or other sample types. Given the timing of the study relative to the pandemic curve in the Houston metropolitan region, donors were at most, 53 days post symptom onset. Additional studies with donors that are later in their convalescence are needed. The sample size was limited by the number of donors recruited for plasma collection and additional studies with larger sample sizes are needed. However, the data represent the most extensive assessment of the correlation between independent live virus neutralization and ELISA assays for anti-SARS-CoV-2 antibodies in convalescent plasma donors to date.

Conclusions

Taken together, the data clearly show that anti-RBD and anti-ECD IgG titers serve as important surrogates for *in vitro* VN activity. A substantial fraction of convalescent plasma donors may have VN titers below the FDA recommended cutoff of ≥1:160. Dyspnea, hospitalization, and

higher disease severity were associated with higher VN titer. Importantly, a small percentage of asymptomatic individuals have virus neutralizing antibodies, including some with a titer of ≥1:160. In the aggregate, it is reasonable to think that our findings provide impetus for widespread implementation of anti-RBD and anti-ECD IgG antibody titer testing programs. The resulting data could be useful in several settings, including, but not limited to, identification of plasma donors for therapeutic uses (e.g., convalescent plasma transfusion and/or source plasma for fractionation in the manufacture of hyperimmune globulin) (7, 13), assessment of recipients of candidate vaccines, assessment of recipients of passive immune therapies, assessment of previously infected individuals, and identification of asymptomatic individuals with antibodies against SARS-CoV-2.

Methods

Convalescent Plasma Donors

Convalescent plasma was obtained by apheresis using the Trima Accel automated blood collection system (Terumo BCT) and processed by standard blood banking protocols. FDA recommendations for COVID-19 convalescent plasma donor collection were followed (7). Each donor had laboratory-confirmed SARS-CoV-2 infection based on a positive RT-PCR test. All plasma was donated by recovered and healthy COVID-19 patients who had been asymptomatic for more than 14 d. Donors were between 18-65 years old. All donors tested negative for SARS-CoV-2 at the time of plasmapheresis. If eligible according to standard blood donor criteria, donors were enrolled in a frequent plasmapheresis program. Donors were documented to be negative for anti-HLA antibodies, hepatitis B, C, HIV, HTLV I/II, Chagas disease, WNV, Zika virus, and syphilis per standard blood banking practices. Disease symptoms (fever, chills, productive or non-productive cough, dyspnea, fatigue, myalgias, headache, runny nose, sore throat, nausea, vomiting, diarrhea, abdominal discomfort, loss of smell or taste, and other), disease severity, hospitalization requirement, and hospitalization course were assessed for each donor. A severity score was assigned as follows: 0 = asymptomatic; 1 = mild disease without dyspnea; 2 = moderate disease with dyspnea that did not require hospitalization; 3 = moderate disease with dyspnea that required hospitalization; 4 = severe disease that required supplemental oxygen; 5 = critical disease that required intensive care unit admission and/or intubation/mechanical ventilation. An aliquot of convalescent plasma product was used for virus microneutralization assays.

Asymptomatic Donors and VN titers

Samples from asymptomatic individuals were obtained from volunteers screened through an IRB-approved community surveillance protocol (22). Analysis of 2,814 asymptomatic adults

found that 73 (2.5%) had an anti-ECD of ≥1:50. These were analyzed for anti-RBD and VN titer (Supplemental Table 1).

Specimens from SARS-CoV-2 Naïve Donors

Ten naïve human plasma specimens (negative controls) were obtained from samples biobanked in Houston well before SARS-CoV-2 was described in China, the United States, or elsewhere.

RT-PCR Testing for SARS-CoV-2 Infection

Symptomatic patients with a high degree of suspicion for COVID-19 disease were tested in the Molecular Diagnostics Laboratory at Houston Methodist Hospital using an assay filed for under Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration (33). The assay follows the protocol published by the World Health Organization (35) and uses a 7500 Fast Dx instrument (Applied Biosystems) and 7500 SDS software (Applied Biosystems). Testing was performed on nasopharyngeal or oropharyngeal swabs immersed in universal transport media (UTM), bronchoalveolar lavage fluid, or sputum treated with dithiothreitol (DTT).

SARS-CoV-2 ELISAs

Detailed ELISA methods have been recently described (36). The ELISA used to measure anti-spike IgG antibodies in donor serum specimens was performed as follows. Briefly, ECD purified recombinant protein used comprises amino acid residues 1-1208, and the RBD comprises amino acids 319-591 of SARS-CoV-2 spike protein (GenBank MN908947). Microtiter plates were coated with either purified recombinant SARS-CoV-2 ECD or RBD. Human mAb CR3022 that targets the RBD of SARS-CoV (37) was used as a positive control. Negative serum control was included on each microtiter plate. Serial dilutions of serum were added, incubated for 1 h, washed, incubated with goat anti-human IgG Fab horseradish peroxidase (Sigma A0293), and

washed. ELISA substrate (1-step Ultra TMB, Thermo Scientific cat# 34028) was added, the plates were developed until the top dilution reached the saturation point, and the reaction was stopped with H_2SO_4. Plates were read at an absorbance of 450 nm.

A similar ELISA was used to study anti-spike ECD antibody titers in serum obtained from surveilled asymptomatic individuals. Recombinant proteins were produced as described above. All samples were tested with an initial screen assay and IgG antibody titers were subsequently performed on positive samples. For the screening assay, patient serum samples and negative control samples were diluted 1:50 in PBS containing 2% nonfat milk prior to addition to the plate. Patient sera that were identified as positive by the screening assay were subsequently titered by 1:3 serial dilutions in PBS-M to create 1:50, 1:150, 1:450, 1:1350, and 1:4050 final dilutions. Titer was defined as the last dilution showing an optical density greater than average negative control plus three standard deviations.

SARS-CoV-2 Microneutralization Assay (VN)

The ability of plasma samples to neutralize SARS-CoV-2 host-cell infection was determined with a traditional VN assay using SARS-CoV-2 strain USA-WA1/2020 (NR-52281-BEI resources), as previously described for SARS-CoV (17). The assay was performed in triplicate, and a series of eight two-fold serial dilutions of the plasma or serum were assessed. Briefly, 100 tissue culture infective dose 50 ($TCID_{50}$) units of SARS-CoV-2 was added to two-fold dilutions of heat inactivated serum or plasma, and incubated for 1 h at 37°C. The virus and plasma mixture was added to Vero E6 cells (ATCC CRL-1586) grown in a 96-well microtiter plate, incubated for 3 d, after which the host cells were treated for 1 h with crystal violet-formaldehyde stain (0.013% crystal violet, 2.5% ethanol, and 10% formaldehyde in 0.01 M PBS). The endpoint of the microneutralization assay was designated as the highest plasma dilution at which all three, or two of three, wells are not protected from virus infection, as assessed by visual examination.

SARS-CoV-2 Microneutralization Assay Two

A second SARS-CoV-2 microneutralization assay (VN2) was adapted from an assay used to study Ebola virus (38). This assay also used SARS-CoV-2 strain WA1. Plasma specimens were heat inactivated in a 56°C waterbath for 30 min to inactivate complement. Heat inactivated plasma specimens were diluted 1:10 in cell culture media (MEM; Corning 10-010, Corning, New York) containing 2% FBS (GE Healthcare Hyclone, Chicago, Illinois), and 3-log dilutions were performed in duplicate. Plasma from naïve and SARS-CoV-2 convalescent individuals was used as a negative and positive control, respectively. Diluted plasma was mixed with the SARS-CoV-2 WA1 strain, incubated at 37°C for 1 h, then added to Vero E6 cells at a target MOI of 0.4. Unbound virus was removed after 1 h incubation at 37°C, and cells were washed once in Dulbecco's Phosphate Buffered Saline without calcium and magnesium (DPBS, Sigma, St. Louis, Minnesota) and culture media (MEM + 5% FBS + 1% Penicillin-Streptomycin) (Gibco ThermoFisher Scientific 15140122, Gaithersburg, MD) was added. Cells were fixed 24 h post-infection, washed three times with DPBS, permeabilized with 1% Triton X-100 (Biorad, Hercules, California), and blocked with Cell Staining Buffer (Biolegend, San Diego, California). The number of infected cells was determined using SARS-CoV-nucleocapsid (N)-specific mAb (Sino Biological 401430-R001, Beijing, China) and goat anti-mouse cross-adsorbed IgG (H&L) Alexa Fluor 488 fluorescently labeled secondary antibody (Catalog #A-11001, Invitrogen ThermoFisher Scientific, Carlsbad, California) and NucBlue™ Live ReadyProbes™ Reagent (Hoechst 33342) (Invitrogen ThermoFisher Scientific, Carlsbad, California). The percent of infected cells was determined with an Operetta high content imaging system (PerkinElmer) and Harmonia software (39). Percent neutralization for each plasma sample at each dilution was determined relative to untreated, virus-only control wells.

SARS-CoV-2 Genome Sequencing and Analysis, and Clade Assignment

Libraries for whole virus genome sequencing were prepared according to version 1 or 3 of the ARTIC nCoV-2019 sequencing protocol (40). Long reads were generated with the LSK-109 sequencing kit, 24 native barcodes (NBD104 and NBD114 kits), and a GridION instrument (Oxford Nanopore). Short reads were generated with the NexteraXT kit and a MiSeq or NextSeq 550 instrument (Illumina). Whole genome alignments of consensus virus genome sequence generated from the ARTIC nCoV-2019 bioinformatics pipeline were trimmed to the start of orf1ab and the end of orf10 and used to generate a phylogenetic tree using RAxML (https://cme.h-its.org/exelixis/web/software/raxml/index.html). Trees were visualized and annotated with CLC Genomics Workbench v20 (Qiagen). SARS-CoV-2 clade assignment was based on procedures described elsewhere (41).

Statistics

To assess the correlation between VNs, anti-RBD, and anti-ECD ELISA titer data, pairwise Pearson correlations were performed using the entire dataset (i.e., individuals with single and repeated measurements) using the *psych* package in R, and a scatter plot of matrices, bivariate scatter plots, histograms, and the Pearson correlation determined with the *pairs.panels* function. To identify the prevalence of donors with VN titers ≥1:160, the frequency distribution of these cases by titer classes critical for RBD, ECD, and VN2 was quantified. Generalized Liner model (GLM), using the first plasma donation data only, was performed between the same variables, as a response, and each of the following predictor factors: dyspnea (yes, no), disease severity (five classes as described above), hospitalization (yes, no) gender (male, female), and age combined into five age groups (≤30, 31-40, 41-50, 51-60 and >60). For variables with more than two factors, a post-hoc t-test (with Bonferroni correction) was used to identify significant pairwise differences. A linear mixed effect (LME) model was used to analyze the relationship between VNs, anti-RBD, and anti-ECD protein titers, as responses, and days since symptoms,

as numerical predictors. Here, we used the whole data set and included the individual's ID as the random factor, to consider multiple sampling. A similar analysis was used for duration of symptoms but using GLM and selecting only the cases at the first visit. Analyses were performed using log2-trasformed numeric data and the R statistical computing platform (42).

Study Approval

Convalescent plasma was obtained and processed by standard blood banking protocols under Houston Methodist human subjects protocol PRO00025121. FDA recommendations for COVID-19 convalescent plasma donor collection were followed (7). All donors provided written informed consent. Studies were conducted with the approval of the Houston Methodist Research Institute ethics review board, and with informed patient or legally-authorized representative consent when applicable.

Author Contributions

Project concept (ES, VK, JM); acquired data (ES, SK, PC, TE, XY, PZ, ZJ, SL, RO, JC, BC, DT, JL, JDG, JC, GI, RN, IB, DG, RR, SS, IP, IC, NJ, LP, KH, AH, JD, VK); analyzed data (VK, SK, IC, JM, ES, PC); wrote manuscript (ES, VK, JM); prepared figures (ES, SK, IC, VK); provided scholarly advice (CL, PH, DB). All authors revised the manuscript and gave final approval for publication.

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Figure legends

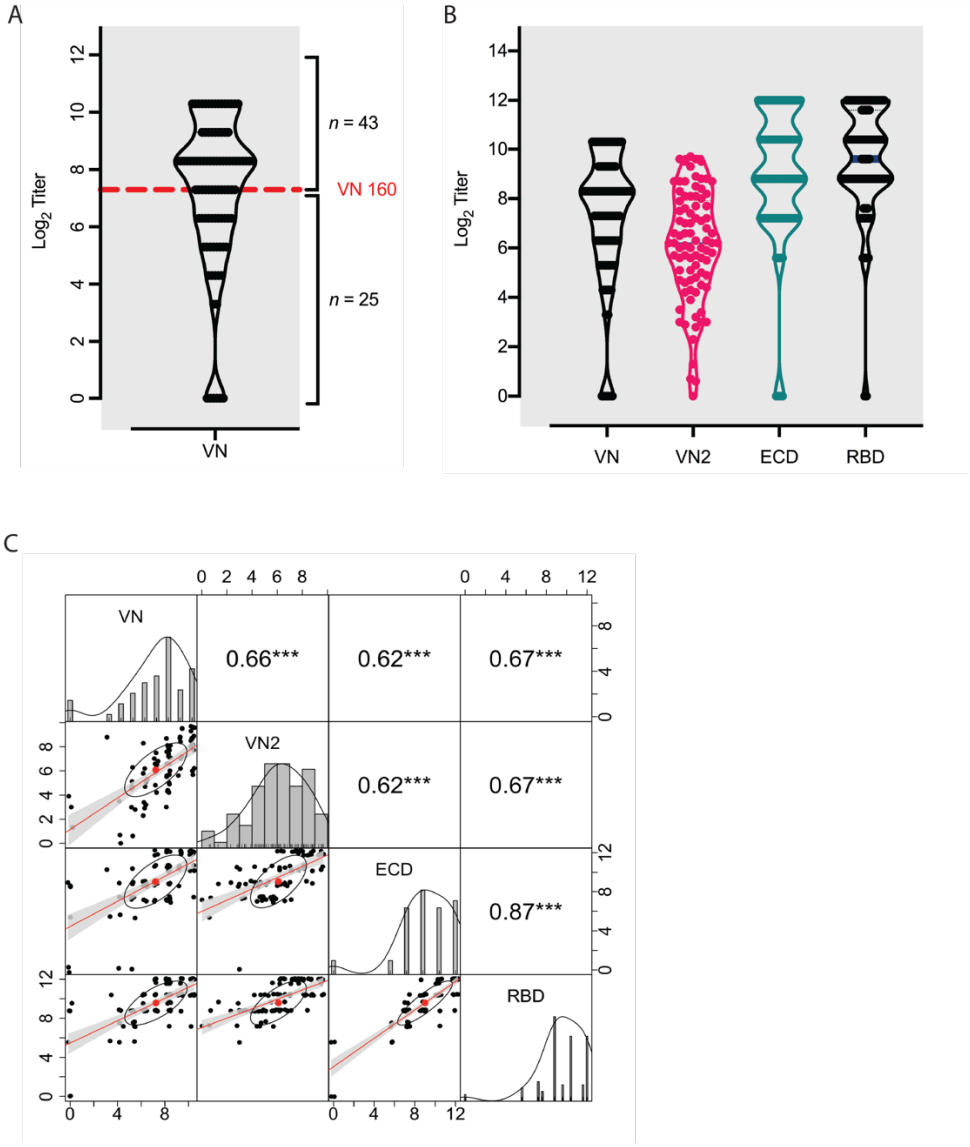


Figure 1. Patterns of VN and ELISA titers. A) Violin plot of distribution of VN titers at initial donation. Number of donor cases (*n*=68) above and below the VN 160 cut off value are reported. B) Violin plots showing similar patterns of distribution of titers at initial donation for the two VN assays, together with the reciprocal ELISA IgG titers for plasma anti-ECD protein (ECD) and anti-RBD IgG (RBD). C) Pair-wise Pearson correlations showing the correlation coefficient (r) and related significant value (***=p<0.001) above the diagonal, and the bivariate scatterplots

(jittered points) with linear regression fit (red line), confidence intervals (grey shading), correlation value (red point) and correlation ellipse (black ellipse) below the diagonal. The density plot (black line) and histogram of each variable is reported along the diagonal. Data are presented in log2-scale of reciprocal titers for VN, anti-ECD IgG and anti-RBD IgG, and in IC50 units for VN2. The sample sizes for which the correlation coefficients were derived are: VN-VN2= 86; VN-ECD=91; VN-RBD=91; VN2-ECD=84; VN2-RBD= 84; ECD-RBD=91.

A

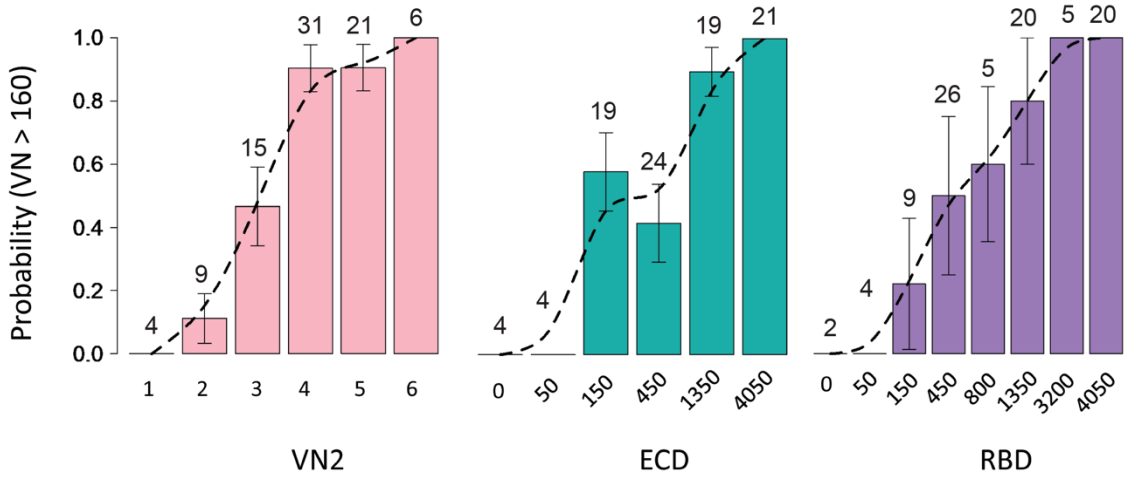


Figure 2. Bar plots reporting the prevalence of donors with VN>160 for VN2, ECD or RBD. Probabilities of VN160 were plotted for six range classes, with an interclass interval of 1.8 log2 IC50 values (class 1 - <2; class 2 - 2,12; class 3 -12,42; class 4 - 42-147; class 5 - 147,512; class 6 - >512) or observed classes for ECD (*n*=6) and RBD (*n*=8) reciprocal ELISA titers. A spline curve (dotted line, smoothness shape=1) has been fitted to the probability values and standard errors (bars) are reported. Numbers of donor samples are shown above the bars.

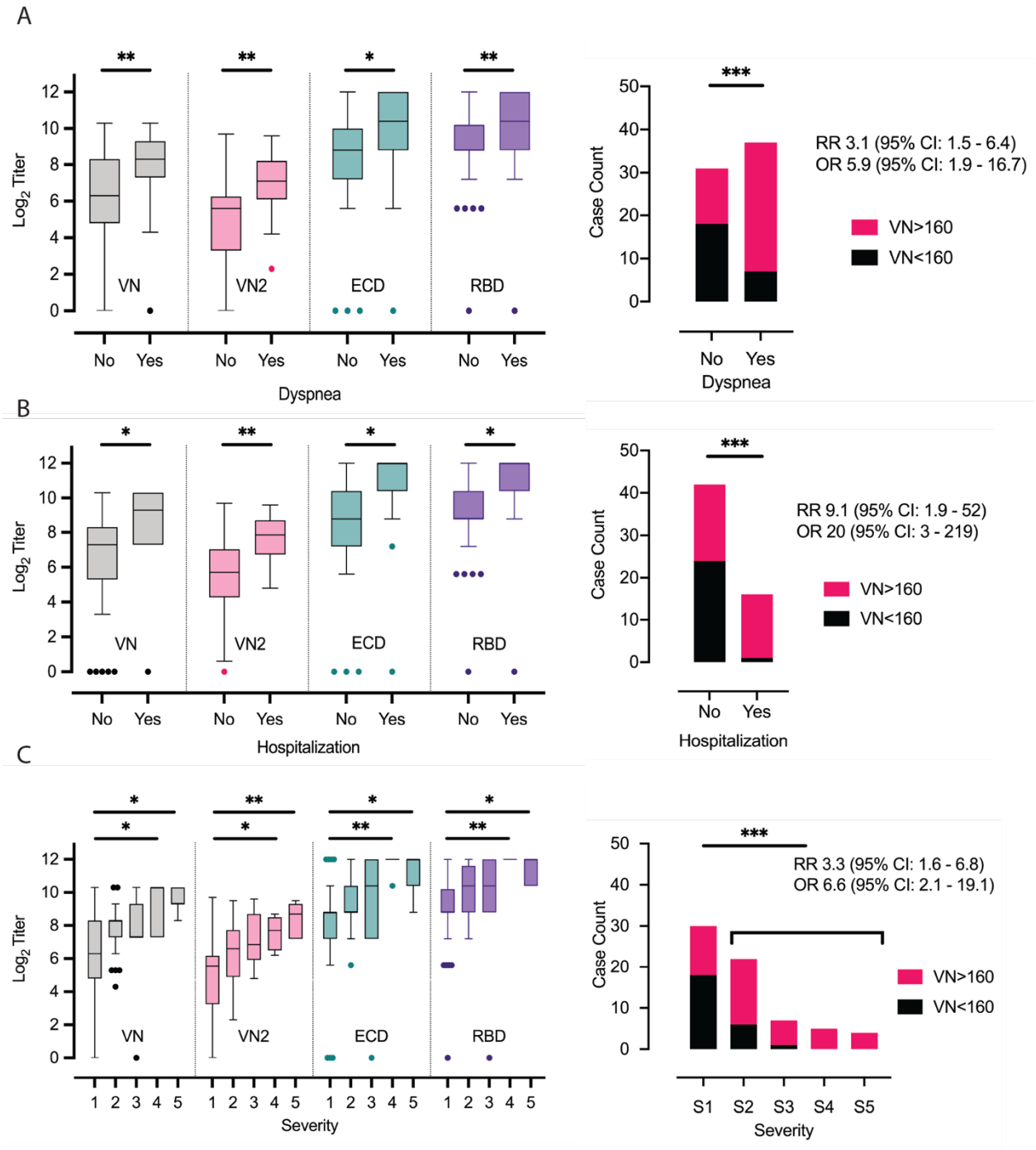
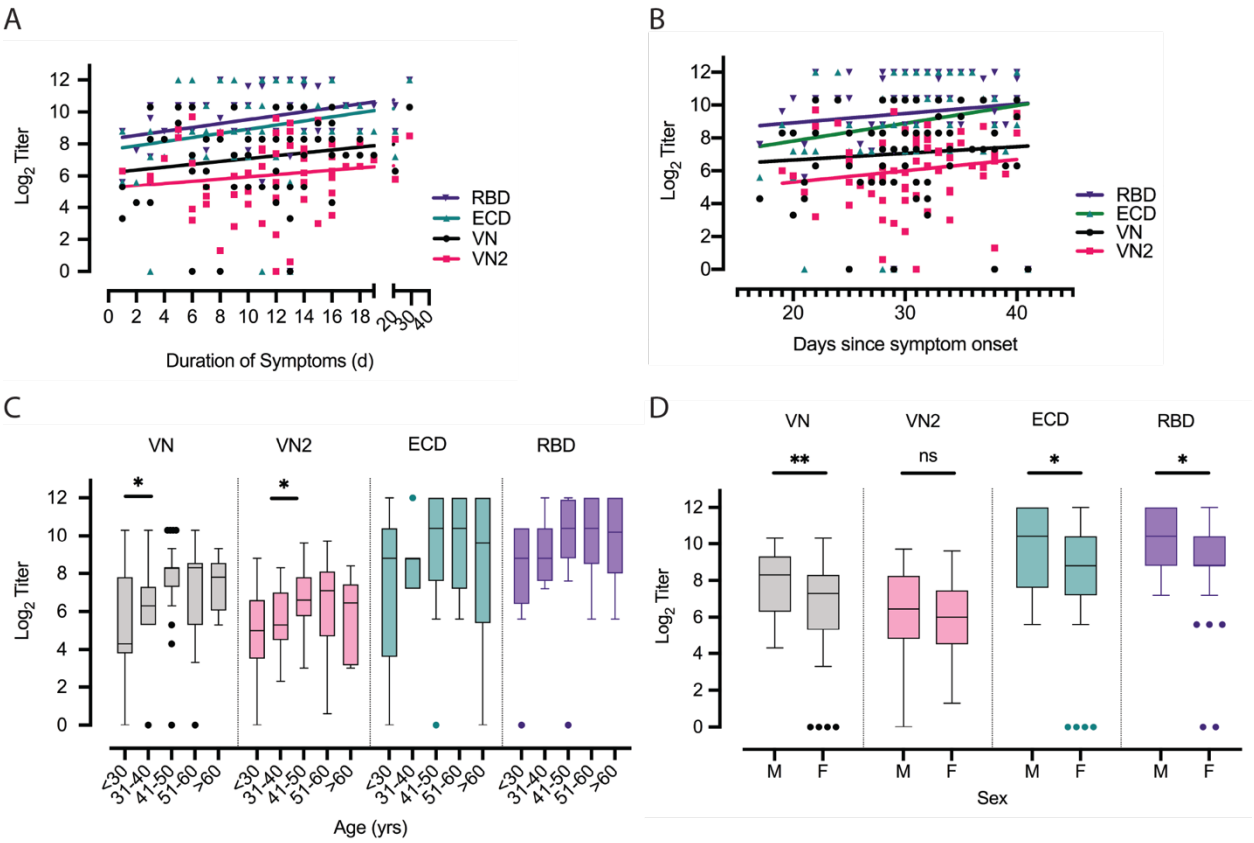
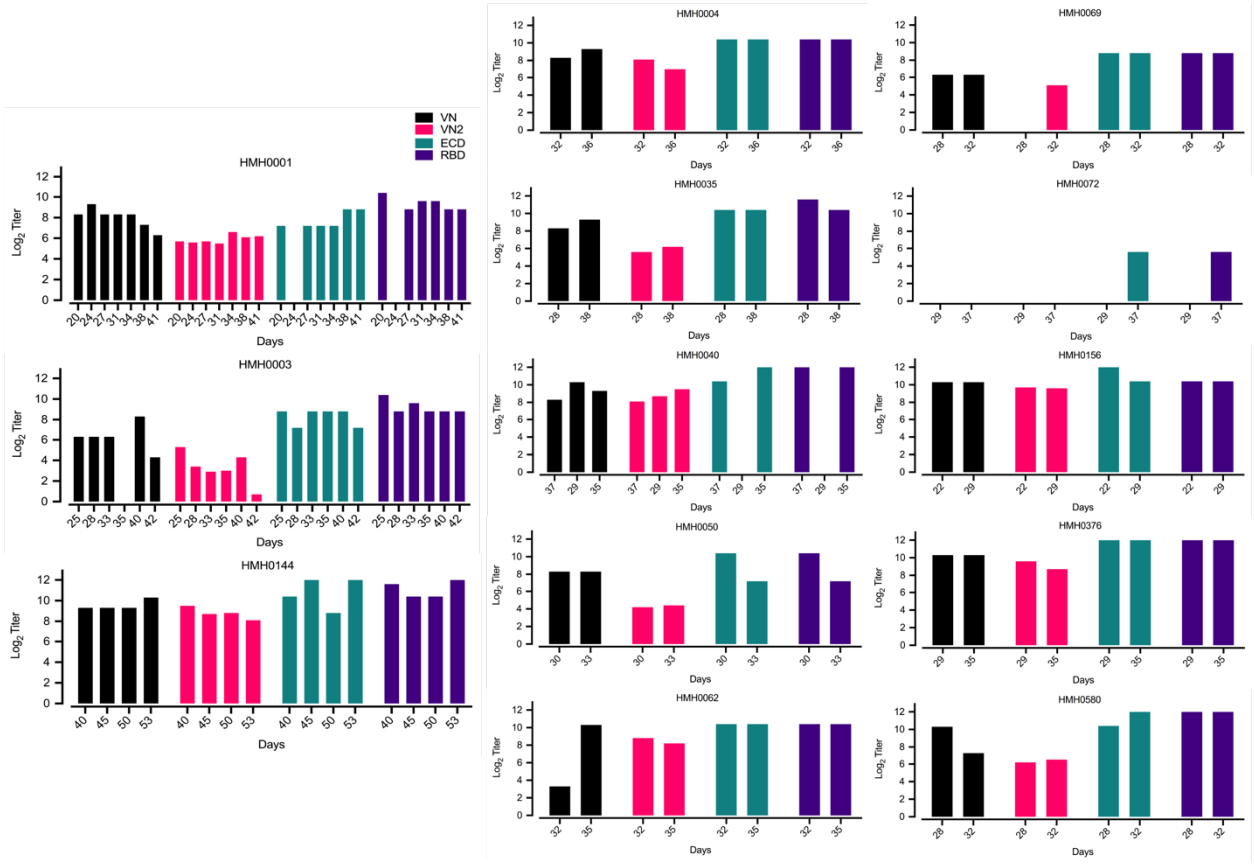


Figure 3. Boxplots of VN, VN2, anti-ECD and anti RBD titers by A) dyspnea, B) hospitalization and C) disease severity (1= low severity, 5 high severity) at initial plasma donation from the 68 individual donors. The median, minimum, maximum, first and third quartile and extreme values are reported. Barplots showing case counts of donors above and below the VN 160 threshold stratified by whether they self-reported A) occurrence of dyspnea during symptomatic phase of

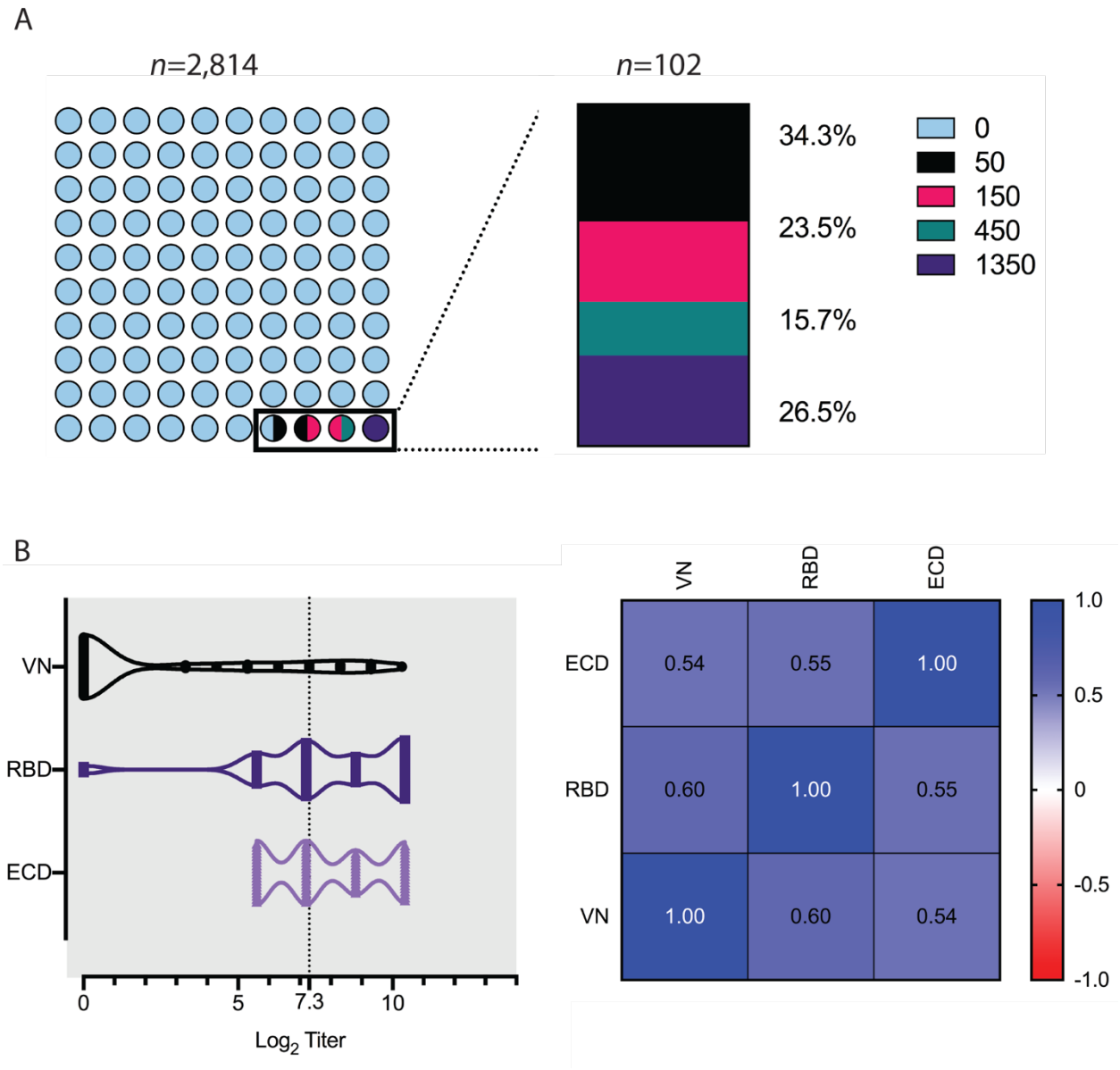
disease; B) hospitalization; and, C) disease severity. Pairwise t-test (significant comparisons (*=$p<0.05$, **= $p<0.01$, ***= $p<0.001$), odds ratio (OR) and relative risk (RR) with confidence intervals (CI) are also reported.



Supplemental Figure 1. Relationships between VN titer, VN2 titer, anti-ECD IgG titer, and anti-RBD titer (n=68) by A) Duration of symptoms and B) Days since symptom onset. A linear regression (line) is fitted to each set of data. C) Boxplots of VN, ECD, and RBD by donor age, or D) sex. The median, minimum, maximum, first and third quartile and extreme values including pairwise significant comparisons (*=p<0.05, **=p<0.01) are reported.



Supplemental Figure 2. Trends in VN, VN2, anti-ECD IgG and anti-RBD IgG titers for donors (n=68) with multiple consecutive donations. VN, ECD, and RBD are reported as log 2 of reciprocal titers whereas VN2 is represented by the log2 of IC50 value. HMH number refers to arbitrary number assigned to each plasma donor.



Supplemental Figure 3. Asymptomatic surveillance sample. A. Counts of ECD titer classes in surveillance screen, left. Percent of individuals in each class are shown, right. B. Boxplots of VN (VNT1), anti-ECD and anti-RBD titers in a subset of samples (*n*=73). C. Pair-wise Pearson correlations showing the correlation coefficient between VN (VNT1), anti-ECD, and anti-RBD titers with P<0.001 for all R values.

Table 1: Demographics and Characteristics of Convalescent Plasma Donors and Samples for Samples 1-49.

Sample #	Subject ID	Gender	Age	Severity	Hospitalization	LOS (Days)	Symptom Duration (Days)	Days Post-symptom Onset	Days Post-symptom Resolution	Visit #	ECD (Titer)	RBD (Titer)	VN (Titer)	VN2 (IC50)	Clade
1	HMH0001	M	44	1	NO	N/A	3	20	17	1	150	1350	320	53.2	A2a
2	HMH0001	M	44	1	NO	N/A	3	24	21	2	N/A	N/A	640	48.6	A2a
3	HMH0001	M	44	1	NO	N/A	3	27	24	3	150	450	320	53.3	A2a
4	HMH0001	M	44	1	NO	N/A	3	31	28	4	150	800	320	46.8	A2a
5	HMH0001	M	44	1	NO	N/A	3	34	31	5	150	800	320	96.3	A2a
6	HMH0001	M	44	1	NO	N/A	3	38	35	6	450	450	160	69.4	A2a
7	HMH0001	M	44	1	NO	N/A	3	41	38	7	450	450	80	71.5	A2a
8	HMH0002	M	54	1	NO	N/A	13	28	15	1	50	150	40	1.5	A2a
9	HMH0003	M	36	1	NO	N/A	7	25	18	1	450	1350	80	38.4	B
10	HMH0003	M	36	1	NO	N/A	7	28	21	2	150	450	80	10.4	B
11	HMH0003	M	36	1	NO	N/A	7	33	26	3	450	800	80	7.6	B
12	HMH0003	M	36	1	NO	N/A	7	35	28	4	450	450	0	8	B
13	HMH0003	M	36	1	NO	N/A	7	40	33	5	450	450	320	19.8	B
14	HMH0003	M	36	1	NO	N/A	7	42	35	6	150	450	20	1.6	B
15	HMH0004	F	54	2	NO	N/A	18	32	14	1	1350	1350	320	270.7	N/A
16	HMH0004	F	54	2	NO	N/A	18	36	18	2	1350	1350	640	128.5	N/A
17	HMH0009	F	38	2	NO	N/A	12	30	18	1	450	450	80	4.8	N/A
18	HMH0011	F	67	1	NO	N/A	11	28	17	1	0	50	40	8	A2a
19	HMH0012	F	46	1	NO	N/A	16	30	14	1	150	450	320	30	N/A
20	HMH0013	F	43	1	NO	N/A	11	28	17	1	1350	3200	320	214.2	A2a
21	HMH0016	F	47	1	NO	N/A	13	32	19	1	4050	4050	320	234.2	A2a
22	HMH0020	F	41	2	NO	N/A	2	17	15	1	50	200	20	N/A	N/A
23	HMH0028	M	23	1	NO	N/A	12	31	19	1	150	150	20	0.6	A2a
24	HMH0029	F	66	1	NO	N/A	6	22	16	1	150	450	80	9.3	A2a
25	HMH0032	M	65	2	NO	N/A	11	25	14	1	450	4050	320	94.8	N/A
26	HMH0035	M	50	2	NO	N/A	14	28	14	1	1350	3200	320	47.3	B
27	HMH0035	M	50	2	NO	N/A	14	38	24	2	1350	1350	640	72	B
28	HMH0040	M	52	2	NO	N/A	12	29	17	1	N/A	N/A	1280	417	A2a
29	HMH0040	M	52	2	NO	N/A	12	35	23	2	4050	4050	640	724.5	A2a
30	HMH0040	M	52	2	NO	N/A	12	37	25	3	1350	4050	320	274	A2a
31	HMH0045	F	23	1	NO	N/A	9	33	24	1	4050	1350	1280	63.8	A2a
32	HMH0049	F	57	1	NO	N/A	12	27	15	1	150	450	320	47	N/A

33	HMH0050	M	41	2	NO	N/A	7	30	23	1	1350	1350	320	18.9	N/A
34	HMH0050	M	41	2	NO	N/A	7	33	26	2	150	150	320	21.4	N/A
35	HMH0051	F	50	1	NO	N/A	16	30	14	1	150	450	160	58.4	N/A
36	HMH0052	F	27	3	YES	2	17	31	14	1	1350	1350	160	100.1	A2a
37	HMH0053	M	29	2	NO	N/A	10	28	18	1	450	450	160	18.9	N/A
38	HMH0055	M	61	3	YES	3	14	33	19	1	1350	3200	320	134.1	B
39	HMH0057	F	44	2	NO	N/A	6	34	28	1	450	450	160	111.5	A2a
40	HMH0062	F	24	1	NO	N/A	13	32	19	1	1350	1350	10	443.9	A2a
41	HMH0062	F	24	1	NO	N/A	13	35	22	2	1350	1350	1280	288.8	A2a
42	HMH0069	F	49	1	NO	N/A	6	28	22	1	450	450	80	NA	A2a
43	HMH0069	F	49	1	NO	N/A	6	32	26	2	450	450	80	35.3	A2a
44	HMH0070	F	37	2	NO	N/A	19	38	19	1	450	1350	160	126.7	A2a
45	HMH0072	F	23	1	NO	N/A	13	29	16	1	0	0	0	N/A	N/A
46	HMH0072	F	23	1	NO	N/A	13	37	24	2	50	50	0	N/A	N/A
47	HMH0088	F	29	1	NO	N/A	3	21	18	1	0	50	20	N/A	A2a
48	HMH0089	F	42	2	NO	N/A	18	38	20	1	450	450	160	95	A2a
49	HMH0090	M	33	1	NO	N/A	3	37	34	1	150	150	1280	51.3	A2a

Abbreviations: F, Female; M, Male; LOS, Length of Stay; ECD, anti-spike ectodomain; RBD, Receptor Binding Domain; VN, Virus Neutralization

Table 2: Demographics and Characteristics of Convalescent Plasma Donors and Samples for Samples 50-93.

Sample #	Subject ID	Gender	Age	Severity	Hospitalization	LOS (Days)	Symptom Duration (Days)	Days Post-symptom Onset	Days Post-symptom Resolution	Visit #	ECD (Titer)	RBD (Titer)	VN (Titer)	VN2 (IC50)	Clade
50	HMH0099	F	54	1	NO	N/A	1	20	19	1	50	50	10	N/A	N/A
51	HMH0112	F	47	1	NO	N/A	1	32	31	1	450	450	40	78.9	A2a
52	HMH0113	F	52	1	NO	N/A	9	29	20	1	1350	150	40	6.9	N/A
53	HMH0116	M	27	1	NO	N/A	16	32	16	1	450	450	20	11.3	N/A
54	HMH0117	F	27	2	NO	N/A	10	30	20	1	1350	1350	320	32.7	N/A
55	HMH0118	F	50	1	NO	N/A	15	34	19	1	1350	450	320	8	N/A
56	HMH0119	F	35	1	NO	N/A	6	25	19	1	450	450	0	47.3	N/A
57	HMH0120	F	41	1	NO	N/A	3	19	16	1	450	800	320	62.3	N/A
58	HMH0121	F	51	1	NO	N/A	7	21	14	1	150	200	40	25.4	N/A
59	HMH0133	M	51	2	NO	N/A	4	25	21	1	150	450	320	138.8	N/A
60	HMH0135	F	47	2	NO	N/A	12	32	20	1	4050	4050	320	174.4	N/A
61	HMH0137	F	53	1	NO	N/A	8	38	30	1	450	800	0	2.5	N/A
62	HMH0143	F	49	2	NO	N/A	10	37	27	1	1350	3200	160	72.4	N/A
63	HMH0144	M	48	5	YES	3	15	40	25	1	1350	3200	640	723	N/A
64	HMH0144	M	48	5	YES	3	15	45	30	2	4050	1350	640	423.1	N/A
65	HMH0144	M	48	5	YES	3	15	50	35	3	450	1350	640	453.8	N/A
66	HMH0144	M	48	5	YES	3	15	53	38	4	4050	4050	1280	278.3	N/A
67	HMH0156	M	59	1	NO	N/A	6	22	16	1	4050	1350	1280	814.8	N/A
68	HMH0156	M	59	1	NO	N/A	6	29	23	2	1350	1350	1280	751.7	N/A
69	HMH0158	M	33	2	NO	N/A	10	26	16	1	150	200	40	33.8	N/A
70	HMH0162	F	51	3	YES	3	9	34	25	1	150	450	160	28.2	N/A
71	HMH0229	M	32	2	NO	N/A	21	40	19	1	450	1350	80	311.3	N/A
72	HMH0234	M	40	2	NO	N/A	12	27	15	1	150	150	40	25	N/A
73	HMH0245	M	51	5	YES	4	14	38	24	1	1350	4050	320	145	N/A
74	HMH0249	M	56	1	NO	N/A	8	22	14	1	4050	4050	320	415.8	N/A
75	HMH0255	M	40	2	NO	N/A	14	31	17	1	450	450	40	22	N/A
76	HMH0260	M	44	2	NO	N/A	5	24	19	1	4050	1350	1280	468.5	N/A
77	HMH0262	F	36	4	YES	2	16	31	15	1	4050	4050	1280	262.8	A2a
78	HMH0265	F	53	2	NO	N/A	11	31	20	1	4050	4050	320	188	N/A
79	HMH0313	M	78	3	YES	1	16	36	20	1	4050	4050	160	77.8	N/A
80	HMH0363	M	56	1	NO	N/A	7	34	27	1	450	1350	80	26.4	N/A
81	HMH0368	F	37	2	NO	N/A	9	29	20	1	450	1350	160	65.4	N/A

82	HMH0369	M	41	1	NO	N/A	21	39	18	1	150	450	80	56.1	N/A
83	HMH0376	M	52	4	YES	7	14	28	14	1	1350	4050	1280	74.8	N/A
84	HMH0376	M	52	4	YES	7	14	32	18	2	4050	4050	160	90.7	N/A
85	HMH0430	M	44	4	YES	4	14	35	21	1	4050	4050	1280	207.4	N/A
86	HMH0576	F	50	3	YES	5	2	41	39	1	0	0	0	N/A	N/A
87	HMH0580	F	43	3	YES	4	12	29	17	1	4050	4050	1280	752.3	A2a
88	HMH0580	F	43	3	YES	4	12	35	23	2	1350	1350	1280	421.4	A2a
89	HMH0598	M	46	4	YES	6	29	30	1	1	4050	4050	1280	365.7	N/A
90	HMH0620	M	59	5	YES	6	13	40	27	1	4050	4050	1280	645.9	N/A
91	HMH0634	M	53	5	YES	13	16	33	17	1	4050	4050	640	148.1	B
92	HMH0699	M	61	3	YES	2	5	35	30	1	4050	450	640	343	N/A
93	HMH0879	M	50	4	YES	5	14	34	20	1	4050	4050	160	178.1	A2a

Abbreviations: F, Female; M, Male; LOS, Length of Stay; ECD, anti-spike ectodomain; RBD, Receptor Binding Domain; VN, Virus Neutralization

Supplemental Table 1: Demographics and Characteristics of Asymptomatic Surveillance Subjects Selected for Study.

Sample #	Subject ID	Sex	Age	S (Titer)	RBD (Titer)	VNT1 (Titer)
94	HMH_Surv_01	F	35	150	1350	1280
95	HMH_Surv_02	F	37	1350	1350	640
96	HMH_Surv_03	F	42	1350	1350	640
97	HMH_Surv_04	F	23	1350	1350	640
98	HMH_Surv_05	F	38	1350	1350	640
99	HMH_Surv_06	F	22	1350	1350	640
100	HMH_Surv_07	M	50	450	1350	320
101	HMH_Surv_08	M	45	1350	1350	320
102	HMH_Surv_09	F	33	1350	1350	320
103	HMH_Surv_10	F	29	1350	1350	320
104	HMH_Surv_11	M	20	1350	1350	160
105	HMH_Surv_12	M	34	1350	1350	160
106	HMH_Surv_13	F	37	50	1350	160
107	HMH_Surv_14	F	38	1350	1350	160
108	HMH_Surv_15	M	32	150	1350	80
109	HMH_Surv_16	F	26	1350	1350	80
110	HMH_Surv_17	F	32	1350	1350	40
111	HMH_Surv_18	F	29	1350	1350	40
112	HMH_Surv_19	F	25	450	450	40
113	HMH_Surv_20	F	40	50	150	40
114	HMH_Surv_21	F	30	150	150	20
115	HMH_Surv_22	F	45	150	150	10
116	HMH_Surv_23	M	38	450	150	10
117	HMH_Surv_24	F	45	450	150	10
118	HMH_Surv_25	F	35	50	1350	0
119	HMH_Surv_26	F	42	1350	1350	0
120	HMH_Surv_27	F	52	50	1350	0
121	HMH_Surv_28	F	45	450	1350	0
122	HMH_Surv_29	F	59	1350	1350	0
123	HMH_Surv_30	F	40	150	1350	0
124	HMH_Surv_31	F	33	150	450	0
125	HMH_Surv_32	F	20	150	450	0
126	HMH_Surv_33	M	39	450	450	0

127	HMH_Surv_34	F	26	150	450	0
128	HMH_Surv_35	F	26	450	450	0
129	HMH_Surv_36	F	32	450	450	0
130	HMH_Surv_37	F	69	150	450	0
131	HMH_Surv_38	F	43	450	450	0
132	HMH_Surv_39	M	46	450	450	0
133	HMH_Surv_40	F	31	450	450	0
134	HMH_Surv_41	F	30	50	150	0
135	HMH_Surv_42	M	43	50	150	0
136	HMH_Surv_43	F	28	50	150	0
137	HMH_Surv_44	M	31	50	150	0
138	HMH_Surv_45	F	34	150	150	0
139	HMH_Surv_46	F	31	50	150	0
140	HMH_Surv_47	F	38	150	150	0
141	HMH_Surv_48	F	36	150	150	0
142	HMH_Surv_49	M	47	150	150	0
143	HMH_Surv_50	F	41	150	150	0
144	HMH_Surv_51	F	42	50	150	0
145	HMH_Surv_52	M	59	450	150	0
146	HMH_Surv_53	F	30	450	150	0
147	HMH_Surv_54	F	25	150	150	0
148	HMH_Surv_55	M	42	150	150	0
149	HMH_Surv_56	M	40	150	150	0
150	HMH_Surv_57	M	31	150	50	0
151	HMH_Surv_58	F	29	50	50	0
152	HMH_Surv_59	F	36	50	50	0
153	HMH_Surv_60	F	27	50	50	0
154	HMH_Surv_61	F	61	1350	50	0
155	HMH_Surv_62	M	28	1350	50	0
156	HMH_Surv_63	F	50	150	50	0
157	HMH_Surv_64	M	46	50	50	0
158	HMH_Surv_65	F	53	50	50	0
159	HMH_Surv_66	F	54	450	50	0
160	HMH_Surv_67	F	51	150	50	0
161	HMH_Surv_68	F	24	50	50	0
162	HMH_Surv_69	F	30	50	0	0

163	HMH_Surv_70	F	40	1350	150	NA
164	HMH_Surv_71	F	51	50	0	NA
165	HMH_Surv_72	F	27	50	0	NA
166	HMH_Surv_73	F	61	50	0	NA

Abbreviations: F, Female; M, Male; ECD, anti-spike ectodomain; RBD, Receptor Binding Domain; VNT1, Virus Neutralization Titer Assay One