# The Impact of Inactive FVIII Antigen in Factor VIII Deficient Plasma on the Measurement of FVIII Inhibitors

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## Introduction

Congenital factor VIII (FVIII) deficient plasma is considered ideal for use in quantifying FVIII or FVIII inhibitors in hemophilia A patients as it provides a substrate that matches the intended patient sample matrix. Access to congenital FVIII deficient plasma is limited and has high donor variability. Manufactured FVIII deficient plasma from chemically or immuno-depleted normal plasma varies in comparison as some lack von Willebrand Factor (VWF) or contain significant inactive FVIII antigen.

## Aim

To evaluate the FVIII and VWF content of nine commercial FVIII deficient plasmas and to compare two manufactured FVIII deficient plasmas, based on their normal VWF content and varying FVIII antigen content (Instrumentation Laboratory and Precision BioLogic Inc.) to the performance of congenital FVIII deficient plasma (George King Bio-Medical, Inc.) in their application as a sample diluent in the Nijmegen Bethesda Assay.

## Method

A single lot of nine FVIII deficient plasmas (Siemens' Coagulation Factor VIII Deficient, Instrumentation Laboratory's two HemosIL Factor VIII Deficient Plasmas, Diagnostica Stago's STA-ImmunoDef VIII and STA Deficient VIII, George King Bio-Medical's Congenital Factor VIII Deficient Plasma, Hyphen Biomed Factor VIII:C Deficient Plasma, Precision BioLogic's CRYO*check*™ Factor VIII Deficient Plasma with VWF and CRYO*check* Congenital Factor VIII Deficient Plasma) were compared, by evaluating the VWF and FVIII content of each.

VWF Activity (VWF:Ac), VWF Antigen (VWF:Ag), FVIII Activity (FVIII:Ac) was assessed in triplicate using Siemens Innovance VWF:Ac Kit on the BCS XP, Stago's VWF LIATEST on the STA-R Evolution, and HemosIL APTT-SP clotting assay with Precision Biologic's CRYO*check* Factor VIII Deficient Plasma on the STA-R Evolution respectively. FVIII Antigen (FVIII:Ag) was assessed in duplicate of two different dilutions (n=4) using Affinity Biologicals' VisuLize FVIII Antigen Kit.

Based on this comparison, two representative FVIII deficient plasmas (CRYO*check* Factor VIII Deficient Plasma with VWF and HemosIL Factor VIII Deficient Plasma (with VWF)) with varying FVIII antigen content were compared to George King's congenital, when used as a sample diluent in the Nijmegan Bethesda Assay to quantify FVIII inhibitor in low (~1.5 BU) and mid (~5 BU) FVIII inhibitor samples. Four replicate dilutions were prepared and each analysed five times for FVIII activity using Stago's CK Prest APTT and Affinity Biologicals' Factor VIII Deficient on the STA-R Evolution. Inhibitor titers were subsequently calculated according to Nijmegen Bethesda Assay for each sample type (n=20) based on residual FVIII activity closest to 50%.

## Results

All evaluated FVIII deficient plasmas were deficient in FVIII activity (<1%), but varied in VWF (activity and antigen) and FVIII antigen content, as displayed in **Table 1** and **Figure 1**. Some products have normal levels of VWF antigen, but lack in normal VWF activity.

Two manufactured FVIII deficient plasmas containing VWF (with varying inactive FVIII antigen) and George King's congenital FVIII deficient plasma were used as a sample diluent in the Nijmegen Bethesda Assay to quantify FVIII inhibitor titers. These deficient plasmas provided variable results as outlined in **Figure 2**. FVIII deficient plasma with significant (>100%) inactive FVIII antigen present provided an underestimation of FVIII inhibitors in comparison to congenital FVIII deficient plasma.

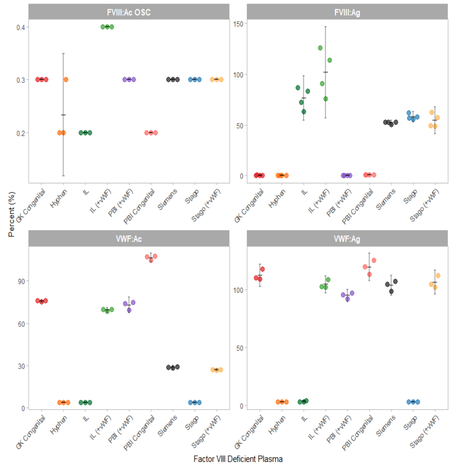
### Table 1

**Comparison of VWF and FVIII content in commercial FVIII deficient plasma products. Mean results displayed (1SD), all assayed in triplicate except FVIII:Ag (n=4).**

| FVIII Deficient Supplier | Catalogue Number | Graph Name | FVIII:Ac (%) | FVIII:Ag (%) | VWF:Ac (%) | VWF:Ag (%) |
|---|---|---|---|---|---|---|
| George King | 0800 | GK Congenital | <1 (0.00) | <1 (0.28) | 76 (0.38) | 112 (4.8) |
| Hyphen Biomed | DP040K | Hyphen | <1 (0.06) | <1 (0.01) | <4 (0.00) | <3 (0.0) |
| Instrumentation Laboratory | 0020012800 | IL (+VWF) | <1 (0.00) | 102 (22.5) | 70 (0.78) | 105 (3.8) |
| | 0020011800 | IL | <1 (0.00) | 76 (10.9) | <4 (0.00) | 3 (0.38) |
| Precision BioLogic | FDP08VWF-15 | PBI (+VWF) | <1 (0.00) | <1 (0.01) | 73 (2.84) | 95 (2.6) |
| | FDP08C-10 | PBI Congenital | <1 (0.00) | <1 (0.12) | 106 (1.55) | 120 (5.9) |
| Siemens | 10446411 | Siemens | <1 (0.00) | 52 (1.3) | 29 (0.60) | 104 (4.3) |
| Diagnostica Stago | 00728 | Stago (+VWF) | <1 (0.00) | 58 (2.6) | 27 (0.32) | 107 (5.2) |
| | 00725 | Stago | <1 (0.00) | 54 (6.7) | <4 (0.0) | <3 (0.0) |

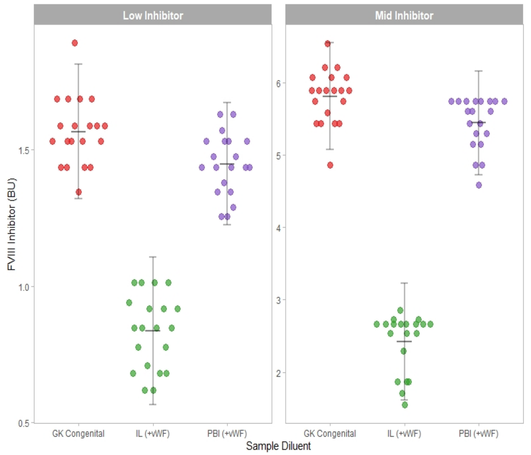
### Figure 1

**Comparison of VWF and FVIII content in commercial FVIII deficient plasma products. All assayed in triplicate except FVIII:Ag (n=4).**




### Figure 2

**The impact of FVIII:Ag in quantification of FVIII inhibitor. Commercial FVIII deficient (with VWF) performance comparison when used as a sample diluent in Nijmegen Bethesda Assay to quantify two FVIII inhibitor titers (n=20).**




## Conclusions

The findings of this study support the view that congenital FVIII deficient plasma is the ideal substrate for FVIII activity and FVIII inhibitor assays.

The suitability of commercially manufactured FVIII deficient plasmas is shown to be quite variable, even in those products containing normal levels of VWF antigen. Of these, the new manufactured FVIII deficient plasma with VWF from Precision BioLogic performed best with the highest proportion of active VWF, the lowest amount of inactive FVIII antigen and the highest recovery of FVIII inhibitor activity relative to congenital FVIII deficient plasma.

The use of other manufactured FVIII deficient plasmas as a sample diluent in the Nijmegen Bethesda Assay could result in an underestimation of FVIII inhibitor due to significant inactive FVIII antigen.

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