Efficient Identification of High-Titer Anti–Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antibody Plasma Samples by Pooling Method

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Context.—The ongoing COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has elicited a surge in demand for serologic testing to identify previously infected individuals. In particular, antibody testing is crucial in identifying COVID-19 convalescent plasma, which has been approved by the Food and Drug Administration as a passive immunotherapy for hospitalized patients infected with COVID-19. Currently, high-titer COVID-19 convalescent plasma can be qualified by Ortho’s Vitros COVID-19 IgG antibody test.

Objective.—To explore the use of an efficient testing method to identify high-titer COVID-19 convalescent plasma for use in treating COVID-19–infected patients and track COVID-19 positivity over time.

Design.—We evaluated an enzyme-linked immunosorbent assay (ELISA)–based method that detects antibodies specific to the SARS-CoV-2 receptor binding domain (RBD) with individual and pooled plasma samples and compared its performance against the Vitros COVID-19 IgG antibody test. Using the pooled RBD-ELISA (P-RE) method, we also screened more than 10,000 longitudinal healthy blood donor samples to assess seroprevalence.

Results.—P-RE demonstrates 100% sensitivity in detecting Food and Drug Administration–defined high-titer samples when compared with the Vitros COVID-19 IgG antibody test. Overall sensitivity of P-RE when compared with the Vitros COVID-19 IgG antibody test and our individual sample RBD-ELISA (I-RE) were 83% and 56%, respectively. When screening 10,218 healthy blood donor samples by P-RE, we found the seroprevalence correlated with the local infection rates with a correlation coefficient of 0.21 (P < .001).

Conclusions.—Pooling plasma samples can be used to efficiently screen large populations for individuals with high-titer anti–RBD antibodies, important for COVID-19 convalescent plasma identification.

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the host cell’s angiotensin-converting enzyme 2 surface receptor.\textsuperscript{15,16} Antibodies against RBD can interfere with binding of the S protein to angiotensin-converting enzyme 2; thus, SARS-CoV-2 RBD serves as a common logical target for the development of antibodies and vaccines.\textsuperscript{16} Some of the therapies being pursued that exploit this relationship include monoclonal antibody,\textsuperscript{17–19} COVID-19 convalescent plasma (CCP),\textsuperscript{20} hyperimmune immunoglobulin,\textsuperscript{21,22} and numerous vaccine strategies.\textsuperscript{23}

CCP was granted Emergency Use Authorization by the FDA on August 2020.\textsuperscript{24} CCP is collected from individuals who have recovered from COVID-19 and have high levels of anti–SARS-CoV-2 neutralizing antibodies in their plasma. Evidence from multiple studies has suggested that transfusion of high anti–SARS-CoV-2 IgG titer CCP into COVID-19 patients reduces mortality.\textsuperscript{25–27} However, these findings are preliminary and have been limited to patients who have received CCP transfusion early in their disease course. Indeed, an earlier prospective randomized controlled study demonstrated no difference in outcome in hospitalized patients with respiratory distress.\textsuperscript{28} The variability in these findings highlights the need to fully characterize not just the patient population being studied, but also the antibody titers that are being evaluated. Although other antibody-based therapies, such as hyperimmune serum and monoclonal antibodies, are being developed to treat individuals with COVID-19, use of CCP remains viable due to supply being easily accessible and more affordable.\textsuperscript{29}

Many platforms have been developed to measure SARS-CoV-2 antibody responses, differing in the isotype being measured (IgG and/or IgM) and the viral antigen being targeted (S, RBD, or nucleocapsid).\textsuperscript{30,31} Ortho’s Vitros Anti-SARS-CoV-2 IgG antibody (VG) assay has been approved by the FDA to identify high-titer CCP for use in passive immune therapy.\textsuperscript{24} In addition, various enzyme-linked immunosorbent assays (ELISAs) being used as laboratory-developed tests at numerous institutions have been shown to be highly effective for semiquantitative detection of anti-SARS-CoV-2 antibodies in previously infected individuals.\textsuperscript{32–34} Currently, to maximize sensitivity most ELISAs are performed on individual plasma specimens. ELISAs performed on individual specimens can be highly sensitive and specific, although false positives dependent on the assay platform are detected in some prepandemic specimens. Being able to pool samples would significantly increase testing capacity, and would allow identification of high-titer potential CCP donors, even if some low-positive specimens are not detected.

We therefore sought to determine the performance characteristics of detecting anti-RBD IgG antibodies by ELISA between pooling (P-RE) and individual (I-RE) sampling methods compared with the VG assay to identify potential high-titer CCP donors. We find that P-RE can be used to efficiently screen for CCP in large plasma specimen populations. When pooled testing methodology is applied to local healthy blood donor products, seropositivity rates were in agreement with the overall community positivity rates for viral testing as reported by our local health department.

**MATERIALS AND METHODS**

**Sample Collection**

We collected 2046 individual discarded/residual ethylenediaminetetraacetic acid whole blood specimens from blood donors from the Stanford Blood Center. These samples represent healthy volunteer blood donors as well as a small subset of CCP donors. Pooled samples were generated by combining the same 2046 individual plasma samples into pools of 6 in equal proportions, resulting in 341 pooled samples. An additional 10 218 samples were collected between April 2020 and July 2020. These 10 218 samples were tested in pools of 6 to determine the seropositivity rate in our blood donor population.

**Ortho Vitros Anti-SARS-CoV-2 IgG**

A subset of our donor samples (1413 individual samples) was tested individually and in parallel using the VG assay, a testing platform granted Emergency Use Authorization by the FDA\textsuperscript{24} for the purpose of defining high-titer in CCP samples. Separate serum samples from the same collection were evaluated on the VG platform. Results were reported as either positive or negative for anti–SARS-CoV-2 IgG antibodies against the spike protein.\textsuperscript{24} In addition to the qualitative antibody result, a signal-to-cutoff value for each sample was also provided for additional reference. In addition, 19 of 1413 individual samples were known CCP donors.

**Anti-RBD IgG ELISA**

The ELISA procedure we used in this study was modified\textsuperscript{35} from a protocol provided by Stadlbauer et al.\textsuperscript{36} Plates were coated with 0.1 μg/well of SARS-CoV-2 RBD, which was expressed from a plasmid kindly provided by Florian Kramer, PhD, and incubated at 4°C overnight. Wells were washed with 300 μL of 0.5% tween in phosphate-buffered saline 3 times and blocked with 0.5% tween phosphate-buffered saline containing 5% non-fat milk powder for 2 hours. Plasma samples were diluted 1:1000 in 0.5% tween phosphate-buffered saline containing 1% non-fat milk powder for individual plasma testing. Of pooled samples, 3 μL were diluted to 100 μL with 1% non-fat milk powder in 0.5% tween phosphate-buffered saline. Wells were washed, and 50 μL of the diluted plasma sample was added to the wells. Plates were sealed and incubated at 37°C for 1 hour. IgG detection antibodies (Invitrogen, Carlsbad, California) were diluted 1:6000, and 50 μL were added to each well following another wash. Plates were incubated at room temperature for 1 hour. Plates were washed again and 100 μL of 3,3',5,5'-Tetramethylbenzidine was added to each well. Wells developed for 12 minutes and stop solution was added.

**Cutoff Determination**

As negative control samples for our assay development, we used 96 serum samples collected from healthy volunteer blood donors in 2018 and tested each sample individually. The threshold for positivity for our individual sample ELISA (0.198) was defined as an optical density (OD) 450 value 3 SDs above the highest OD450 in the sample set.

For pooled testing, the same 96 samples were pooled into 16 pools of 6 and tested using ELISA. Similar to the individual testing, the positivity threshold for P-RE (0.218) was defined as 3 SDs above the highest OD450 value in our negative control pooled sample set.

**RESULTS**

**Anti-RBD IgG Antibody Detection Using P-RE and 13211-RE Methods**

Two thousand forty-six blood donor samples were assayed individually by RBD ELISA (I-RE), and 57 samples were determined to be positive for anti-RBD IgG. These 2046 samples were assayed in parallel in 341 pools of 6 specimens by RBD ELISA (P-RE). Every positive pool was confirmed by testing the individual samples it contained. Of 57 positive samples identified by I-RE, 32 were detected by P-RE, equating into a sensitivity of 56% (Figure 1, A and B). To confirm that the P-RE false negatives were indeed...
due to low-positive samples by I-RE that were diluted when pooled, we compared I-RE OD values of samples that were positive by both P-RE and I-RE against those that were positive by I-RE but negative by P-RE. Positive individual samples in positive P-RE pools had a significantly higher average OD value compared with positive individual samples in false-negative P-RE pools (0.77 versus 0.34; \( P < .001 \); Figure 1, A), confirming the lower sensitivity of P-RE testing. Of 284 individual plasma samples that tested negative, 2 came from positive pools resulting in a specificity of 99% (Figure 1, B). The 2 pooled samples that were negative by I-RE had relatively low P-RE

Figure 1. Performance comparison between individual (I-RE) and pooled (P-RE) receptor binding domain–enzyme-linked immunosorbent assay. A, Boxplot comparing I-RE–positive absorbance values by P-RE results. Fifty-seven I-RE–positive samples were separately pooled with negative plasma and assayed using P-RE. Cutoff values were 0.19 for I-RE and 0.22 for P-RE. Average absorbance values for individual samples that were positive for both assays were 0.77, whereas average absorbance values for samples positive only by I-RE were 0.34 (\( P < .001 \)). B, This confusion matrix shows the number of agreeing (blue boxes) and disagreeing (red boxes) results between the P-RE and I-RE assays.

Figure 2. Performance comparison between Vitros IgG antibody test (VG) and pooled receptor binding domain–enzyme-linked immunosorbent assay (P-RE). A, This confusion matrix shows the number of agreeing (blue boxes) and disagreeing (red boxes) results between the P-RE and individual-RE assays. B, This dot plot demonstrates how VG-positive sample data correlate between P-RE and VG. The \( R^2 \) value is 0.60 and the \( P \) value < .001. C, The boxplot compares VG-positive sample optical density (OD) values based on P-RE results.
absorbance values (0.27 and 0.33); we individually tested the 12 samples comprising these 2 pools and identified 1 individual sample in each pool with relatively high yet negative absorbances (0.11 and 0.12).

Comparison of RBD-ELISA With Ortho’s Vitros Anti-SARS-CoV-2 IgG Antibody Test Method

To further evaluate the performance characteristics of P-RE, we compared it against individual samples tested on the VG antibody platform, which has been recognized by the FDA to identify high-titer CCP donors. One thousand three hundred and ninety-four individual samples were assayed using I-RE and VG (Figure 3, A). We observed a $R^2$ value of 0.83 when comparing the I-RE absorbance values with the signal-to-cutoff values derived from VG ($P < 0.001$, Figure 2, B). In addition, we evaluated positive VG individual samples that were not detected in P-RE testing. Similar to the P-RE missing low-positive I-RE above, we found that P-RE also missed low-level positives by VG (Figure 2, C). Of note, for the 18 samples that were above the VG cutoff to be considered as high titer (signal-to-cutoff value $> 9.5$), all were detected on P-RE, indicating that pooling can accurately detect potential high-titer CCP donations. Of note, 1 sample was identified as positive by P-RE, and subsequently confirmed by I-RE, but negative by VG. This sample had a P-RE signal of 0.22.

We also compared the performance characteristics between I-RE and VG. One thousand three hundred and ninety-four individual samples were assayed using I-RE and VG (Figure 3, A). We observed a $R^2$ value of 0.82 when

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**Figure 3.** Performance comparison between individual receptor binding domain–enzyme-linked immunosorbent assay (I-RE) and Vitros IgG antibody test (VG). A, This confusion matrix shows the number of agreeing (blue boxes) and disagreeing (red boxes) results between the VG and I-RE assays. B, The 1413 samples were plotted according to their VG absorbance value on the y-axis and I-RE absorbance value on the x-axis. The $R^2$ value is 0.83 and the $P$ value $< 0.01$. The horizontal dotted line denotes VG cutoff threshold and the vertical dotted line denotes I-RE cutoff threshold. C, The boxplot compares the VG absorbance values of the 1413 samples by their VG and I-RE outcomes along the x-axis. The dotted line denotes the VG cutoff threshold. D, The boxplot compares the I-RE absorbance values of the 1413 samples by their VG and I-RE outcome along the x-axis. The dotted line denotes the I-RE cutoff threshold.

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examining correlation between the values measured for individual samples in the 2 assays (Figure 3, B). Furthermore, 10 positives were identified through VG. Of these, 9 were also positive with I-RE testing, as well as an additional 12 positive specimens (Figure 3, A, C, and D).

**P-RE Accurately Detects High-Titer CCP Donations**

The recent FDA guidance further outlined the requirement for high-titer CCP in COVID-19 patients. Because P-RE misses low-level positive samples but can detect high-titer plasma samples from healthy blood donors, we next evaluated whether P-RE could serve as an effective strategy to screen for CCP donors. At Stanford Blood Center, individuals self-identify and register as possible CCP donors based on a previous laboratory-confirmed diagnosis of COVID-19 and having been asymptomatic for at least 2 weeks. Using VG, 19 CCP samples were assayed and 18 positives were identified, indicating a sensitivity of 95%. We tested 31 known CCP samples by I-RE and 32 CCP samples in pools by P-RE and were able to detect 94% (29 positives) and 88% (28 positives) of the CCP samples, respectively (Figure 4, A). In addition, 28 CCP samples were tested using both I-RE and P-RE to directly compare their abilities in detecting CCP. I-RE proved to be more sensitive by detecting 2 more CCP than P-RE (Figure 4, B).

**Implementation of P-RE in a Healthy Blood Donor Population**

We applied the P-RE methodology to 1703 pools collected from 10,218 healthy blood donors from April 19, 2020, to July 17, 2020. Positivity rate was tracked over time (Figure 5, A) and compared with virology testing data collected by Santa Clara County (SCC). Within the timeframe of our study, SCC observed weekly positivity rates.
rates reach as high as 10.81%; our study, on the other hand, had weekly positivity rates that peaked at 0.92% (Figure 5, A). Although the magnitude of the positivity rates differed, the peaks and troughs between the county data and ours coincided temporally. Overall, 43 of 1703 non-CCP pools tested positive which equates to an individual sample positivity rate of 0.4%. Furthermore, we observed a $R^2$ value of 0.21 when examining correlation between the positivity rates of the 2 populations (Figure 5, B).

**DISCUSSION**

COVID-19 continues to spread widely and cause morbidity and mortality worldwide, with therapeutics only showing modest effectiveness in a significant amount of cases.8-11 One promising form of treatment is the use of high-titer CCP for passive immunity in hospitalized COVID-19 patients. Even with effective vaccines beginning to be approved and distributed, manufacturing and resource constraints mean that it will be months to years before vaccines are available worldwide, and sizeable fractions of the population in some countries may decline to be vaccinated.8 CCP will continue to be a treatment option in many parts of the world for the foreseeable future. Here, we have validated an efficient pooling and ELISA testing methodology to screen large numbers of samples for high titers of neutralizing antibodies to identify potential CCP donors.

In testing of 2046 individual samples in 341 pools, we found that only 56% of I-RE–positive individuals were also identified by P-RE, showing the expected decreased sensitivity of pool testing. The ELISA OD values of positive I-RE samples that were missed by P-RE were lower than those of samples that were positive by both methods. P-RE performed well, with 100% sensitivity in detecting individuals with high titers to RBD, which is the positive I-RE samples that were missed by P-RE were found that only 56% of I-RE–positive individuals were also donors.

We tested an additional 1722 samples by P-RE to track SARS-CoV-2 exposure over time and compared our results with our local county’s public health COVID-19 testing data. Within the timeframe of our study, we compared the rate of viral nucleic acid test positivity rate in SCC with our own serologically positive blood donor population by P-RE. Although the serologic positivity rate of the blood donor population was significantly lower than the viral positivity rate of SCC, both rates trended similarly through time, suggesting that pooled testing of the blood donor population is a potential method to monitor trends in COVID infectivity rates in the general local population.

Our findings indicate that P-RE can be used to efficiently screen large healthy populations for high-titer SARS-CoV-2 antibody CCP donor identification, saving time and resources. Use of high-titer CCP in passive immune therapy is likely to remain a viable treatment option, particularly in lower resource regions.12

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**References**

1. Kamenidou IE, Stavrianea A, Liava C. Achieving a COVID-19 free country: citizens preventive measures and communication pathways. *Int J Environ Res Public Health*. 2020;17(13):4613. doi:10.3390/ijerph17114633

2. Ali I, Alharbi OML. COVID-19: disease, management, treatment, and social impact. *Sci Total Environ.* 2020;728:138861. doi:10.1016/j.scitotenv.2020.138861

3. Gold MS, Sekaye D, Gabrielli S, Zhang X, McCusker C, Ben-Shoshan M. SARS-CoV-2 clinical features and comorbidities: a systematic review and meta-analysis. *Postgrad Med.* 2020;132(8):749–755. doi:10.1080/00325481.2020.1788694

4. Perez-Saez J, Lauer SA, Kaiser L, et al. Serology-verified estimates of SARS-CoV-2 infection fatality risk in Geneva, Switzerland. *Lancet Infect Dis.* 2021;21:e56–e70. doi:10.1016/S1473-3099(20)30548-3

5. Pradhan D, Biswasroy P, Kumar Naik P, Ghosh G, Rath G. A Review of current interventions for COVID-19 prevention. *Arch Med Res.* 2020;51(5):363–374. doi:10.1016/j.arcmed.2020.04.020

6. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* 2020;30(3):269–271. doi:10.1038/s41422-020-0282-0

7. Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat Rev Drug Discov.* 2020;19(3):149–150. doi:10.1038/d41573-020-00016-0

8. Serafin MB, Bottega A, Foletto VS, da Rosa TF, Hörner A, Hörner R. Drug repositioning is an alternative for the treatment of coronavirus COVID-19. *Int J Antimicrob Agents.* 2020;55(6):105969. doi:10.1016/j.ijantimicag.2020.105969

9. RECOVERY Collaborative Group, Homer P, Lim WS, et al. Dexamethasone in hospitalized patients with Covid-19. *N Engl J Med.* 2021;384(18):693–704. doi:10.1056/NEJMoa2104146

10. Ortúñano C, Pasteroa EA. Hydroxychloroquine and dexamethasone in COVID-19: who won and who lost? *Clin Mol Allergy.* 2020;18:17. doi:10.1186/s12948-020-00132-7

11. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of Covid-19: Final Report. *N Engl J Med.* 2020;383(19):1813–1826. doi:10.1056/NEJMoa2007764

12. Li Z, Wang X, Cao D, Sun R, Li C, Li G. Rapid review for the anti-coronavirus effect of remdesivir. *Drug Discov Ther.* 2020;14(2):73–76. doi:10.5582/ddt2020.01015

13. Samwat SK, Tharappel AM, Li Z, Li H. Prospect of SARS-CoV-2 spike protein: potential role in vaccine and therapeutic development. *Viruses Res.* 2020;288:198141. doi:10.1016/j.viruses.2020.198141

14. Hussain A, Hasan A, Nejadi Babadaree MM, et al. Targeting SARS-CoV-2 spike protein receptor binding domain by therapeutic antibodies. *Biomed Pharmacother.* 2020;130:110559. doi:10.1016/j.biopha.2020.110559

15. Li F. Structure, function, and evolution of coronavirus spike proteins. *Annu Rev Virol.* 2016;3(1):237–261. doi:10.1146/annurev-virology-110615-042301

16. Tai W, He L, Zhang X, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cell Mol Immunol.* 2020;17(6):613–620. doi:10.1007/s12282-020-00404-4

17. Wang C, Li W, Drabek D, et al. A human monoclonal antibody blocking SARS-CoV-2 infection. [published correction appears in Nat Commun. 2020; 11(1):2511]. *Nat Commun.* 2020;11(1):2251. doi:10.1038/s41467-020-16256-y

18. Weinreich DM, Sivapalasingam S, Norton T, et al. REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19. *N Engl J Med.* 2021;384(15):1483–1491. doi:10.1056/NEJMoa2030502

19. Tacconi M, Ferraro S, Convertino I, et al. Anti-SARS-CoV-2 neutralizing monoclonal antibodies: clinical pipeline. *Mabs.* 2020;12(1):1854149. doi:10.1080/19420862.2020.1854149
20. Wooding DJ, Bach H. Treatment of COVID-19 with convalescent plasma: lessons from past coronavirus outbreaks. Clin Microbiol Infect. 2020;26(10):1436–1446. doi:10.1016/j.cmi.2020.08.005

21. Nguyen AA, Habiballah SB, Platt CD, Geha RS, Chou JS, McDonald DR. Immunoglobulins in the treatment of COVID-19 infection: proceed with caution! Clin Immunol. 2020;216:108439. doi:10.1016/j.clim.2020.108439

22. Chai KL, Valk SJ, Piechotta V, et al. Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review. Cochrane Database Syst Rev. 2020;10:CD013600. doi:10.1002/14651858.CD013600.pub3

23. Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. Nat Rev Immunol. 2020;20(10):615–632. doi:10.1038/s41577-020-00434-6

24. EUA 26382: Emergency Use Authorization (EUA) Request (original request 8/12/20; amended request 8/23/20). Food and Drug Administration Web site. https://www.fda.gov/media/141480/download. Accessed September 20, 2020.

25. Salazar E, Christensen PA, Graviss EA, et al. Treatment of coronavirus disease 2019 patients with convalescent plasma reveals a signal of significantly decreased mortality. Am J Pathol. 2020;190(11):2290–2303. doi:10.1016/j.ajpath.2020.08.007

26. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci U S A. 2020;117(17):9490–9496. doi:10.1073/pnas.2004168117

27. Zhang B, Liu S, Tan T, et al. Treatment with convalescent plasma for critically ill patients with severe acute respiratory syndrome coronavirus 2 infection. Chest. 2020;158(1):e9–e13. doi:10.1016/j.chest.2020.03.039

28. Shen C, Wang Z, Zhao F, et al. Treatment of 3 critically ill patients with COVID-19 with convalescent plasma. JAMA. 2020;323(16):1582–1589. doi:10.1001/jama.2020.4783

29. Hegerova L, Cooley TA, Sweerus KA, et al. Use of convalescent plasma in hospitalized patients with COVID-19: case series. Blood. 2020;136(6):759–762. doi:10.1182/blood.2020006964

30. Focosi D, Tuccori M, Franchini M. The road towards polyclonal anti-SARS-CoV-2 immunoglobulins (hyperimmune serum) for passive immunization in COVID-19. Life (Basel). 2021;11(2):144. doi:10.3390/life11020144

31. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020;26(6):845–848. doi:10.1038/s41591-020-0897-1

32. Weisberg SP, Connors T, Zhu Y, et al. Antibody responses to SARS-CoV2 are distinct in children with MIS-C compared to adults with COVID-19. medRxiv. 2020;2020.07.12.20151068. doi:10.1101/2020.07.12.20151068

33. Beavis KG, Matushek SM, Abeleda AF, et al. Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. J Clin Virol. 2020;129:104468. doi:10.1016/j.jcv.2020.104468

34. Liu W, Liu L, Kou G, et al. Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2. J Clin Microbiol. 2020;58(6):e00461–20. doi:10.1128/JCM.00461-20

35. Röltgen K, Wirz OF, Stevens BA, et al. SARS-CoV-2 antibody responses correlate with resolution of rmRNAemia but are absent-lives in patients with mild illness. medRxiv. 2020;2020.08.15.20175794. doi:10.1101/2020.08.15.20175794

36. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. Curr Protoc Microbiol. 2020;57(1):e00100. doi:10.1002/cpmc.100

37. Bloch EM, Goel R, Montemayor C, Cohn C, Tobian AAR. Promoting access to COVID-19 convalescent plasma in low- and middle-income countries. Transl Infect Dis. 2021;60(1):102957. doi:10.1016/j.tranid.2020.102957