Analysis of Canadian Dried Serum from World War II: Advancing Freeze-Dried Plasma for Prehospital Management of Haemorrhage

UNIVERSITY OF TORONTO

THE UNIVERSITY OF BRITISH COLUMBIA

The 12th Norman Bethune Symposium

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Introduction

During World War II, Charles Best employed Charles Drew's technique of isolating and dehydrating plasma to lead Canada's initiative in providing dried serum for treating frontline casualties. The use of serum was preferred over plasma due to its capacity to increase volume without clotting during extended storage. Upon rehydrating the dried serum in 1943, we found that it contained intact albumin, anti-thrombin, plasminogen, protein C, and protein S activities. We obtained an original unopened bottle containing dried serum from the Connaught Campus of Sanofi Canada (Toronto, Canada), produced on 19 February 1943 from voluntary Canadian donors by the University of Toronto/ Canadian Red Cross Society. The 1943 sample was obtained by drying a large donor pool containing 48 L of serum and subsequently aliquoted into individual bottles [1, 2]. Our goals were to reconstitute and analyse the content of Canadian dried serum for the presence of hepatitis B virus (HBV) and assess its haematological properties. We aimed to highlight the advancements of freeze-drying technology using the latest iteration of Canadian freeze-dried plasma (CFDP; Terumo BCT) [3], and its importance in managing modern prehospital haemorrhage.

Methods

We investigated Canadian dried serum from 1943, Canadian freeze-dried plasma (CFDP), and freshly prepared pooled human serum (n=10) for control. Samples were tested using rotational thromboelastometry (ROTEM) and Stago to analyze coagulation and fibrinolysis parameters. Prothrombin time (PT) and partial thromboplastin time (PTT) were determined. The 1943 sample and serum control were tested for HBV DNA, antigen, antibodies, and further examined using tandem mass spectrometry (LC MS/MS) proteomics.

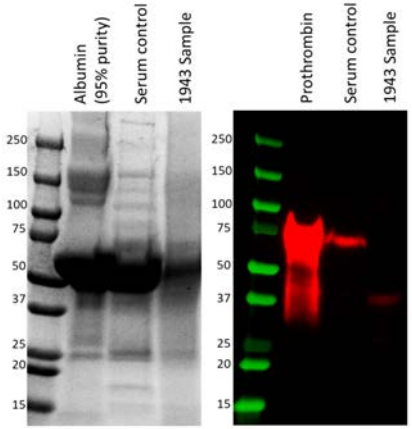


Figure 2. Comprehensive analysis of the 1943 sample using Coomassie blue staining method (left panel), and anti-prothrombin Western blot (right panel). Coomassie controls: human albumin and serum control. Western blot controls: recombinant prothrombin, serum control. SDS-PAGE (4-20%) under reducing conditions.

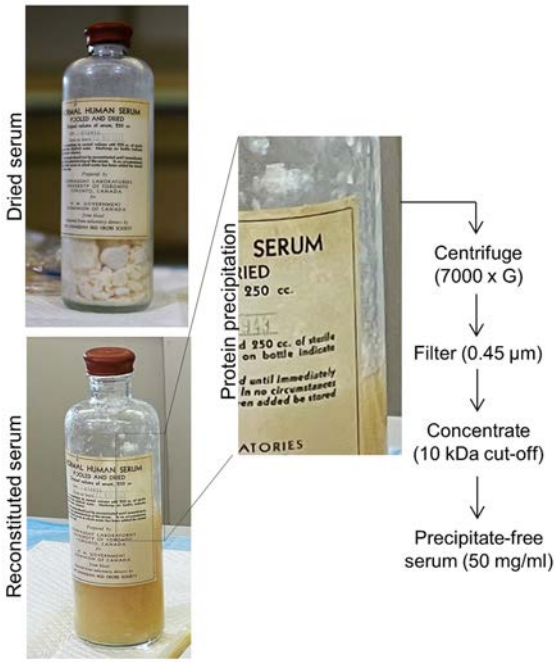
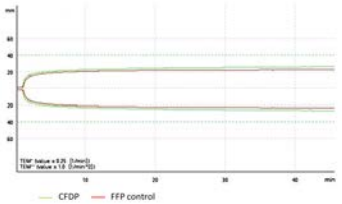


Figure 1. Images of the 1943 sample before and after reconstitution. The 1943 sample was reconstituted with 250 mL of sterile water. Reconstitution resulted in a heterogeneous solution with approximately 50% (w/v) protein precipitation. Soluble portion of the mixture was isolated (50 mg/mL) for further analyses [1].



EXTEM parameters	CFDP	FFP Control	1943 Sample	Serum Control
CT (s)	54	59	-	-
CFT (s)	173	128	-	-
α (°)	83	80	-	-
A10 (mm)	23	21	-	-
A20 (mm)	25	22	-	-
MCF (mm)	26	23	-	-
LI30 (%)	100	100	-	-

Figure 3. Extrinsic pathway (EXTEM) analysis of Canadian freeze-dried plasma (CFDP). EXTEM profile (left) and acquired values (right) of CFDP prototype and fresh frozen plasma (FFP) control. EXTEM profiles of 1943 sample and serum control were below detection limits [1].

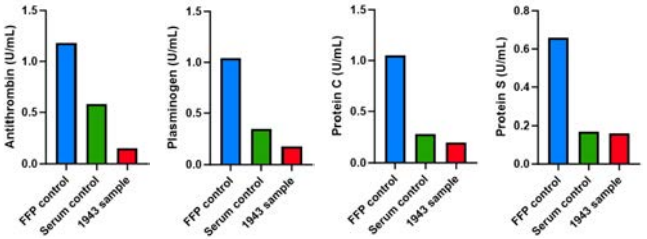


Figure 4. PT, PTT, fibrinogen, FVIII, anti-thrombin, plasminogen, protein C and protein S of 1943 sample was tested using Stago hemostasis analyzer. Pooled serum and FFP were used as controls. PT, PTT, fibrinogen and FVIII values of 1943 sample and serum control were below detection limits. FFP, fresh frozen plasma; PT, prothrombin time; PTT, partial thromboplastin time.

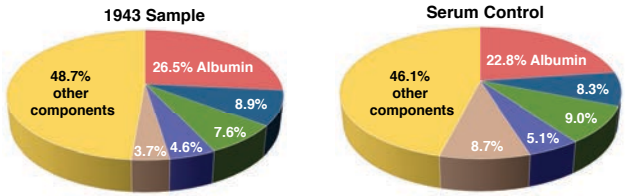


Figure 5. A total of 71 proteins were identified through proteomics analysis. The pie charts highlights the top five proteins, with albumin (red) being the most predominant, followed by serotransferrin (blue), complement C3 (green), component C4-B, alpha-2-macroglobulin (purple), hemopexin (wheat) [1].

Conclusions

Serological testing confirmed hepatitis B. Stago hemostasis analysis showed similar levels of anti-thrombin, plasminogen, protein C, and protein S in the 1943 sample and serum control, lower than the FFP control. PT, PTT, fibrinogen, and FVIII parameters were undetectable in both the 1943 sample and serum control. The CFDP's extrinsic clotting capacity was compared to fresh plasma, serum controls, and the 1943 sample, with the CFDP showing a similar profile to the FFP control. Our analysis shows CFDP clotting capacity is comparable to FFP after 4 years of storage. Our findings demonstrate the advantages of CFDP technology and its potential for managing bleeding diathesis.

References

[1] Singh K, Peng HT, Moes K, Kretz CA, Beckett AN. *Br J Haematol.* 2024: 1 - 8. DOI: 10.1111/bjh.19298.
[2] Best CH, Solandt DY, Ridout JH. *Blood Substitutes and Blood Transfusion.* 1942. Ch. 26, 235 - 241.
[3] Sheffield WP, Singh K, Beckett AN, and Devine, DV. *Transfusion Med. Reviews.* 2024: 38 (1) 150807.

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