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# Temporal Analysis of Serial Donations Reveals Decrease in Neutralizing Capacity and Justifies Revised Qualifying Criteria for Coronavirus Disease 2019 Convalescent Plasma

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**(See the Editorial Commentary by Bloch et al, on pages 740–2.)**

***Background.*** Coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) received an Emergency Use Authorization by the US Food and Drug Administration (FDA). CCP with a signal-to-cutoff ratio of ≥12 using the Ortho VITROS severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin G (IgG) test (OVSARS2IgG) is permitted to be labeled "high titer." Little is known about the relationship between OVSARS2IgG ratio and neutralizing capacity of plasma/sera against genuine SARS-CoV-2.

***Methods.*** Nine hundred eighty-one samples from 196 repeat CCP donors 0–119 days post–initial donation (DPID) were analyzed. Neutralizing capacity was assessed for 50% ($PRNT_{50}$) and 90% ($PRNT_{90}$) reduction of infectious virus using the gold standard plaque reduction neutralization test (PRNT). A subset of 91 donations was evaluated by OVSARS2IgG and compared to PRNT titers for diagnostic accuracy.

***Results.*** Of donations, 32.7%/79.5% ($PRNT_{90}$/$PRNT_{50}$) met a 1:80 titer initially but only 14.0%/48.8% ($PRNT_{90}$/$PRNT_{50}$) met this cutoff ≥85 DPID. Correlation of OVSARS2IgG results to neutralizing capacity allowed extrapolation to CCP therapy results. CCP with OVSARS2IgG ratios equivalent to a therapeutically beneficial group had neutralizing titers of ≥1:640 ($PRNT_{50}$) and/or ≥1:80 ($PRNT_{90}$). Specificity and positive predictive value of the OVSARS2IgG for qualifying highly neutralizing CCP was optimal using ratios significantly greater than the FDA cutoff.

***Conclusions.*** This information provides a basis for refining the recommended properties of CCP used to treat COVID-19.

**Keywords.** COVID-19; convalescent plasma; neutralizing antibody.

The emergence of coronavirus disease 2019 (COVID-19) disease, caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has precipitated a global public health crisis. Treatment of COVID-19 primarily consists of supportive care, although several experimental therapies are being tested in clinical trials. Convalescent plasma has long been used as a therapeutic for viral infections when other effective drugs or therapies are absent and has been used to treat severe acute respiratory syndrome coronavirus infections [1, 2]. Through the Expanded Access Program (EAP), 92 283 patients in the United States (US) were transfused with COVID-19 convalescent plasma (CCP) before the US Food and Drug Administration (FDA) authorized emergency use [3]. The efficacy of CCP has been difficult to establish, but it is thought that CCP has an acceptable safety profile and may provide therapeutic benefit [4–7].

Protective correlates of immunity have not been definitively established for COVID-19, but vaccine trials in nonhuman primates have shown correlation between neutralizing antibody response and protection from SARS-CoV-2 challenge [8–10]. The potential therapeutic benefit of CCP is thought to be primarily dependent on the ability of antibodies present in the plasma to neutralize SARS-CoV-2 and block infection, although other mechanisms of therapeutic benefit are possible. Neutralizing antibodies from recovered COVID-19 patients compete with angiotensin-converting enzyme 2 for binding of receptor binding domain (RBD) on the trimeric spike protein of SARS-CoV-2 [11]. Several serological assays have been developed to measure the binding of antibodies in sera/plasma to a variety of SARS-CoV-2 spike antigens, including RBD, the S1 subunit of spike, and trimeric conformations of the spike ectodomain [12, 13]. An absolute neutralizing titer (NT) associated with therapeutically beneficial CCP has been difficult to

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define, as many larger published studies characterized none or few of the CCP units for NT with live SARS-CoV-2 virus [4, 6, 10]. Instead, serological assays that measure the titer of anti-RBD or anti-spike antibodies have served as surrogates, and a lack of widely available standardized reagents and control sera make direct comparison across laboratories difficult. Several different titers have been recommended after comparison of customized enzyme-linked immunosorbent assays to varied microneutralization assays that use SARS-CoV-2, but the relationship between these assays and the neutralizing capacity of sera/plasma as measured by the gold-standard plaque reduction neutralization test (PRNT) is not always clear [12–14]. This point becomes particularly crucial when selecting CCP with the highest neutralizing capacity and/or avoiding the transfusion of units of CCP with insufficient neutralizing capacity [15]. Recently, the FDA declared that units of CCP can be qualified as high titer if they have a signal-to-cutoff ratio of ≥12 using the Ortho VITROS SARS-CoV-2 immunoglobulin G (IgG) test (OVSARS2IgG) [16]. Like other surrogate neutralization assays, the OVSARS2IgG measures antibody binding to the SARS-CoV-2 S1 antigen. A single report has shown concordance between OVSARS2IgG ratios and pseudovirus neutralization, but this test has not been compared to PRNT using genuine SARS-CoV-2 [17].

Symptom severity has been reported to positively correlate with neutralizing capacity in convalescent COVID-19 patients, as do male sex and age [18, 19]. It has been suggested that these criteria can be used to select the CCP donors most likely to contribute CCP with high neutralizing capacity. However, a specific optimal window post–symptom resolution for CCP collection has not been defined [18]. There is debate about the stability of neutralizing antibody responses in recovered COVID-19 patients, with conflicting results from different studies. Prior studies lacked serial specimens from the same individual, completed analyses on a small number of serial specimens within a limited time frame, or did not assess neutralizing capacity of paired specimens [14, 19–21]. There is great interest in understanding the durability of neutralizing antibody responses in convalescent COVID-19 patients, as there is concern that rapid decay in neutralizing capacity could result in susceptibility to reinfection with SARS-CoV-2. Additionally, significant decreases in the neutralizing antibody capacity of convalescent COVID-19 patients would render time post–symptom resolution a critical factor in CCP donor selection.

The goals of this study are to characterize the neutralizing capacity of serial donations of CCP using PRNT with genuine SARS-CoV-2, to evaluate the CCP-qualifying criteria of the OVSARS2IgG test at different levels of neutralizing capacity, and to correlate PRNT titer and OVSARS2IgG ratios to data on the therapeutic efficacy of CCP compiled by the Mayo Clinic/US Expanded Access Program COVID-19 Plasma Consortium (US-EAP-CPC).

## MATERIALS AND METHODS

### Study Specimens

Specimens were collected from individuals who met all FDA donor eligibility requirements (21 Code of Federal Regulations [CFR] 630.10 and 21 CFR 630.15) and qualifications in accordance with the guidelines of the New York Blood Center (NYBC). Donors were required to present documentation of a positive SARS-CoV-2 polymerase chain reaction diagnostic test or positive serologic test after recovery and to have been symptom free for at least 2 weeks. Donors contributed at will CCP for use as a therapeutic and sera for evaluation of antibody levels, with a minimum of 7 days between serial contributions. Specimens were stored at –20°C until tested. Testing of sera at the Wadsworth Center was done under protocol 20-021 with approval from the New York State Department of Health Institutional Review Board.

### PRNT Analysis

Participants were selected at random from a de-identified list of residual clinical specimens submitted for anti–SARS-CoV-2 antibody testing at the Wadsworth Center Laboratory. Groups of donors with varying intervals between initial donation and final donation were retrospectively selected for the study: 14–35 days (n = 45), 36–60 days (n = 27), 61–75 days (n = 55), and >75 days (n = 69). Sample selection and testing was blinded to clinical data including age, sex, and SARS-CoV-2 antibody test results from the Wadsworth Center clinical assay. Once the study was complete, a retrospective analysis of patient demographics (age and sex) revealed that the study cohort of 196 individuals was similar to the repeat donor pool, although the repeat donor pool studied here had a slightly lower proportion of female participants (Supplementary Figure 1).

PRNT analysis was conducted by mixing 100 µL of 200 plaque-forming units of SARS-CoV-2, isolate USA-WA1/2020 (BEI Resources, NR181 52281) with 100 µL of 2-fold serially diluted test sera and incubating at 37°C in 5% carbon dioxide ($CO_2$) for 1 hour. Confluent Vero E6 cells (C1008, ATCC CRL-1586) seeded in 6-well plates were inoculated with 100 µL of the virus:serum mixture and adsorption proceeded for 1 hour at 37°C in 5% $CO_2$. A 0.6% agar overlay prepared in maintenance medium (Eagle's minimal essential medium, 2% heat-inactivated fetal bovine serum, 100 µg/mL penicillin G, 100 U/mL streptomycin) was added after adsorption and the assay was incubated at 37°C in 5% $CO_2$. A second agar overlay with 0.2% Neutral red added was added 2 days postinfection. After an additional day of incubation, the number of plaques in each well were recorded. The titer was reported as the inverse of the highest dilutions of sera providing 50% ($PRNT_{50}$) or 90% ($PRNT_{90}$) viral plaque reduction relative to virus-only infection. Normal human serum was used as a negative control, and previously characterized COVID-19 patient sera was used as a positive control in each assay.

### Ortho VITROS SARS-CoV-2 IgG Testing

A subset of approximately 10% of donor sera was selected for analysis using the Ortho VITROS SARS-CoV-2 IgG test (OVSARS2IgG). Specimens with missing or indeterminate PRNT titers were excluded from analysis (n = 3, Supplementary Figure 1), but selection was blinded to the sex and age of participants. The subset was selected to approximate the total study population with regard to neutralization at/above the FDA minimal 1:80 titer at the $PRNT_{50}$ level (total study population = 73%, subset = 68.1%). Additionally, the subset included 11 matched pairs with steady PRNT titers and 26 matched pairs with decreases in PRNT titers (late vs early donation). Two selected donations had insufficient volumes for testing, so were replaced with specimens with equivalent titers selected at random from NYBC specimens subjected to PRNT. The OVSARS2IgG test was run on the Ortho VITROS 5600 instrument according to the manufacturer's instructions and the signal-to-cutoff ratio was automatically calculated by the system. This test is an automated chemiluminescent immunoassay for the quantitative detection of anti–SARS-CoV-2 spike subunit 1 IgG antibodies in human sera. Undiluted human sera (20 μL) are added to a reaction cup containing immobilized spike protein, and unbound antibody is washed away before horseradish peroxidase–labeled antihuman IgG is added. Unbound secondary antibody is washed away, and signal detection enhancer and detection reagent are added and chemiluminescence is detected. The laboratorians completing the OVSARS2IgG were blinded to clinical information on the specimens, including age, sex, SARS-CoV-2 antibody test results from the Wadsworth Center clinical assay, PRNT results, and date of collection.

### Statistical Analyses

Correlation between age and NT was assessed with a 2-tailed Spearman *r* test. Statistical significance of correlations and comparisons between PRNT titers, days post–initial donation (DPID), and OVSARS2IgG ratios were computed using the Kruskal–Wallis test with Dunn correction for multiple comparisons when comparing 3 or more groups. The Mann–Whitney test was applied when comparing 2 groups of a continuous variable, and the Kolmogorov–Smirnov test was used when comparing 2 groups of a discrete variable. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using the Wilson–Brown method for calculating 95% confidence intervals. A 2-sided Fisher exact test was used to assess significance of the effect sizes calculated.

## RESULTS

### Durability of the Neutralizing Capacity of CCP Donors

Of the 981 specimens analyzed ranging up to 119 DPID, 61.1% were contributed by males, 38.4% were contributed by females, and 0.5% were contributed by individuals who did not specify sex. Specimens contributed by

**Table 1. Distribution of $PRNT_{50}$ Titers of Coronavirus Disease 2019 Convalescent Plasma Donations Over Time The number of donations in each category is specified in parentheses following the percentage of specimens in each category. N = 978 for the entire cohort.**

| | Initial Donation | Days Post–Initial Donation | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $PRNT_{50}$ | 0 d | 1–14 d | 15–28 d | 29–42 d | 43–56 d | 57–70 d | 71–84 d | ≥85 d |
| <20 | 2.1 (4) | 1.4 (3) | 2.8 (4) | 1.8 (2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 20 | 3.1 (6) | 6.0 (13) | 5.5 (8) | 9.0 (10) | 8.0 (9) | 12.2 (10) | 16.7 (12) | 11.6 (5) |
| 40 | 15.4 (30) | 16.5 (36) | 15.2 (22) | 16.2 (18) | 23.2 (26) | 17.1 (14) | 20.8 (15) | 39.5 (17) |
| 80 | 15.9 (31) | 17.4 (38) | 24.8 (36) | 23.4 (26) | 23.2 (26) | 35.4 (29) | 25.0 (18) | 16.3 (7) |
| 160 | 17.4 (34) | 22.0 (48) | 15.9 (23) | 21.6 (24) | 24.1 (27) | 13.4 (11) | 18.1 (13) | 16.3 (7) |
| 320 | 12.3 (24) | 12.8 (28) | 11.7 (17) | 12.6 (14) | 11.6 (13) | 8.5 (7) | 6.9 (5) | 11.6 (5) |
| 640 | 7.7 (15) | 4.6 (10) | 5.5 (8) | 6.3 (7) | 4.5 (5) | 11.0 (9) | 9.7 (7) | 2.3 (1) |
| >640 | 26.2 (51) | 19.3 (42) | 18.6 (27) | 9.0 (10) | 5.4 (6) | 2.4 (2) | 2.8 (2) | 2.3 (1) |
| ≥80 | 79.5 (155) | 76.1 (166) | 76.6 (111) | 73.0 (81) | 68.8 (77) | 70.7 (58) | 62.5 (45) | 48.8 (21) |
| ≥160 | 63.6 (124) | 58.7 (128) | 51.7 (75) | 49.5 (55) | 45.5 (51) | 35.4 (29) | 37.5 (27) | 32.6 (14) |

Abbreviation: $PRNT_{50}$, Plaque Reduction Neutralization Test 50.

males had significantly greater mean neutralizing capacities than those contributed by females (mean $PRNT_{50}$ titer, 263.8 and 171.5, respectively; $P < .0001$). The mean age of the study population was 48 years. Age was weakly, positively correlated with NT ($PRNT_{50}$: Spearman $r = 0.2129$, $P < .0001$; $PRNT_{90}$: Spearman $r = 0.1988$, $P < .0001$). Neutralizing antibodies were not detected in 1.3% of CCP tested at the minimum dilution (1:20) screened in this study. As a population, 25.8% ($PRNT_{90}$) to 73.0% ($PRNT_{50}$) of donations had a PRNT titer of ≥1:80. Comparatively, 9.5% ($PRNT_{90}$) to 51.4% ($PRNT_{50}$) of all donations had a PRNT titer of ≥1:160.

Neutralizing capacities of donations were compared in 2-week intervals at the $PRNT_{50}$ (Table 1) and $PRNT_{90}$ levels (Supplementary Table 1). The proportion of specimens that met the FDA minimal 1:80 and recommended 1:160 cutoffs decreased over time at both levels of neutralization. Analyses of the titers from individual donors revealed that a significant proportion (23.4%) decreased ≥4-fold in $PRNT_{50}$, while fewer (8.5%) had a decreased $PRNT_{90}$ titer vs their initial draw (Table 2, Supplementary Table 2, and Supplementary Figure 3). Mean $PRNT_{50}$ NTs decreased significantly over time ($P < .0001$) (Figure 1A and 1B). The most significant decreases in $PRNT_{50}$ titer occurred at ≥43 DPID, suggesting that a 6-week window is optimal for maximizing collection of high titer CCP. This window likely corresponds to 3–9 weeks post–symptom onset as estimated from the donor eligibility criteria. While the decrease in $PRNT_{90}$ NTs was not significant ($P = .0661$) (Supplementary Figure 2*A* and 2*B*), all donors with titers ≥1:320 experienced decreases in $PRNT_{90}$ titer by their final draw (Supplementary Figure 2*B*). While 9.2% (18/196) of CCP donors converted from positive to negative at a dilution of 1:20 for neutralizing activity at the $PRNT_{90}$ level, none of the donors converted to negative for neutralizing activity at the $PRNT_{50}$ level at any time point. Loss of neutralizing capacity at $PRNT_{90}$ level occurred at ≥61 DPID for 15 of 18 donors. While low a $PRNT_{50}$ titer may render a unit of CCP undesirable for use as a COVID-19 therapeutic, these results suggest that individuals who produce neutralizing antibody retain some SARS-CoV-2 neutralizing capacity for up to 119 DPID.

### Ortho VITROS SARS-CoV-2 IgG Test and Neutralizing Capacity of Sera

A subset of specimens was evaluated using the FDA-approved OVSARS2IgG test at FDA and Mayo Clinic/US-EAP-CPC–derived cutoffs. DPID ranged from 0 to 114 days for this subset, which was 52.8% male and 47.2% female and had an average age of 48.8 years (Supplementary Figure 1). The OVSARS2IgG ratios of sera from initial draws were compared to the OVSARS2IgG ratios of a subsequent draw where NT remained constant or decreased. While most sera pairs with decreases in neutralizing capacity also had decreases in OVSARS2IgG ratios, the difference was not significant (Figure 1C). Sera with less than the FDA-mandated OVSARS2IgG ratio (<12) had significantly lower $PRNT_{50}$ and $PRNT_{90}$ titers than sera with a ratio ≥12 (Figure 2A and 2B). Next, the distribution of PRNT titers was analyzed according to the OVSARS2IgG ratio ranges used by the Mayo Clinic/US-EAP-CPC in a recent report on CCP efficacy [4]. The $PRNT_{50}$ and $PRNT_{90}$ titers of sera with an OVSARS2IgG ratio >18.45 were significantly higher than those with ratios <4.62 or 4.62–18.45 (Figure 3A and 3C).

The accuracy of the OVSARS2IgG test to qualify high-titer CCP was compared to the reference standard PRNT at both levels of neutralizing capacity. The FDA-established OVSARS2IgG ratio of ≥12 and the Mayo Clinic/US-EAP-CPC–derived ratio of >18.45 were used in the performance assessment. The ability of the OVSARS2IgG test to correctly identify sera with any neutralizing capacity (NT ≥20), or those with titers at the FDA-recommended levels (NT ≥80 or NT ≥160) at the $PRNT_{50}$ (Table 3) and $PRNT_{90}$ levels, was determined (Supplementary Table 3). The FDA cutoff of 12 resulted in 100% specificity and PPV for NT ≥20 and NT ≥80 at the $PRNT_{50}$ level. Sensitivity and NPV improved when capturing specimens with NT ≥80 or NT ≥160 compared to NT ≥20 at the $PRNT_{50}$ level.

CCP with an OVSARS2IgG ratio >18.45 significantly correlated with improved outcomes in patients transfused shortly after hospitalization compared to patients transfused with CCP units with lower OVSARS2IgG ratios [4]. While our results support the use of an OVSARS2IgG ratio ≥12 to exclude CCP with no neutralizing capacity from therapeutic use, we determined whether an OVSARS2IgG ratio >18.45 provided additional discriminatory power. Significantly, the >18.45 cutoff had improved specificity and PPV for specimens with

**Table 2. Coronavirus Disease 2019 Convalescent Plasma Donations With a ≥4-Fold Decrease in $PRNT_{50}$ Titer Presented in 2-Week Intervals (n = 783)**

| | Days Post–Initial Donation | | | | | | |
|---|---|---|---|---|---|---|---|
| $PRNT_{50}$ | 1–14 d | 15–28 d | 29–42 d | 43–56 d | 57–70 d | 71–84 d | ≥85 d |
| No change | 87.2 (190) | 81.4 (118) | 73.0 (81) | 67.9 (76) | 67.1 (55) | 66.7 (48) | 53.5 (23) |
| ≥4-fold decrease | 10.1 (22) | 17.2 (25) | 27.0 (30) | 32.1 (36) | 31.7 (26) | 33.3 (24) | 46.5 (20) |
| ≥2-fold increase | 2.8 (6) | 1.4 (2) | 0 (0) | 0 (0) | 1.2 (1) | 0 (0) | 0 (0) |

Changes in neutralizing titer at the indicated levels were monitored by comparing the titer of the initial donation to subsequent donations. The number of donations in each category is specified in parentheses following the percentage of specimens in each category. One hundred ninety-five donations exhibited a 2-fold decrease in neutralizing titer versus the initial donation (data not shown).

Abbreviation: $PRNT_{50}$, Plaque Reduction Neutralization Test 50.

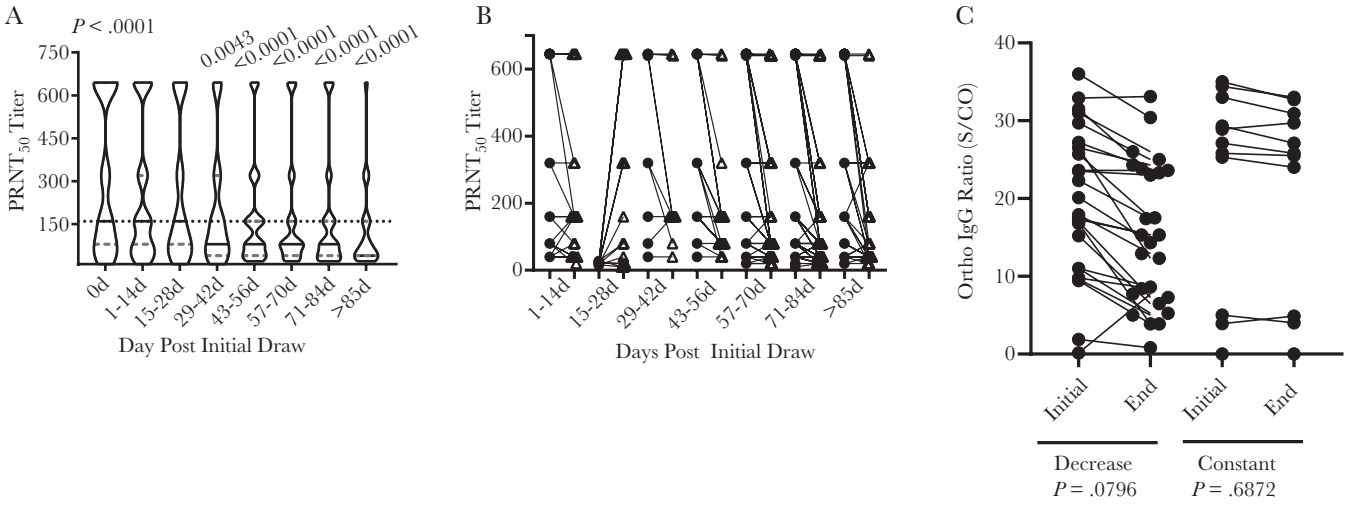


**Figure 1.** Neutralizing capacity of coronavirus disease 2019 convalescent plasma (CCP) donations decreases significantly over time. *A*, Distribution of Plaque Reduction Neutralization Test 50 ($PRNT_{50}$) titers in 2-week intervals (days [d]) of all analyzed CCP donations. The Kruskal–Wallis test with Dunn correction for multiple comparisons was applied. Each of the 2-week time periods was compared to initial collection (0 d). n = 978. *B*, Before and after plot of initial 0 d donation (circles) and final donation (triangles) $PRNT_{50}$ titers for all donors. n = 196 pairs. *C*, Subset of 37 donors was assessed using the Ortho VITROS severe acute respiratory syndrome coronavirus 2 immunoglobulin G (IgG) test and are grouped by specimen pairs where the second specimen had decrease in PRNT titer (decrease) or those that were constant (constant) vs the first donation. The Mann–Whitney test was applied.

NT ≥160 (Table 3). It also had better specificity and PPV than a cutoff of ≥12 for specimens with an NT ≥20 or NT ≥80 at the $PRNT_{90}$ level (Supplementary Table 4). The mean $PRNT_{50}$ titer of the >18.45 group is 1:523.5 (mode >1:640), while the mean $PRNT_{90}$ titer is 1:190.4 (mode 1:80) (Figure 3A and 3C). Analysis of the distribution of NTs compared to the OVSARS2IgG ratios and the ranges associated with efficacy by the Mayo Clinic/US-EAP-CPC revealed that sera with the highest neutralizing capacity have OVSARS2IgG ratios that are significantly higher than sera with low neutralizing capacities (Figure 3B and 3D). These direct comparisons between the neutralizing capacity of sera, their corresponding OVSARS2IgG ratios, and the patient outcome data released by the Mayo Clinic/US-EAP-CPC support updating recommendations for CCP use to specify that a $PRNT_{90}$ titer ≥1:80 and/or a $PRNT_{50}$ titer ≥1:640 qualify as high-titer CCP.

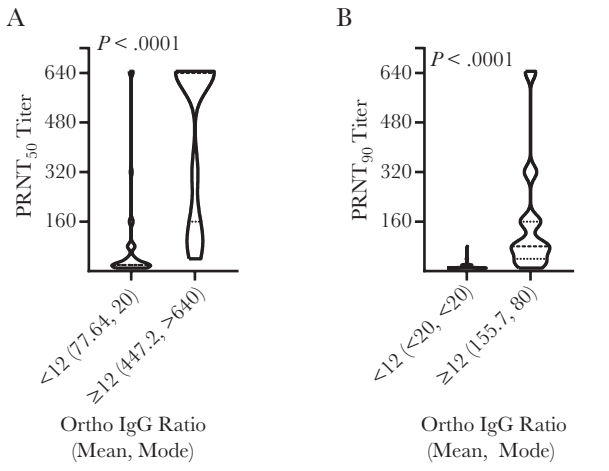


**Figure 2.** Comparison of the Ortho VITROS severe acute respiratory syndrome coronavirus 2 immunoglobulin G test (Ortho IgG) to the neutralizing capacity of coronavirus disease 2019 convalescent plasma at the US Food and Drug Administration (FDA) cutoff. *A*, Distribution of Plaque Reduction Neutralization Test 50 ($PRNT_{50}$) titers in a subset of 91 donations groups with Ortho IgG ratios using the FDA cutoff. The Mann–Whitney test was applied. *B*, As in *A*, but for Plaque Reduction Neutralization Test 90 ($PRNT_{90}$) titers.

Receiver operating characteristic curve analysis using various PRNT titers to delineate diagnostic groups was completed to empirically identify optimal OVSARS2IgG ratios. An area under the curve value for each PRNT group was considered acceptable for diagnostic use if it was ≥0.900. By this standard, the OVSARS2IgG test has enough diagnostic power to assess specimens for $PRNT_{50}$ titers of ≥1:80, ≥1:160, ≥1:320, and ≥1:640 (Supplementary Figure 4*A*). Comparatively, the OVSARS2IgG test can accurately assess specimens for a $PRNT_{90}$ titer of ≥1:20 and ≥1:80 but does not have enough diagnostic power to accurately assess specimens for a $PRNT_{90}$ titer ≥1:160 (Supplementary Figure 4*B*). An ideal OVSARS2IgG ratio cutoff was defined as having ≥89% sensitivity and specificity, an acceptable cutoff had approximately equal sensitivity and specificity that were ≥80%, and a stringent cutoff had ≥90% specificity but a substantial decrease in sensitivity. This assessment revealed that OVSARS2IgG ratio cutoffs >9 but <12 are sufficient for identifying specimens with $PRNT_{90}$ titers ≥1:20 and $PRNT_{50}$ titers ≥1:80 (Table 4). Cutoffs for identifying high-titer CCP ranged from >16.05 to >24.65 and suggest that an OVSARS2IgG ratio significantly greater than the FDA-mandated ratio of ≥12 more accurately characterizes high-titer CCP (Table 4).

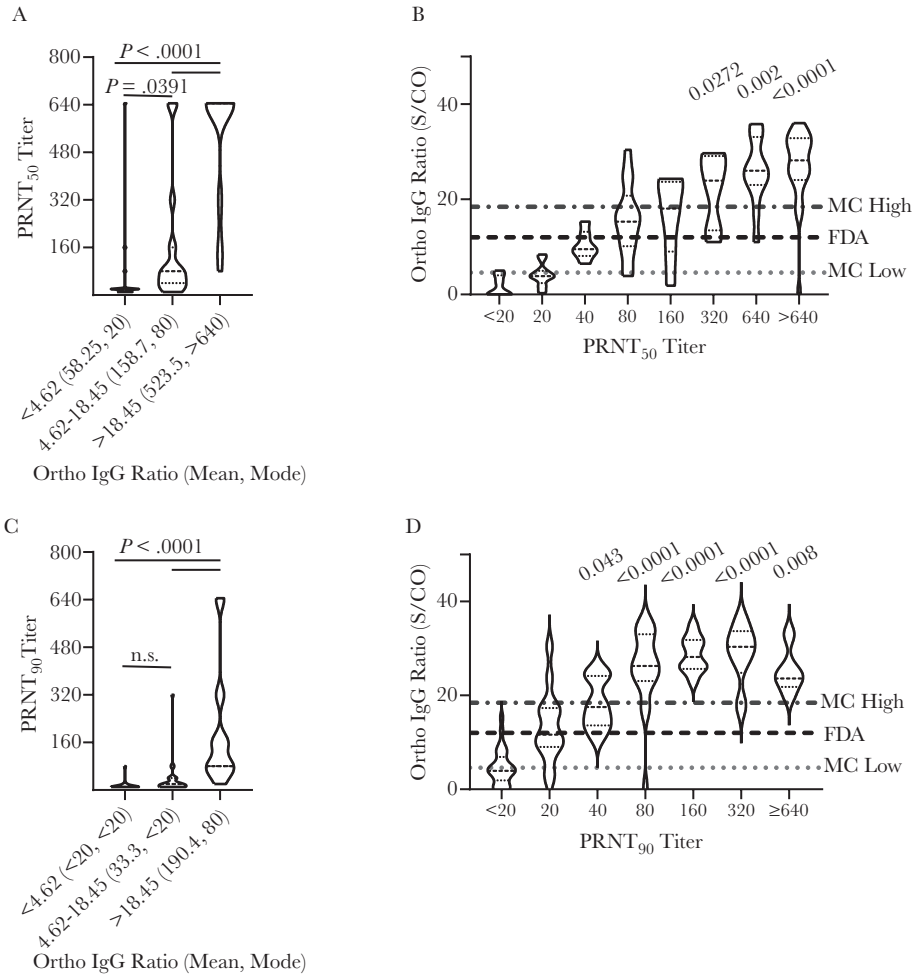


**Figure 3.** Comparison of the Ortho VITROS severe acute respiratory syndrome coronavirus 2 immunoglobulin G test (Ortho IgG) to the neutralizing capacity of coronavirus disease 2019 convalescent plasma. *A*, Distribution of Plaque Reduction Neutralization Test 50 ($PRNT_{50}$) titers in groups with Ortho IgG ratios defined by the Mayo Clinic (MC)/United States Expanded Access Program for Convalescent Plasma (US-EAP-CPC). The Kruskal–Wallis test with Dunn correction for multiple comparisons was applied. *B*, Distribution of $PRNT_{50}$ titers across all Ortho IgG test ratios. The Kruskal–Wallis test with Dunn correction for multiple comparisons was used to compare $PRNT_{50}$ ≥20 to groups with $PRNT_{50}$ <20. Dashed lines indicate ratio cutoffs used by the MC and US Food and Drug Administration (FDA). *C*, As in *A*, but for Plaque Reduction Neutralization Test 90 ($PRNT_{90}$) titers. *D*, As in *B*, but for $PRNT_{90}$ titers. n = 91 for all panels.

## DISCUSSION

Although protective correlates of immunity to SARS-CoV-2 have not been firmly established, there has been much emphasis on measuring and characterizing the antibody response to COVID-19. Neutralizing antibodies are a subset of the antibody response to infection that are expected to significantly contribute to immunity against reinfection with SARS-CoV-2 and are the basis for treating COVID-19 patients with CCP. Prior reports have found generally low neutralizing antibody titers in convalescent COVID-19 patients, but there has been significant debate about the longevity of COVID-19 neutralizing antibody responses [14, 19–22]. In this study, we found that a significant proportion of repeat CCP donors experience declines in their neutralizing antibody titers. While some individuals lost neutralizing capacity at a $PRNT_{90}$ titer of 20, all individuals that met a $PRNT_{50}$ titer of 20 retained this neutralizing capacity for the duration of the study (estimated >12 weeks post symptom onset). The mechanistic and biological consequences of the different levels of neutralizing capacity measured by $PRNT_{50}$ and $PRNT_{90}$ titers are unknown, but we observed differential stability between these 2 levels of neutralizing capacity, which suggests that they may represent the effects of distinct categories of neutralizing antibodies. Whether the levels of neutralizing antibodies measured here would protect against reinfection is unknown, but vaccinated nonhuman primates were protected against SARS-CoV-2 challenge and had low levels of neutralizing antibodies [8]. Cell-mediated immunity may offer significant protection even if neutralizing antibodies are not abundant, as has been suggested by immune profiling of COVID-19 patients with mild disease.

The limitations of this study include the lack of information regarding the days postonset at initial donation of CCP/sera and the lack of information on the COVID-19 disease severity of the studied CCP donors. However, at the time that these

**Table 3. Diagnostic Accuracy Profiles of the Ortho VITROS Severe Acute Respiratory Syndrome Coronavirus 2 Immunoglobulin G Test at 2 Cutoffs Versus the Gold-Standard $PRNT_{50}$ Titer (n = 91)**

| $PRNT_{50}$ | NT ≥20, OVSARS2IgG Ratio ≥12 | NT ≥20, OVSARS2IgG Ratio >18.45 | NT ≥80, OVSARS2IgG Ratio ≥12 | NT ≥80, OVSARS2IgG Ratio >18.45 | NT ≥160, OVSARS2IgG Ratio ≥12 | NT ≥160, OVSARS2IgG Ratio >18.45 |
|---|---|---|---|---|---|---|
| Sensitivity | 65.5% (54.8%–74.8%) | 48.8% (38.4%–59.3%) | 85.5% (74.7%–92.2%) | 66.1% (53.7%–76.7%) | 89.8% (78.2%–95.6%) | 77.6% (64.1%–87.0%) |
| Specificity | 100.0% (64.6%–100.0%) | 100.0% (64.5%–100.0%) | 93.1% (78.0%–98.8%) | 100.0% (88.3%–100.0%) | 73.8% (58.9%–84.7%) | 92.9% (81.9%–95.7%) |
| PPV | 100.0% (93.5%–100.0%) | 100.0% (91.4%–100.0%) | 96.4% (87.7%–99.4%) | 100.0% (91.4%–100.0%) | 80.0% (67.6%–88.5%) | 92.7% (80.6%–97.5%) |
| NPV | 19.4% (9.8%–35.0%) | 14.0% (7.0%–26.1%) | 75.0% (58.9%–86.3%) | 58.0% (44.2%–70.6%) | 86.1% (71.3%–93.9%) | 78.0% (64.8%–87.3%) |
| *P* value (Fisher exact test) | .0010 | .0151 | <.0001 | <.0001 | <.0001 | <.0001 |

The 95% confidence intervals for all comparisons of diagnostic accuracy are reported in parentheses.

Abbreviations: NPV, negative predictive value; NT, neutralization titer; OVSARSIgG, Ortho VITROS severe acute respiratory syndrome coronavirus 2 immunoglobulin G test; PPV, positive predictive value; $PRNT_{50}$, Plaque Reduction Neutralization Test 50.

**Table 4. Ortho VITROS Severe Acute Respiratory Syndrome Coronavirus 2 Immunoglobulin G Test Cutoffs Derived From Receiver Operating Characteristic Curve Analysis at Different Neutralizing Capacities (n = 91)**

| | $PRNT_{90}$ ≥1:20 | $PRNT_{90}$ ≥1:80 | $PRNT_{50}$ ≥1:80 | $PRNT_{50}$ ≥1:160 | $PRNT_{50}$ ≥1:160 | $PRNT_{50}$ ≥1:320 | $PRNT_{50}$ ≥1:320 | $PRNT_{50}$ ≥1:640 | $PRNT_{50}$ ≥1:640 |
|---|---|---|---|---|---|---|---|---|---|
| Cutoff ratio | >10.15 | >19.40 | >9.71 | >16.05 | >17.35 | >17.70 | >23.40 | >21.20 | >24.65 |
| Sensitivity | 90.3% | 89.7% | 91.9% | 85.7% | 83.7% | 85.4% | 75.6% | 82.7% | 71.4% |
| Specificity | 93.1% | 90.3% | 93.1% | 85.7% | 90.5% | 86.0% | 90.0% | 82.1% | 91.1% |
| Category | Ideal | Ideal | Ideal | Acceptable | Stringent | Acceptable | Stringent | Acceptable | Stringent |

The PRNT was used as the gold standard for sensitivity and specificity for each category as indicated.

Abbreviation: $PRNT_{50}$, Plaque Reduction Neutralization Test 50; $PRNT_{90}$, Plaque Reduction Neutralization Test 90.

donors were recruited by the NYBC to donate CCP, donors were being scheduled for their initial draw right at or close to the 2-week symptom-free waiting period, which has allowed us to make a rough estimate relating to a probable ideal time from post–symptom onset for CCP collection. The samples analyzed by OVSARS2IgG included an approximate representation of the distribution of NTs of the larger study population, but performance characteristics of this test could be altered if a greater proportion of specimens with very high or very low NTs were assayed instead.

Significant decreases in neutralizing antibody titer may render a CCP donation less desirable for therapeutic use. However, the tremendous variation in surrogate neutralization assays used to evaluate CCP and their unclear or untested correlations with a gold-standard reference method for evaluating neutralization capacity against live SARS-CoV-2 has complicated the rigorous assessment of CCP in practice. The use of CCP has varied reports of efficacy in COVID-19 patients, but improvements in clinical outcomes are thus far associated with early treatment with units of CCP with high NTs [5–7, 10, 23, 24]. This trend parallels the finding that passive transfer of humoral immunity in NHPs provided the greatest reduction SARS-CoV-2 viral load when larger doses of neutralizing antibody were administered [25]. Dilution of neutralizing antibodies in CCP upon transfusion likely contributes to the comparatively high level of neutralizing activity required for positive therapeutic outcomes.

The analysis presented herein clarifies the functional relationships between live SARS-CoV-2 virus NTs and the only current FDA-approved surrogate neutralization test for qualifying CCP. While an OVSARS2IgG test ratio ≥12 excluded all tested specimens with an NT <20 at the $PRNT_{50}$ level, this ratio does not exclude specimens with low neutralizing capacity and uncertain therapeutic efficacy from being labeled high-titer CCP. Rather, high-titer CCP donations are better characterized by an OVSARS2IgG test ratio >18.45, which maximizes specificity and PPV at both levels of neutralizing capacity measured. This recommendation is supported by our observation that CCP in a therapeutically beneficial treatment group is most likely to have $PRNT_{90}$ titer ≥1:80 and/or a $PRNT_{50}$ titer ≥1:640. Furthermore, our recommendation is bolstered by findings in a recent retrospective analysis of the OVSARS2IgG test ratios of CCP that was therapeutically effective [26]. Along with previously established guidelines for donor selection [18], consideration of time since disease resolution will further refine donor selection to yield high-titer CCP, particularly when CCP donations cannot be tested for their neutralizing capacity using live SARS-CoV-2.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

***Author contributions.*** Study design: R. C. G. Experimentation: R. C. G., A. D. P., A. F. P., T. J. S. Materials/support: M. P., D. S., K. A. M. Data analysis: R. C. G. All authors contributed to the writing and/or editing of the manuscript.

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