

Efficacy of Thawing COVID-Convalescent Plasma using ZipThaw™202

Background

The use of convalescent plasma (CP) collected from previously infected individuals to passively transfer antibodies in order to protect or treat humans dates back almost 100 years. CP has been used both as post-exposure prophylaxis as well as treatment for diverse infectious diseases, notably in times of outbreaks (e.g. polio) and pandemics (e.g. Spanish flu and now COVID-19). Other examples of CP use include rabies, hepatitis B, measles, influenza, Ebola and hemorrhagic fevers. Results from small case series during the prior MERS and SARS coronavirus outbreaks suggested that CP is safe and may confer clinical benefits, including faster viral clearance, particularly when administered early in the disease course.

The vast majority of patients who recover from COVID-19 illness develop circulating antibodies to various SARS-CoV-2 proteins 2-3 weeks following infection. These antibodies appear to be protective, based on several primate studies showing animals could not be re-infected with SARSCoV-2 weeks to months later. Multiple studies have now reported the use of COVID-19 convalescent plasma (CCP) to treat severely or critically ill COVID-19 patients, without unexpected or serious adverse events (see below). Most of these studies have been observational and non-randomized, complicated by evolution of additional treatment interventions over time, such as steroids, antivirals and other drugs; patient heterogeneity; and a lack of detailed analyses of neutralizing antibody content of infused units. Most recently, preliminary efficacy results from 35,000 patients enrolled in a US FDA-sponsored expanded access program coordinated by Mayo Clinic were made available on a preprint server. While many patients improved clinically, the specific role of CCP is unclear, because all patients received at least one additional therapy, including antivirals, antibiotics or antifungals, and/or corticosteroids. Mortality was lower in patients who received CCP within 3 days of diagnosis of COVID-19, and in those who received units of CCP with higher specific IgG levels.

There is only incomplete and very limited randomized controlled trial (RCT) data available to date but they do provide some encouraging evidence for CCP efficacy. All suggest that CCP, preferably with high antibody titer, should be given early, prior to intubation and development of life-threatening inflammatory end organ failure, in order to expedite viral clearance and prevent further tissue damage. Thus, maintaining antibody titer levels in CCP prior to administration will be critical.

What are the potential risks of convalescent plasma for COVID-19 and EUA?

Over 72,000 people have received CCP in the US and many more worldwide: initial safety data has been published for the first 5,000 patients in the US receiving CCP via the expanded access program. Convalescent plasma appears to be a relatively safe intervention. The incidence of severe adverse events was less than 1%, most of which were deemed to be unrelated to CCP, but rather to known general risks of plasma transfusion. On August 23, 2020, the US FDA announced emergency use authorization (EUA) of CCP in hospitalized people with COVID-19.

An EUA is issued when a treatment is deemed safe and showing sufficient potential benefit to justify urgent access to a potentially-lifesaving treatment outside of a clinical trial. The current EUA applies only to hospitalized patients with COVID-19. The EUA language suggests treatment early in disease course, and the use of “high titer” CCP units as measured by specific anti-viral Ig testing and titer threshold criteria. Those CCP units (including those collected prior to the EUA) that have not undergone such testing, or do not meet the titer threshold, will be considered “low titer”, but can still be administered under the EUA per the judgement of the treating clinician.

The logistics of CCP procurement are complex, with plasmapheresis primarily used as the means to collect large volumes of plasma. Importantly, CCP is frozen and processed as FFP (Fresh Frozen Plasma) and PF24 (Plasma Frozen within 24 hrs), both of which are FDA cleared for thawing with ZipThaw™202.

Current limitations

Clinical use of CCP is limited by the following variables:

- ❖ Antibody levels will vary from patient to patient, thus convalescent plasma samples would not all be the same and preservation of existing levels will be important;
- ❖ Currently, Covid-19 Antibody positive plasma is RARE and therefore precious. It cannot be wasted; and
- ❖ Methods that avoid protein degradation and preserve levels of circulating antibody in every plasma sample are lacking.

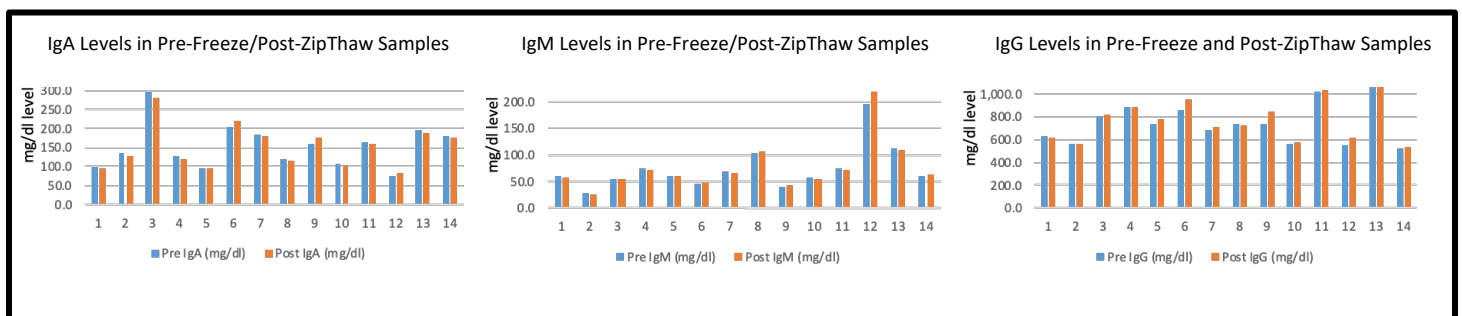
Thus, reliable recovery of antibody from each CCP specimen is necessary to achieve clinical benefit, namely immunization in severe clinical cases. When FFP and PF24 CCP needs to be thawed before transfusing to the patient, prolonged or uncontrolled thawing can denature plasma immunoglobulins. The potential risk of contamination by wet thawing is also a point of concern appreciated by all.

ZipThaw™202 for CCP

ZipThaw™202 is a dual chamber, light weight, hand portable device that is easy to use in nearly any setting from blood/bio bank to point of care to laboratory. The device works with ZipSleeve™, a disposable anti-cross-contamination thawing pouch uniquely equipped with a RFID sensor to safely and accurately measure the actual temperature of the substance being thawed without risk of contamination or exposure to bacteria or hazardous waste.

In 2019, the ZipThaw™202 system was shown to reproducibly and reliably thaw FFP and PF24 with preservation of coagulation factors for clinical use (data submitted to FDA and presented at ASCO and AABB 2019 conferences). We conducted over 1,782 tests on plasma for the following factors: Prothrombin time (PT), International Normalized Ratios (INR), Activated Partial Thromboplastin time (aPTT) Factor VIII activity, Factor V activity, Protein C activity, Protein S antigen, Von Willebrand activity and Thrombin Antithrombin Complex (TAT) concentration. Results demonstrated 100% concordance with no statistically significant differences between the pre-freeze and post-ZipThaw groups. Moreover, the results supported substantial equivalence claims leading to FDA clearance issuance.

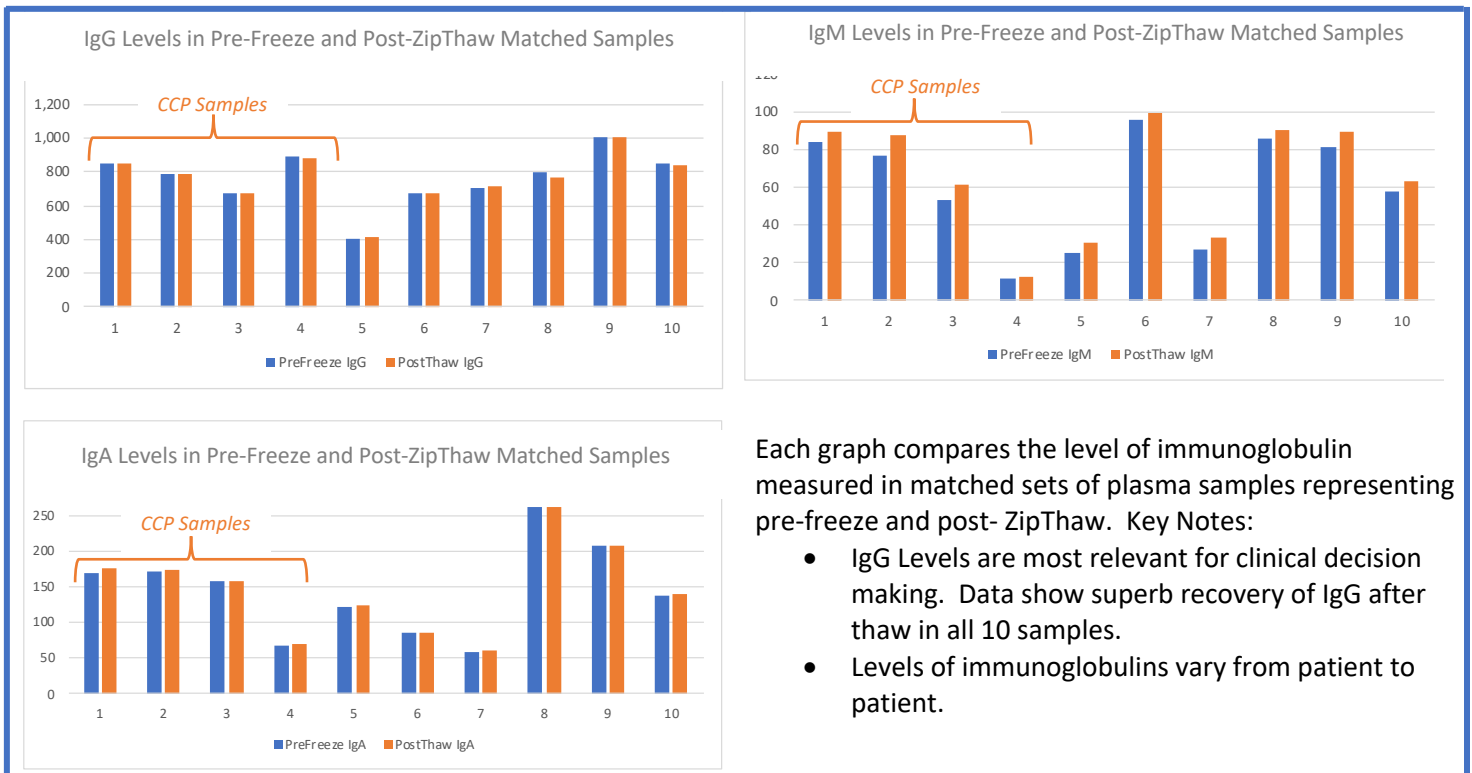
In 2020, and with the emergence of COVID-19, the ZipThaw™202 system was used to thaw convalescent plasma to determine if immunoglobulins could be reliably recovered from frozen plasma adequate for clinical transfusion. In the first study, and in collaboration with the San Diego Blood Bank, 14 grade A plasma specimens (280-350g) from healthy individuals were collected and divided into pre-freeze and post-ZipThaw groups. IgA, IgM, and IgG mg/dl levels were measured across all samples by the CAP accredited UCSD Clinical Chemistry Lab. The graphs below demonstrate 100% concordance with all samples yielding equivalent levels of Immunoglobulins recovered in post-ZipThaw samples as compared to pre-freeze levels.



Less than 1-4% difference was observed between all matched sample pairs of pre (blue) freeze- and post (orange)-ZipThaw samples for each of the immunoglobulins, IgA, IgG and IgM. These data provide proof of concept for reliable recovery of normally circulating antibodies in plasma frozen for clinical transfusion.

In a second study, 10 grade A plasma specimens (222-375g) from COVID-19 recovered (Samples 1-4) and healthy (Samples 5-10) patients were collected and divided into pre-freeze and post-ZipThaw groups as in the first study but blinded to status of COVID. Similarly, IgA, IgM and IgG mg/dl levels were measured across all samples by the UCSD Clinical Chemistry Lab. Samples were then unblinded and analyzed.

The graphs below demonstrate equivalence across all three immunoglobulin levels among each paired set for both the CCP (#1-4) and normal (#5-10) samples.



Each graph compares the level of immunoglobulin measured in matched sets of plasma samples representing pre-freeze and post- ZipThaw. Key Notes:

- IgG Levels are most relevant for clinical decision making. Data show superb recovery of IgG after thaw in all 10 samples.
- Levels of immunoglobulins vary from patient to patient.

Conclusion:

ZipThaw™202 system is demonstrated to be reliable for thawing of precious frozen plasma samples. Whether for standard transfusion or for antibody therapy, the technology is applicable across sample types and enables healthcare works to thaw plasma safely when and where it’s needed, avoiding the need for pre-thawing frozen plasma units which often leads to potential waste and degradation of life saving blood components.

References:

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