Comparative Analysis of Commercial Factor VIII (FVIII) Deficient Plasmas With and Without von Willebrand Factor for FVIII One-Stage Clotting Assay (OSA)

Abstract ID: 455

Stephanie Henneberry, Mushfiqur Rahman, Amanda Wood, K. Maria Notini,* Karen M. Black, Ali Sadeghi-Khomami
Precision BioLogic Inc., Dartmouth, Nova Scotia, Canada
*Clinical Laboratories, Duke University Medical Center, Durham, North Carolina, USA

Presented at ISLH 2025
May 7–9, 2025

PrecisionBioLogic

Background

Commercial FVIII-deficient plasmas serve as substitutes for congenital severe hemophilia A (HA) plasma in the FVIII-OSA. These plasmas are produced using various controlled methods, including immunodepletion, cryoprecipitation, and chemical treatment. Ideally, substrate plasma should contain normal levels of all coagulation factors except the analyte of interest, ensuring compatibility with APTT reagents, including contact factor activators and phospholipids.

A FVIII:C level of <1% alone is an insufficient criterion to mimic FVIII-deficient plasma. Variations in plasma matrix composition can influence clotting performance, leading to discrepancies in OSA measurements. Previously, we reported underestimated FVIII-inhibitor titer measurements by modified Nijmegen-Bethesda assays if noticeable inactive FVIII (FVIII:Ag) remains in the FVIII-deficient plasma.[1]

Here, we employ a multidisciplinary analytical approach to compare the composition of commercial FVIII-deficient plasmas and identify their differences.

Objective

To characterize multiple commercial FVIII-deficient plasmas, with and without von Willebrand factor (VWF), for potential analytical markers including routine coagulation assays, factor activity measurements, pH, osmolality, albumin, citrate concentration and elemental composition.

Methods

We analyzed both lyophilized [Siemens, Werfen (IL)] and frozen [George King, HRF, and Precision BioLogic Inc. (PBI)] FVIII-deficient plasma products **(Table 1)**.

Testing included routine coagulation assays (HemosIL RecombiPlasTin 2G PT, aPTT-SP, Stago STA-Fibrinogen) and various coagulation factor activities using PBI's factor deficient plasmas and CRYO*check*™ Normal Reference Plasma (calibrator) on a Stago STA-R Max3 analyzer (Stago OK diluent, 25 mM $CaCl_2$).

Citrate concentration of plasma samples was quantified by an optimized Megazyme Citric Acid kit. Albumin, measured by Beckman Coulter Unicel DxC 600/800, Bromocresol Purple timed endpoint, and Osmolality, measured by Osmometer to detect the osmotic freezing point depression, were performed by Duke University, Durham, NC. Quantification of elements of interest (Ca^{2+}, Mg^{2+}, Cu^{2+}, Na^{+}, K^{+} and Cl^{-}) was measured by ICP-OES at Dalhousie University, Halifax, NS.

Recovery of FVIII replacement products spiked in HRF pooled congenital severe HA plasmas were measured by three FVIII-deficient substrate plasmas (PBI FDP08, PBI FDP08VWF and HRF pooled severe HA plasma) using IL SynthASil and 20 mM $CaCl_2$ on the ACL TOP 700 analyzer (neat dilution). The assays were calibrated using PBI CRYO*check* Normal Reference Plasma (CCNRP), IL factor diluent and the corresponding FVIII-deficient substrate plasma following IL's FVIII test configuration.[2]

Results

All examined FVIII-deficient products had FVIII activity <1% or IU/dL, with pH ranging from 7.7 to 8.2.

Analysis of multiple lots of products indicated that Siemens lyophilized FVIII-deficient plasma had the lowest overall coagulation factor activities (ranging from ~57–100%, FV ~57%), while IL lyophilized FVIII-deficient plasma had an exceptionally prolonged PT (~20 sec) and lowest FV activity (~50%) **(Figure 1)**.

Lyophilized plasma products had higher osmolality than frozen plasmas (450 vs. 350 mOs/kg), probably due to the inclusion of lyophilizing agents, while albumin levels remained consistent (~3.1 g/dL) **(Table 2)**.

The elemental analysis revealed distinct characteristics of the IL lyophilized FVIII-deficient plasma compared to other samples: low Ca^{2+} (3 vs. 80 mg/L), Mg^{2+} (5 vs. 17 mg/L), and Na^{+} (800 vs. 4000 mg/L), but elevated K^{+} (3000 vs. 150 mg/L) levels, a signature of K-EDTA plasma.[3] The citrate concentration of IL's product was near the citrate assay's limit of quantification (~0.5 mM), significantly lower than Siemens' (~9 mM), HRF and PBI (~11 mM). These findings were supported by qualitative NMR and Mass spectrometry (data not presented).

Recovery of FVIII replacement products spiked in HRF congenital severe HA plasma (5–100 IU/dL) showed similar performance between HRF pooled congenital severe HA plasma and PBI's FVIII-deficient substrate plasma products on the ACL TOP/SynthASil assay system, with Altuviiio and Afstyla being underestimated **(Figure 2)**.

The VWF content in FVIII-deficient substrate plasma could dose-dependently impact accurate activity measurements of some FVIII replacement therapies depending on the molecular motifs of these FVIII products. For instance, at peak FVIII levels, VWF could boost (Jivi), suppress (Eloctate and Obizur) or not influence (Altuviiio, Afstyla, Wilate, Advate, Novoeight) activity measurements **(Figure 3)**.

Conclusions

- Werfen-IL lyophilized FVIII-deficient with VWF plasma product is not a citrated plasma, has prolonged PT, reduced FV activity and low total calcium concentration. It is possibly treated with EDTA, and was previously determined to contain residual FVIII:Ag >50%[1], which interferes with FVIII-inhibitor assays.
- Siemens lyophilized FVIII-deficient plasma (FVIII:Ag >50%, VWF:Ac <30%) showed multiple mid range factor activities, posing challenges for calibration reproducibility at low FVIII levels on BCS® XP/Actin FSL.
- PBI's FDP08VWF (FVIII:Ac and Ag <1%) with VWF:Ac and Ag ≥50%, more closely resembles congenital severe HA plasma substrates, making it a promising candidate for FVIII-OSA on various analyzers.
- Further standardization of FVIII-OSA and defined specifications for FVIII-deficient substrate plasma are needed to improve assay harmonization.

References

1. Wood A, Kesavan N, Sadeghi-Khomami A, Black K, Boylan M, Ni R, Della Maestra J, Erb P, Foulon D, Hoogendoorn H. (2021). The impact of inactive FVIII antigen in factor VIII deficient plasma on the measurement of FVIII inhibitors [Poster presentation PB0340]. ISTH 2021 Congress.
2. Kesavan N, Rahman M, Sadeghi-Khomami A, Black K, Wood A. (2022). Performance of factor VIII deficient plasma with VWF in the activity measurement of FVIII replacement products in plasma samples using an OSC assay [Poster presentation PB1177]. ISTH 2022 Congress, London, England.
3. Lippi G, Salvagno GL, Adcock DM, Gelati M, Guidi GC, Favaloro EJ. Right or wrong sample received for coagulation testing? Tentative algorithms for detection of an incorrect type of sample. (2010). International Journal of Laboratory Hematology, 32:1p2, 132–138.

Table 1

FVIII-deficient plasma used in each analysis.

Analysis	Plasma	Supplier	Part No. (CDN)	Lot No.	Format
Coagulation Assay (**also for FVIII recovery assay)	Pooled congenital severe HA donors	HRF (5 unique donors)	Internally prepared	Pool 1 Pool 2**	Frozen
	Congenital HA Plasma	George King BioMedical	0800	6941	Frozen
	HemosIL FVIII Deficient Plasma (normal VWF)	Werfen (IL)	00020012800	N0320811 N0101339	Lyophilized
	Factor VIII Deficient Plasma	Siemens	10446411	560835 560838 560856	Lyophilized
	Factor VIII Deficient Plasma	Precision BioLogic	FDP08	D8-98**	Frozen
	Factor VIII Deficient Plasma with VWF	Precision BioLogic	FDP08VWF	D8W-07** 11-178 11-179	Frozen
Other Analytes	Pooled congenital severe HA donors	HRF (5 unique donors)	Internally prepared	Pool 2	Frozen
	HemosIL FVIII Deficient Plasma (normal VWF)	Werfen (IL)	00020012800	No138549	Lyophilized
	Factor VIII Deficient Plasma	Precision BioLogic	FDP08	D8-98	Frozen
	Factor VIII Deficient Plasma with VWF	Precision BioLogic	FDP08VWF	D8W-07	Frozen
	Factor VIII Deficient Plasma	Siemens	10446411	560849	Lyophilized

Figure 1

Grand mean of routine coagulation and factor activity assay measurements for four FVIII-deficient plasmas (PBI FDP08VWF, PBI FDP08, Siemens FVIII-deficient, and IL FVIII-deficient with VWF) and two congenital severe HA FVIII-deficient plasmas (George King congenital FVIII-deficient and HRF pooled congenital severe HA plasmas). Each lot was tested in triplicate (N=3). Error bars denote ±SD of grand mean.

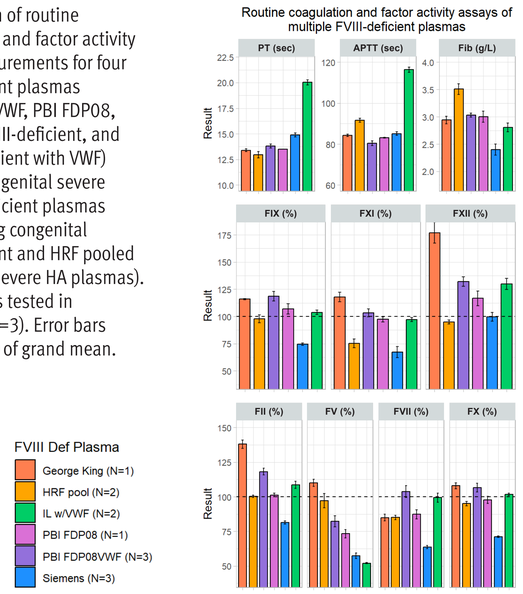


Figure 2

Grand mean ±SD recovery of seven doses (5-10-20-40-60-80-100 IU/dL) of FVIII replacement products spiked in a pool of congenital severe HA plasma and assayed in triplicate (N=3×7) using IL SynthASil on the ACL TOP 700 analyzer (neat dilution) with various FVIII-deficient substrate: Precision BioLogic FDP08 (no VWF), FDP08VWF, and HRF pooled severe HA plasmas. Dashed lines indicate 100 ±25% grand mean recovery in the range of 5–100 IU/dL.

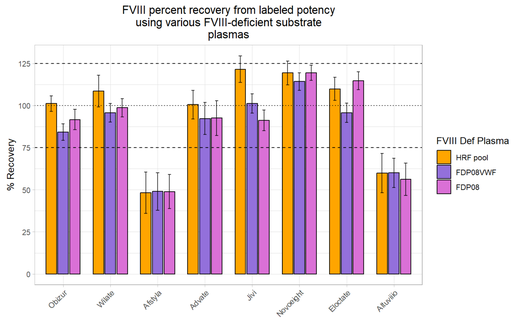
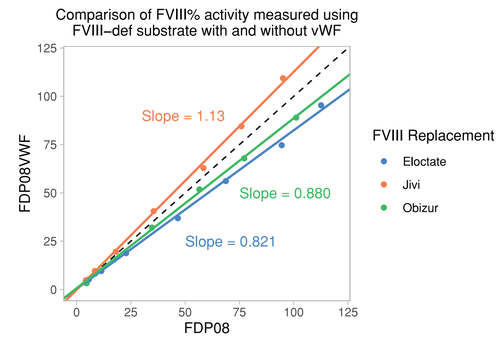


Figure 3

Comparison of FVIII% activity measured for three FVIII replacement therapies using IL SynthASil on the ACL TOP 700 analyzer with PBI FVIII deficient plasma, with and without VWF (FDP08VWF and FDP08, respectively). Plasma samples containing Eloctate, Jivi and Obizur in a pool of congenital severe HA plasma at seven doses (5-10-20-40-60-80-100 IU/dL) were measured by neat dilution (N=3). The black dashed line indicates the identity line.



Plasma	pH	Albumin (g/dL)	Osmolality (mOsm/kg)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Cu^{2+} (mg/L)	Na^{+} (mg/L)	K^{+} (mg/L)	Cl^{-} (mg/L)	Citrate (mM)
Pooled HRF	8.2	NA	NA	88.8	17.2	0.86	3901	153	3527	10.83
IL w/ VWF	7.9	3.1	448	2.71	4.4	0.88	772	3139	3013	0.46
Siemens	7.7	3.1	418	74.9	14.4	0.84	3623	126	2978	8.94
PBI	8.2	3	340	86.2	16.2	1.02	4506	140	4591	10.89
PBI w/ VWF	8.1	3	303	86.4	16.9	1.12	4039	148	3643	11.35

Table 2

Analytical measurements for four commercial FVIII-deficient plasmas and one pooled congenital severe HA plasma.