Comparative Recovery of FVIII Antigen with Various FVIII Therapeutic Concentrates Using an Improved 2nd Generation ELISA Kit

Abstract: PB0471

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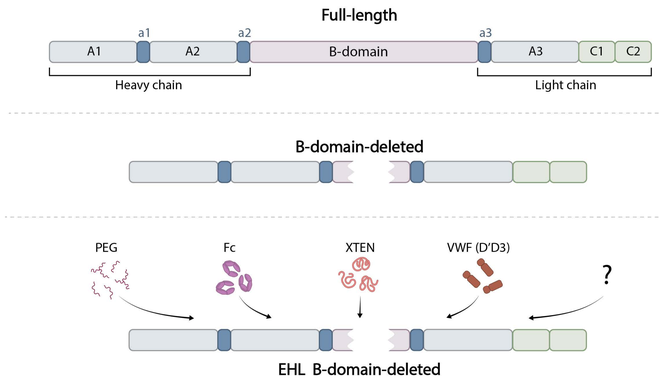
Presented at ISTH 2021
July 17–21, 2021

Introduction

Treatment of hemophilia A has trended towards the use of truncated forms of Factor VIII (FVIII) where most or all of the B-domain has been deleted (BDD-FVIII), while FVIII coagulant activity is retained. More recently, extended half-life (EHL) versions of BDD-FVIII have become available, employing the strategic additions of polyethylene glycol (PEG), dimeric Fc, XTen linker, and von Willebrand factor (VWF)-binding fragment to improve the *in vivo* activity and survival.

Figure 1



The use of various BDD-FVIII constructs present challenges for existing commercial FVIII antigen kits, in that their recognition can be significantly reduced. A new 2nd generation ELISA kit (VisuLize™ Factor VIII Antigen PLUS Kit) was developed with the intent to provide a more uniform recognition of native, full-length, and BDD-FVIII preparations, using a WHO-traceable plasma-based calibrator. The method and assay performance of the 1st (VisuLize Factor VIII Antigen Kit) and 2nd generation kits (VisuLize Factor VIII Antigen PLUS Kit) are similar with regard to detection sensitivity (0.008 IU/mL) and non-interference by VWF. The distinguishing property of the new kit is the improved recognition of BDD-FVIII.

Aim

We have previously shown that the 2nd generation ELISA kit (VisuLize Factor VIII Antigen PLUS Kit) accurately recovered a full-length FVIII (Kogenate®) and a BDD-FVIII (Xyntha®) (Haemophilia. 2021 Feb;27(S2):23). In this study, we assessed the FVIII recognition of various FVIII therapeutic products available, including those with PEG and dimeric Fc modifications, in both the 1st and 2nd generation FVIII antigen kits.

Methods and Results

Plasma-derived (pdFVIII), recombinant full-length (rflFVIII) and BDD-FVIII replacement products were reconstituted and spiked into congenital FVIII-deficient plasma to achieve activity levels of 1.0, 0.3, and 0.05 IU/mL based on the product assigned activity potency. FVIII antigen in normal pooled plasma (NPP) and spiked samples was measured in both the 1st and 2nd generation antigen kits. FVIII activity of NPP was measured by one-stage clotting assay. Results were expressed as the ratio of FVIII antigen to activity.

The antigen:activity (Ag:Ac) ratios obtained for NPP were elevated; consistent with the ratio (1.53) of FVIII antigen to activity assigned to the WHO 6th International Standard for FVIII. For the pdFVIII and rflFVIII products, similar recoveries were obtained by both kits and were consistent with the expected activity. Not surprisingly, both kits did not recognize porcine rFVIII. The BDD-FVIII products tested in the 1st generation kit were significantly under-recovered, in contrast, these samples tested in the 2nd generation kit (VisuLize Factor VIII Antigen PLUS Kit) demonstrated considerably higher recoveries, with antigen values more in line with the expected activity values.

Conclusions

Different constructs and molecular modifications to the FVIII molecule can alter immunoassay recognition and lead to under-recovery when measured relative to a plasma calibrator.

Even though we have not yet reached perfection in the journey towards harmonizing the quantification of all therapeutic FVIII products, we verified significantly improved recognition of BDD-FVIII products using the VisuLize Factor VIII Antigen PLUS Kit and demonstrated its versatility in modern hemophilia research.

Table 1

Ratio of FVIII Antigen Determined by VisuLize Factor VIII Antigen Kit (1st gen kit) and VisuLize Factor VIII Antigen PLUS Kit (2nd gen kit) to Assigned FVIII Activity Potency for Normal Pooled Plasma, Full-Length and B-Domain Deleted FVIII Replacement Product-Spiked Samples.

FVIII Sample	Description	FVIII Ag:Ac Ratio by 1st Gen Kit (VisuLize Factor VIII Antigen Kit)			FVIII Ag:Ac Ratio by *NEW* 2nd Gen Kit (VisuLize Factor VIII Antigen PLUS Kit)		
NPP	Normal Pooled Plasma	1.50			1.33		
Spiked Activity based on Assigned Potency		**1 IU/mL**	**0.3 IU/mL**	**0.05 IU/mL**	**1 IU/mL**	**0.3 IU/mL**	**0.05 IU/mL**
Wilate®	Plasma-Derived FVIII/VWF Complex	1.20	1.07	1.04	1.32	1.13	1.16
Humate-P®	Plasma-Derived FVIII/VWF Complex	0.72	0.63	0.62	1.04	0.83	0.84
Kovaltry®	Recombinant Full-Length FVIII	1.57	1.50	1.26	1.24	1.00	0.86
Adynovate®	PEGylated Recombinant Full-Length FVIII	1.37	1.26	1.06	1.22	0.97	0.90
Obizur®	Recombinant Porcine BDD-FVIII	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Eloctate®	Recombinant BDD-FVIII with Dimeric Fc	0.26	0.21	0.20	0.81	0.67	0.66
Nuwiq®	Recombinant BDD-FVIII	0.26	0.20	0.20	0.78	0.60	0.52
Novoeight®	Recombinant BDD-FVIII	0.32	0.28	0.24	0.87	0.70	0.66
Afstyla®	Recombinant BDD-FVIII	0.27	0.22	0.20	0.71	0.57	0.56