


determine both cellular composition and residual WBC/RBC count of a blood product with one method and one measurement. As mentioned in the Editorial by Mack and Vasallo,<sup>3</sup> this use of multifunctional instruments, already widely employed by blood banks, promises increased efficiency and cost reduction.

### CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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### REFERENCES

1. Blanco RA, Cavagnetto C, Wilmott L, et al. The use of hematology analyzer with a new generation of software as an alternative to flow cytometry for enumerating residual white blood cells in blood components. *Transfusion*. 2020;60:155–164.
2. Lagerberg J, Hoederdaal R, Blanco RA, et al. Counting of residual white and red cells in blood components using blood bank mode on Sysmex XN1000 haematology analyser. *Vox Sang*. 2019;114(Suppl 2):63–64.
3. Mack SM, Vasallo RR. Component residual white blood cell counting made easy? *Transfusion*. 2020;60:4–6.

## Anti-SARS-CoV-2 spike antibodies are stable in convalescent plasma when stored at 4° Celsius for at least 6 weeks

To the Editor

As nations around the world continue to address the public health crisis of Coronavirus Disease 2019 (COVID19), the global medical and scientific communities continue to search for and develop therapeutic strategies for patients. To date, remdesivir presents one such option and can shorten the duration of hospitalization in patients with severe disease.<sup>1</sup> Convalescent plasma (CP) has presented another option, with its use and safety supported by numerous case series and retrospective studies.<sup>2–4</sup> While its degree of effectiveness has been variable, there is general agreement that it acts as a supportive adjunctive therapy in patients with moderate to severe disease.<sup>2,4</sup>

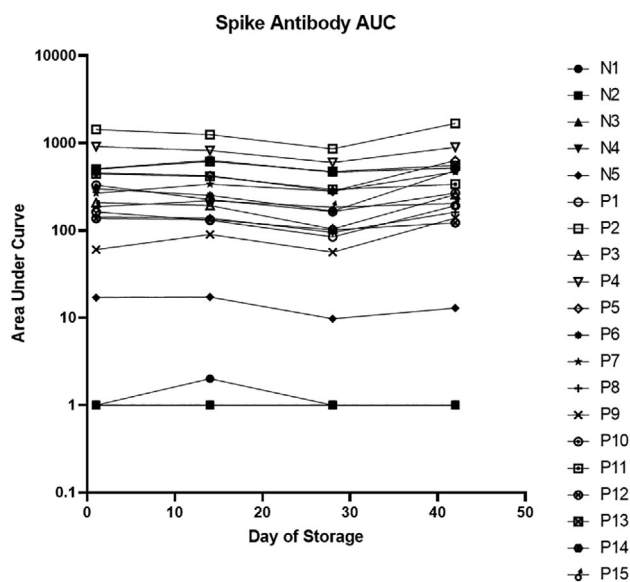
Current CP protocols such as the Mayo Clinic Early Access Program (EAP) specify that once thawed, CP may be stored for up to 5 days at 4°C. Presumably, this is intended to mimic current guidelines which apply to thawed plasma (TPL), which specify a similar shelf-life. However, the rationale for this guideline stems from the fact that under normal circumstances, donor plasma is transfused or exchanged therapeutically for the purposes of correcting or preventing a coagulation factor deficiency, to replace deficient factors necessary for hemostatic balance such as ADAMTS13, or to restore complement-regulatory factors in the setting of atypical hemolytic uremic syndrome. For these purposes, TPL is largely considered equivalent to fresh frozen plasma, with the exception of decreases in Factors V and VIII activity levels, and several

other components.<sup>5,6</sup> However, the therapeutic goal of CP transfusion is to deliver passive immunity to the patient via antibodies. Studies demonstrating antibody stability under refrigerated conditions have largely focused on peripheral blood samples stored for several days.<sup>7,8</sup> However, studies describing the stability of antibodies in refrigerated donor plasma over longer periods are lacking. Here, we demonstrate the long-term stability of anti-SARS-CoV-2 spike antibodies in donor CP samples collected at a local blood donor center for transfusion.

After thawing 15 CP units, segments were sampled and anti-spike antibody titers were determined via enzyme-linked immunosorbent assays (ELISAs).<sup>9</sup> Segments from five non-CP units were sampled as a negative control. All samples were then stored at 4°C, and plasma endpoint titers were re-evaluated at 14, 28, and 42 days. We detected no reduction in antibody titers for any of the samples (Table 1). The anti-spike ELISA assay demonstrates a between-run %CV ranging from 6.3% at an antibody concentration of 0.3 mcg/mL, to 23% at a concentration of 0.01875 mcg/mL. Therefore, the perceived decrease in titer for most samples between days 1 and 7, and the perceived increase between days 28 and 42 reflects the serial dilution cutoff values of the ELISA assay itself and run-to-run variation, and are not reflective of a true change. This is supported by the results obtained when the area under the curve (AUC) values for raw absorbance data are calculated, and show no significant

Sample	Titer: day 1	Titer: day 14	Titer: day 28	Titer: day 42
N1	50	100	50	50
N2	Negative	Negative	Negative	Negative
N3	Negative	Negative	Negative	Negative
N4	Negative	Negative	Negative	Negative
N5	200	200	100	100
P1	800	400	400	800
P2	3200	3200	1600	3200
P3	800	800	400	800
P4	3200	1600	1600	3200
P5	1600	1600	800	1600
P6	1600	800	800	1600
P7	1600	1600	800	1600
P8	800	400	400	800
P9	400	400	400	400
P10	800	800	800	800
P11	1600	1600	800	800
P12	400	400	400	400
P13	1600	1600	1600	1600
P14	1600	1600	1600	1600
P15	800	800	800	800

**TABLE 1** Spike antibody endpoint titers at days 1, 14, 28, and 42 of storage at 4°C, as determined by ELISA. Samples N1-N5 represent negative control plasma from random donor units and P1-P15 represent CP samples. We note the presence of one negative control unit that demonstrates detectable titers and one with titers at the lower limit of detection; this may reflect a COVID-convalescent donor in the general population that did not donate convalescent plasma specifically



**FIGURE 1** Spike antibody levels at days 1, 14, 28, and 42 of storage at 4°C, as determined by ELISA, displayed as AUC values, to account for inter-run variability of the assay. Samples N1-N5 represent negative control plasma from random donor units, and samples P1-15 represent CP samples

variation over time (Figure 1). While our study does not address the neutralization capacity of these antibodies, previous studies demonstrate significant correlation

between spike antibody titers and neutralization capacity of plasma and serum samples.<sup>9</sup>

We do note that our findings are limited by several factors. First, while limited case series have identified that CP may be effective in reducing morbidity and overall mortality, its efficacy has not been formally evaluated in large randomized clinical trials. Similarly, while there is correlation between spike antibody titers and neutralization capacity, as well as historical basis for the antibodies themselves as a cause of therapeutic benefit in CP, it is still unknown in the setting of COVID-19 if the anti-spike antibodies are the source of therapeutic benefit. It is well-established at this point that COVID-19 is associated with significant coagulopathy,<sup>10</sup> and it may be possible that coagulation or other factors provided in plasma are what provide therapeutic benefit, which may have limited stability past 5 days. This would need to be formally determined by a randomized trial comparing CP to standard frozen plasma. There may also be a slight increase in risk for septic reactions, though we believe this risk remains low, given the estimated incidence of septic reactions from red cell units of 1 in 250 000-500 000,<sup>11</sup> a product which in some cases is also stored for up to 42 days at 4°C.

Our findings demonstrate long-term stability of anti-SARS-CoV-2 spike antibodies in donor CP for at least 42 days when stored under refrigerated conditions. This finding may support a rationale for therapeutic use of CP

beyond 5 days post-thaw, as antibody levels are maintained. It should be noted that extension of the storage window to 42 days would require amendment to the current Emergency IND protocol established by the United States FDA or the Mayo Clinic EAP, but could be of significant value as it would prevent wastage of this precious resource in situations where planned CP transfusions are canceled after the product has been thawed. Additionally, it may allow for transport to more remote locations where monitored freezer facilities are limited or unavailable, potentially making this important treatment option available to patient populations with limited access to CP.

### CONFLICT OF INTEREST

F.K., D.S. and F.A. are listed as inventors of the anti-SARS-CoV2 immunoassay used in this work, which Mount Sinai Hospital has licensed to commercial entities. I.B., K.L., K.J., J.S.J. and S.A.O. have declared no conflicts of interest.

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### REFERENCES

1. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. 2020;395(10236):1569–1578.
2. Salazar E, Perez KK, Ashraf M, et al. Treatment of COVID-19 patients with convalescent plasma. *Am J Pathol*. 2020;190(8):1680–1690.
3. Joyner MJ, Wright RS, Fairweather D, et al. Early safety indicators of COVID-19 convalescent plasma in 5,000 patients. *J Clin Invest*. 2020;130(9):4791–4797.
4. Liu ST, Lin HM, Baine I, et al. Convalescent plasma treatment of severe COVID-19: a matched control study. *Nat Med*. 2020. <https://doi.org/10.1038/s41591-020-1088-9>.
5. Scott E, Puca K, Heraly J, Gottschall J, Friedman K. Evaluation and comparison of coagulation factor activity in fresh-frozen plasma and 24-hour plasma at thaw and after 120 hours of 1 to 6 degrees C storage. *Transfusion*. 2009;49(8):1584–1591.
6. Scott EA, Puca KE, Pietz BC, Duchateau BK, Friedman KD. Comparison and stability of ADAMTS13 activity in therapeutic plasma products. *Transfusion*. 2007;47(1):120–125.
7. Gonzalez-Garcia J, Gutierrez-Nicolas F, Nazco-Casariago GJ, Vina-Romero MM. Long-term stability of trastuzumab in plasma and whole blood samples stored under different conditions. *Farm Hosp*. 2019;43(2):50–52.
8. Vieillard V, Astier A, Sauzay C, Paul M. One-month stability study of a biosimilar of infliximab (Remsima<sup>®</sup>) after dilution and storage at 4 degrees C and 25 degrees C. *Ann Pharm Fr*. 2017;75(1):17–29.
9. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med*. 2020;26:1033–1036.
10. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood*. 2020;135(23):2033–2040.
11. Hillyer CD, Josephson CD, Blajchman MA, Vostal JG, Epstein JS, Goodman JL. Bacterial contamination of blood components: risks, strategies, and regulation: joint ASH and AABB educational session in transfusion medicine. *Hematology Am Soc Hematol Educ Program*. 2003;2003:575–589.

## Impact of primary culture: A true positive culture rate mystery

We read with interest the article by Kundrapu and colleagues.<sup>1</sup> The major findings of the study were (a) there was no change in the culture positive rate or

septic rate of apheresis platelets (PLTs) before or after the introduction of primary culture and (b) there was a decline both the culture-positive rate and the septic