

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

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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 *crvocheck*™ Normal Reference Plasma (calibrator) on a Stago STA-R Max3 analyzer (Stago OK diluent, 25 mM CaCl₂).

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 (Ca²⁺, Mg²⁺, Cu²⁺, 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 CaCl₂ on the ACL TOP 700 analyzer (neat dilution). The assays were calibrated using PBI *crvocheck* 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 Ca²⁺ (3 vs. 80 mg/L), Mg²⁺ (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 Altuviio 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 (Altuviio, 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%, 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

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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.

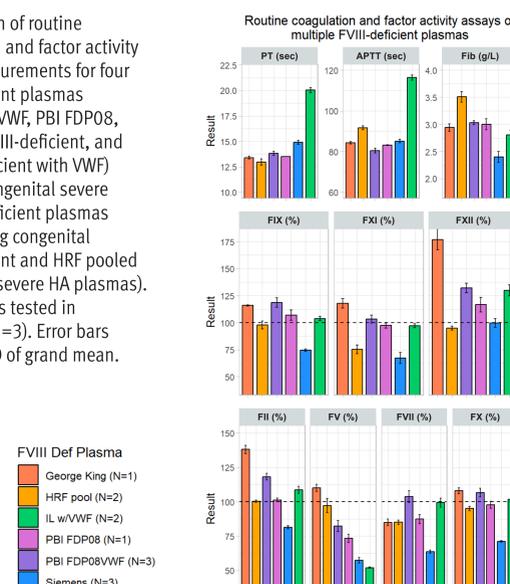


Figure 2

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=3x7) 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.

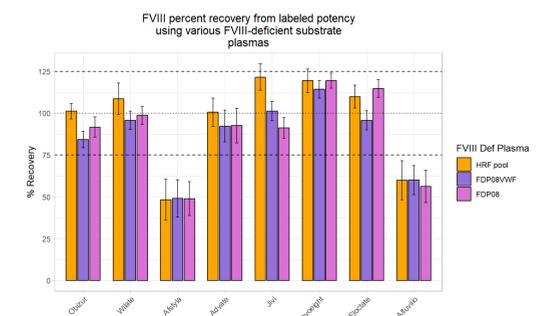
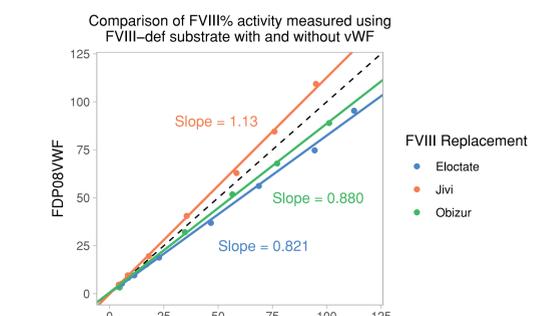


Figure 3

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.