

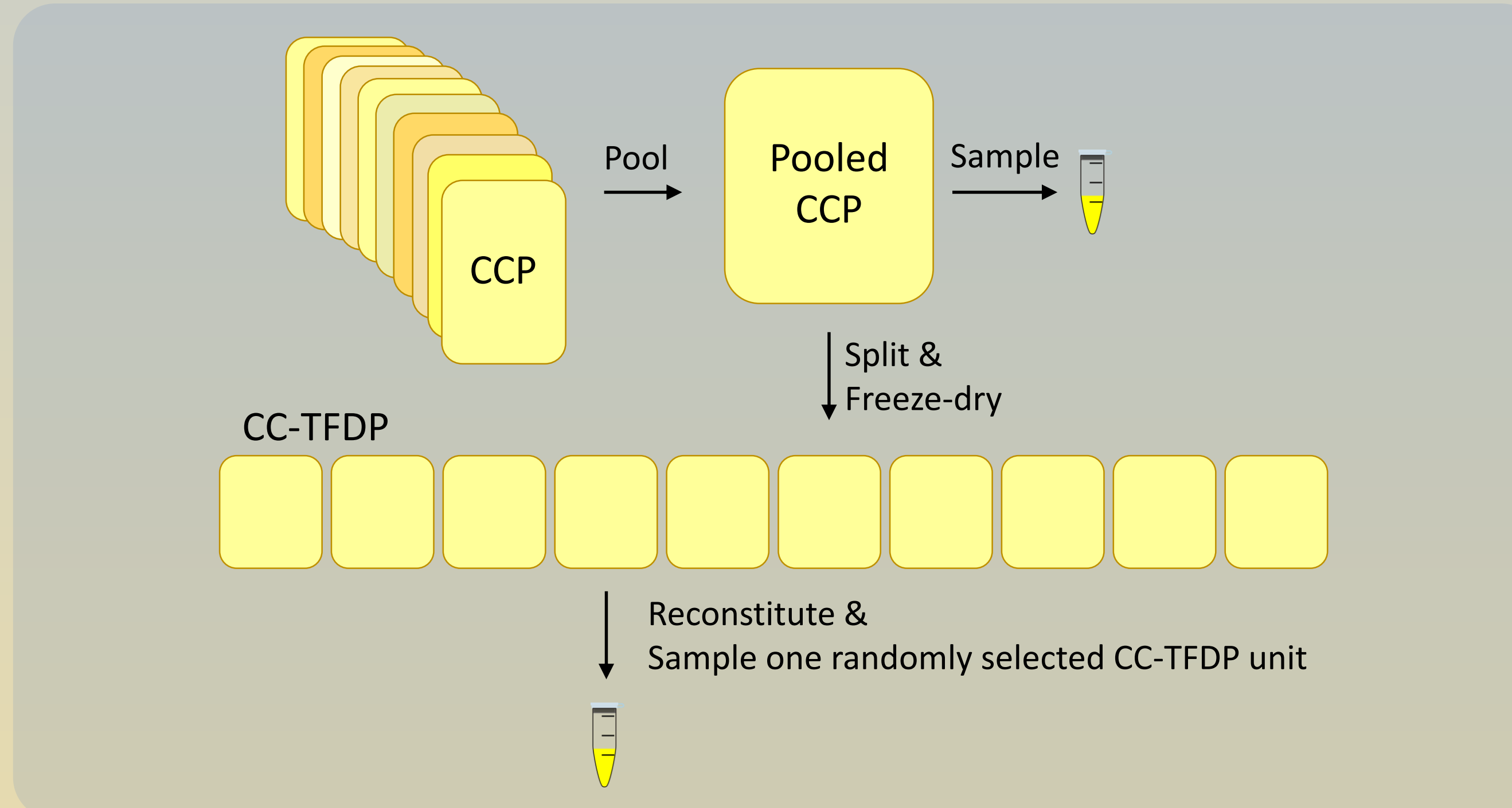
## Background

- Convalescent plasma (CCP) has been anticipated in civilian and military settings as a therapy for COVID-19 patients in early stage disease.
- Utilizing Terumo BCT's freeze-dried plasma (TFDP) technology on CCP may prolong shelf-life and storage logistics.
- The impact of using TFDP technology on neutralizing and protein binding antibody levels remains unknown

## Objective

To determine whether lyophilization affects functional antibody levels and ultimately the availability of CCP for therapeutic use.

## Methods



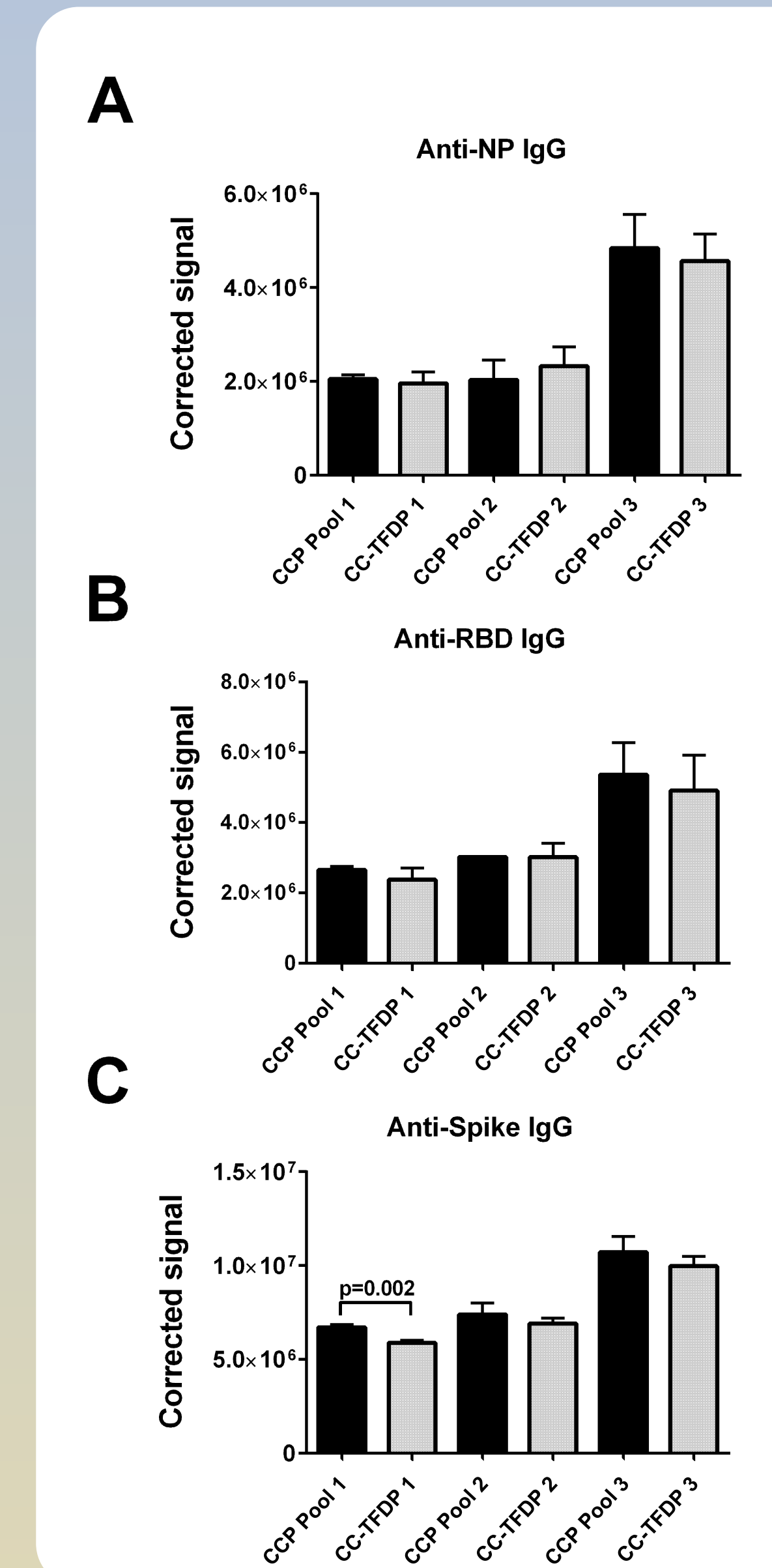
Ten units of CCP were pooled, split, and lyophilized with Terumo's pre-market FDP system to make CC-TFDP. Three pools were examined.

Pooled CCP and reconstituted CC-TFDP were assessed for:

- Anti-SARS-CoV-2 antibody levels to (A) nucleocapsid protein, (B) receptor binding domain of S, and (C) spike glycoprotein by enzyme immunoassay.
- SARS-CoV-2 neutralizing capacity of antibodies by plaque reduction neutralization-50 (PRNT<sub>50</sub>) assays.
- Functional coagulation factor levels and evidence of Complement activation using enzyme immunoassays.

## Results & Discussion

### 1 Anti-SARS-CoV-2 antibody levels



Comparative antibody levels against SARS-CoV-2 nucleocapsid (NP) (A), receptor binding domain (RBD) (B), or spike (C) proteins in CCP pools (black bars) or derived CC-TFDP (grey bars). Error bars indicate mean  $\pm$  SD of three determinations for plasma diluted 1:640.

For the most part, antibody levels were not affected by the lyophilization process (grey versus black bars).

### 2 Anti-SARS-CoV-2 neutralizing capacity

Median titers in the PRNT<sub>50</sub> assays were unaffected by freeze-drying in 2 of three data sets. The third pair of pooled CCP and CC-TFDP had a median titer of 1:80 before freeze-drying and 1:40 after, which given the 4-fold variability of this assay can be discounted. (CCP units were selected for study only if their SARS-CoV-2 PRNT<sub>50</sub> neutralizing antibody titer was at least 1:80.)

### 3 Quality parameters of CCP before and after the lyophilization process

	Pooled CCP <sup>a</sup>	CC-TFDP <sup>a</sup>	% Difference	p value <sup>b,c</sup>
<b>Coagulation factors and coagulation-related protein activities</b>				
Prothrombin (U/mL)	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	-10	NS
Fibrinogen (g/L)	3.0 $\pm$ 0.2	2.9 $\pm$ 0.3	-3.3	NS
Factor V (U/mL)	1.09 $\pm$ 0.08	1.0 $\pm$ 0.1	-8.3	NS
Factor VIII (U/mL)	1.1 $\pm$ 0.3	0.9 $\pm$ 0.3	-18	0.034
Protein S (U/mL)	0.81 $\pm$ 0.06	0.74 $\pm$ 0.08	-8.6	NS
Antithrombin (U/mL)	0.94 $\pm$ 0.03	0.87 $\pm$ 0.03	-7.4	0.0021
<b>Hemostasis screening tests</b>				
PT (sec)	12.8 $\pm$ 0.4	13.2 $\pm$ 0.4	+3.1	NS
APTT (sec)	30 $\pm$ 1	32 $\pm$ 1	+6.6	0.0049
<b>Complement factors</b>				
C3a (ng/ml)	170 $\pm$ 20	200 $\pm$ 30	+18	NS
C5a (ng/ml)	7 $\pm$ 1	6 $\pm$ 2	-14	NS

<sup>a</sup>n=3 (mean  $\pm$  SD), <sup>b</sup>by paired t test, <sup>c</sup>NS; not significant

Coagulation factor activity decreased between 2 and 10% post-lyophilization, except for factor VIII, which decreased by 18%. Prothrombin (PT) and activated partial thromboplastin times (aPTT) were slightly prolonged post-lyophilization (<7%), likely reflecting the small coagulation factor losses. C3a levels slightly increased (NS) post-lyophilization possibly indicating minor complement activation during the process.

## Conclusions

- Anti-SARS-CoV-2 antibody levels (including neutralizing capacity) in CCP remained relatively stable after the freeze-drying process.
- Complement or coagulation protein activities were slightly reduced or in keeping with current standards for transfusion.
- We only investigated SARS-CoV-2 antibodies, however, freeze-dried plasma technology could potentially be applied to convalescent plasma specific for other illnesses.

*Applying freeze-dried plasma technology to COVID-19 Convalescent Plasma allows for maintenance of anti-SARS-CoV-2 antibody levels while simultaneously enabling storage outside of frozen temperatures to facilitate shipment and use both in developing countries and for deployed troops.*

## Acknowledgements

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