Hemostatic Stability of Dried Blood Products During Simulated Arctic Deployment and Extreme Temperature Cycling

Arctic Stability of Dried Blood Products for Combat Casualty Care

Background

As military operations expand into Arctic and other extreme cold regions, there is an urgent need to ensure that medical supplies essential for casualty care remain functional and reliable under harsh environmental conditions. Many critical items—such as blood products, hemostatic dressings, and tourniquets—are developed for temperate climates, and their performance after freezing or repeated freeze–thaw exposures is not well characterized.

An environmental test chamber offers a controlled and repeatable setting to simulate Arctic as well as tropical and humid conditions, enabling comprehensive evaluation and validation of medical products and technologies prior to field deployment in austere environments.

This study examined the hemostatic stability and protein integrity of three dried blood products (DBPs)—freeze-dried plasma (FDP), fibrinogen concentrate (FC) and prothrombin complex concentrate (PCC) following a simulated Arctic deployment and repeated extreme temperature cycling, respectively. These dried blood products have logistic advantages over liquid blood products for prehospital transfusion such as extended shelf-life and storage stability, high portability, no requirement for cold-chain logistics, rapid reconstitution and administration with small infusion volumes, and have been clinically approved for military trauma resuscitation [1, 2].

Materials and Methods

FDP (OctaPlasLG Powder, Octapharma), FC (FIBRYGA, Octapharma) and PCC (Octaplex, Octapharma) were exposed to a simulated Arctic deployment profile over 25 days, including temperatures as low as -30°C and nine freeze–thaw cycles using a programmable environmental chamber (Figure 1 and 2). Furthermore, FIBRYGA and Octaplex were exposed to extreme temperature cycles between +45°C and −55°C, with each temperature held for one day and one-hour ramps between temperature transitions (Figure 3). Products in their original packaging were removed after 6, 15 and 23 cycles for evaluation. Unexposed products stored at 4°C served as controls. All products were reconstituted according to manufacturer instructions.

Hemostatic function was assessed by using rotational thromboelastometry (ROTEM) performed with reconstituted plasma for OctaPlasLG Powder, human plasma with a low concentration of fibrinogen for FIBRYGA and human plasma containing low concentrations of clotting factors II, VII, IX, and X for Octaplex. All tests (NATEM and EXTEM) were run for at least 60 min. The ROTEM variables assessed included clotting time (CT), Alpha angle, maximum clot firmness (MCF) and clot lysis index LI30. Protein integrity was evaluated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis for each sample diluted by 100 times with deionized water and mixed with SDS buffer at an equal volume with or without 5% dithiothreitol (DTT) as a sulfide bond reducer. Total amount of proteins in each sample was quantified by commercial bicinchoninic acid (BCA) protein assay kit according to manufacturer's procedure.

Data are presented as mean ± standard deviation (SD), with n = 3 unless otherwise stated. Parametric data were analyzed using one-way ANOVA and t-tests. All statistical analyses were conducted using SPSS Statistics 28 (IBM Corporation, Armonk, NY, USA). Statistical significance was defined as $p < 0.05$. Values between 0.05 and 0.10 were considered trends.

Results and Discussion

Temperature chamber programming successfully reproduced the variability and extremes recorded during Arctic field trials, including large fluctuations characteristic of operational cold-weather conditions. It is noted that even within the same deployment in the field, the samples were exposed to a large variation in temperatures (as indicated by the big standard deviation). This really highlights the variable field conditions and the importance for a well-controlled lab study.

No glass breakage or package damage noted following exposure in a simulated deployment over 25 days. Reconstitution was completed within 5 minutes for FIBRYGA and 2 minutes for OctaPlasLG Powder and Octaplex, consistent with baseline performance. The ROTEM analyses demonstrated no meaningful changes in clotting time, clot formation rate, clot strength, or fibrinolysis parameters for FDP, FC or PCC after the simulated Arctic deployment (Table 1). Gel electrophoresis revealed identical banding patterns before and after exposure for both FC and PCC products (Figure 4). Minor differences in band intensity were observed but did not indicate significant protein degradation. The non-reduced samples show mainly bands corresponding to very high molecular weights above 250 kDa. The reduced samples exhibit better-resolved bands as indicated by the typical triplet of bands corresponding to α-, β- and γ-chains of fibrinogen [3], and number of bands with molecular weights both higher and lower than the therapeutic proteins [4].

We conducted an accelerated study involving extreme temperature cycling between 45°C and -55°C. No significant changes in reconstitution were observed over the study period. Hemostatic function remained largely unchanged until 23 cycles, when FIBRYGA demonstrated a trend toward reduced clot strength (MCF) and Octaplex showed a trend toward prolonged clotting time (CT) (Table 1). Consistent with these findings, a decrease in total protein content was observed in FIBRYGA after 23 cycles. Notably, three of five glass bottles containing water for FIBRYGA reconstitution fractured during the study.

Figure 1. Programmable temperature test chamber.

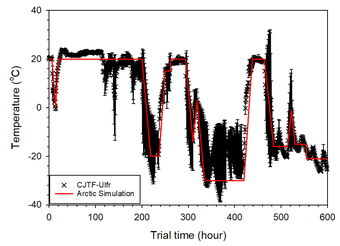

Figure 2. Temperature profiles in the backpack carried by medic during an Arctic deployment (JTFUlfr) and corresponding simulation using the temperature test chamber. Experimental data represent mean and standard deviation (n=2).

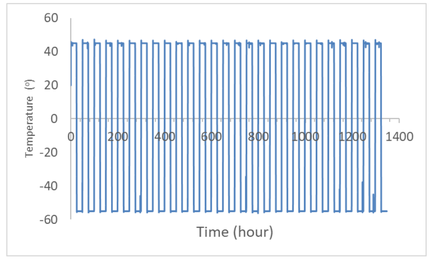

Figure 3. Simulation of extreme temperature cycles between +45°C and −55°C, with each temperature held for one day and one-hour ramps between temperature transitions.

Table 1. Hemostatic properties of dried blood products exposed to a simulated Arctic deployment and extreme temperature cycling conditions.

Products	Exposure conditions	ROTEM				Total amount of proteins (mg/ml)
		CT (sec)	Alpha (°)	MCF (mm)	LI30 (%)	
OctaPlasLG Powder	4°C (Control)	67.0 ± 7.0	75.5 ± 0.6	21.3 ± 2.1	100.0 ± 0.0	43.7 ± 4.5
	Simulated Arctic deployment	67.7 ± 5.1	78.0 ± 1.0	21.0 ± 1.0	100.0 ± 0.0	36.7 ± 3.9
FIBRYGA	4°C (Control)	529.0 ± 40.0	30.0 ± 2.8	8.3 ± 1.2	100.0 ± 0.0	26.6 ± 0.4
	Simulated Arctic deployment	513.7 ± 21.1	29.0 ± 1.7	9.0 ± 0.0	100.0 ± 0.0	27.5 ± 0.4
	Extreme temperatures 15 cycles	481.3 ± 62.1	NA	8.3 ± 2.1	99.7 ± 0.6	25.7 ± 0.9
	Extreme temperatures 23 cycles	501.7 ± 36.1	NA	**6.7 ± 0.6***	100.0 ± 0.0	**16.4 ± 2.2#**
Octaplex	4°C (Control)	46.0 ± 1.4	70.0 ± 5.7	14.0 ± 1.4	100.0 ± 0.0	20.3 ± 0.7
	Simulated Arctic deployment	49.5 ± 3.5	69.0 ± 1.4	12.5 ± 0.7	100.0 ± 0.0	19.8 ± 0.3
	Extreme temperatures 15 cycles	49.7 ± 3.1	73.3 ± 2.5	14.0 ± 0.1	100.0 ± 0.0	18.1 ± 1.6
	Extreme temperatures 23 cycles	**56.8 ± 6.4***	70.2 ± 2.2	13.4 ± 0.5	99.8 ± 0.4	19.2 ± 0.3

ROTEM tests (NATEM and EXTEM) were performed with reconstituted plasma for OctaPlasLG Powder, human plasma with a low concentration of fibrinogen for FIBRYGA and human plasma containing low concentrations of clotting factors II, VII, IX, and X for Octaplex. $p < 0.10$ versus control (*); $p < 0.05$ versus control (#).

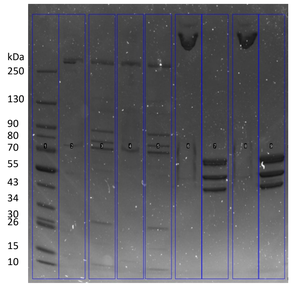

Figure 4. Coomassie-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of FIBRIGA and Octaplex before and after simulated Arctic deployment. Samples were analyzed under non-reducing and reducing conditions (5% DTT). Lanes from left to right: molecular weight markers, Octaplex control, reduced Octaplex control, Arctic-exposed Octaplex, reduced Arctic-exposed Octaplex, FIBRYGA control, reduced FIBRYGA control, Arctic-exposed FIBRYGA, and reduced Arctic-exposed FIBRYGA.

Main Findings

The key finding of this study is that commercially available dried blood products, including OctaPlasLG Powder, FIBRYGA, and Octaplex, maintained their hemostatic function following a simulated Arctic deployment.

These products (FIBRYGA and Octaplex) remained hemostatically stable through up to 23 extreme freeze-heat cycles, with only modest trends toward reduced clot strength (MCF), prolonged clotting time (CT), and decreased total protein content observed after prolonged exposure.

The glass bottles containing water for reconstitution were more vulnerable to extreme freeze-heat cycles than the dried products themselves. Reliable thawing strategies for reconstitution water require further investigation.

While hemostatic function remained largely preserved, other product characteristics may be affected by repeated temperature cycling and warrant further evaluation.

Conclusions

- Dried blood products, including freeze-dried plasma, fibrinogen concentrate and prothrombin complex concentrate, maintained hemostatic function and protein integrity following a simulated 25-day Arctic deployment and through 15 freeze-heat cycles.
- These preliminary findings support the robustness of dried clotting factor concentrates for use in extreme cold environments. Ongoing studies are extending testing to longer exposure durations and additional Arctic stress profiles to generate comprehensive stability data that will help inform cold-weather military medical logistics and trauma resuscitation planning.
- Further investigations are warranted to evaluate the effects of Arctic environmental conditions on a broader range of medical products and technologies intended for operational deployment.

References

1 Peng, H.; Rhind, S.; Kanwal, S.; Zhang, J.; Beckett, A. Transfusion strategies for trauma resuscitation: A historical and comparative analysis. DRDC–RDDC–2026–R2066.
2 Peng, H. T. (2020). Hemostatic agents for prehospital hemorrhage control: a narrative review. Mil Med Res 7(1), Art 13.
3 Schulz, P.M.; Gehringer, W.; Nöhring, S.; Müller, S.; Schmidt, T.; Kekeiss-Schertler, S.; Solomon, C.; Pock, K.; Römisch, J. Biochemical characterization, stability, and pathogen safety of a new fibrinogen concentrate (fibryga®). Biologicals 2018, 52, 72–77.
4 Kalina, U.; Bickhard, H.; Schulte, S. Biochemical comparison of seven commercially available prothrombin complex concentrates. Int J Clin Pract 2008, 62, 1614–1622.

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