Improved Recognition of B-Domain Deleted FVIII in Plasma with a New Commercially Available VisuLize™ F8Plus Antigen Kit

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Background

The 1st generation of VisuLize Factor VIII Antigen Kit was found to under-recover a recombinant B-domain deleted (BDD) FVIII antigen (Xyntha®) relative to plasma FVIII. As BDD forms of FVIII are becoming the most administered replacement products in Hemophilia A, there is a need for a commercially available FVIII antigen kit to reliably measure these truncated forms in plasma, using a WHO-traceable, plasma-based calibrator.

Methods

The 1st generation ELISA (VisuLize Factor VIII Antigen Kit) was reoptimized to select for conditions that improved the recovery of BDD-FVIII (Xyntha). The resulting 2nd generation of VisuLize Factor VIII Antigen Plus (F8PLUS Antigen) Kit still uses a normal plasma calibrator and plasma controls.

Objective

To provide a research tool for harmonizing the quantification of recombinant full-length and BDD-FVIIIs relative to a plasma-based calibrator.

Conclusions

The 2nd generation of VisuLize F8PLUS Antigen Kit demonstrates significantly improved recognition of BDD-FVIII (Xyntha®), while preserving the recognition of plasma FVIII and recombinant full-length FVIII, as well as the limit of detection and the insensitivity to von Willebrand Factor shown with the original VisuLize Factor VIII Antigen Kit. This is a positive step towards the harmonization of antigen measurements of native FVIII and various recombinant FVIII constructs.

Table 1: Key Feature Summary of VisuLize F8PLUS Antigen Kit

Procedure	**Sandwich ELISA**
Intended Use	Antigen Quantification of Full-Length Human FVIII and B-Domain Deleted FVIII Constructs in Plasma
Capture and Detection Antibodies	Sheep Polyclonal Antibodies to Human FVIII (Affinity Biologicals)
Calibrator	Pooled Citrated Plasma from Normal Human Donors That is Traceable to WHO Standard
Curve Fit / Regression	Non-Linear / 4 Parameter Logistic
Sample Matrix	Citrated Plasma
Dilution Factors	1/8 and 1/16 Dilutions for Samples Containing Normal Levels of FVIII Antigen
	1/4 and 1/8 Dilutions for Samples Containing Low Levels of FVIII Antigen
Detection Limit	0.008 IU/mL
Shelf Life	Whole Kit Stability of 24 Months at 2-8 °C
No Interferences to:	**Maximal Concentration Tested:**
Albumin	60 g/L
Bicarbonate	40 mM
Bilirubin	0.4 g/L
Hemoglobin	5 g/L
L-Ascorbic Acid	3 mg/dL
Lipemia	5 g/L
von Willebrand Factor	20 µg/mL
Limitations	Interference May be Observed in Samples Containing Antibodies to FVIII or Heterophilic Antibodies
Recommendations	Interference Should be Suspected When FVIII Antigen is Discordant with FVIII Activity
	Pre-Treat Samples with Suspected Interfering Antibodies Using a Validated Heterophilic Antibody Blocking Reagent Prior to Re-Testing

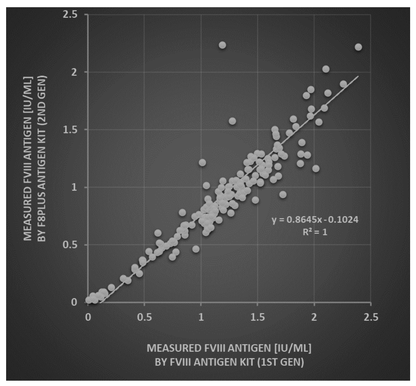
Table 2: Precision Summary of VisuLize F8PLUS Antigen Kit

	Mean (IU/mL)	N	Within-Run	Within-Lot
Normal FVIII Sample	0.811	240	3.8%	5.2%
Mid-Level FVIII Sample	0.394	240	3.3%	4.3%
Low-Level FVIII Sample	0.109	240	3.2%	4.5%
	Between-Run	**Between-Day**	**Lot-to-Lot**	**Within-Lab (Total)**
Normal FVIII Sample	3.6%	0.0%	2.3%	5.7%
Mid-Level FVIII Sample	2.8%	0.0%	1.1%	4.5%
Low-Level FVIII Sample	3.0%	1.0%	5.9%	7.4%

Table 3: Example of Calibration Curve Summary of VisuLize F8PLUS Antigen Kit

Stats of 10 Runs	Standard Concentration (IU/mL)							
	0.960	**0.480**	**0.240**	**0.120**	**0.060**	**0.030**	**0.015**	**0.008**
Mean	0.960	0.481	0.240	0.120	0.061	0.030	0.015	0.008
SD	0.003	0.004	0.004	0.001	0.001	0.001	0.000	0.001
CV	0.3%	0.9%	1.6%	0.9%	1.7%	2.6%	2.1%	7.2%
RE	0.2%	0.2%	0.0%	0.0%	1.3%	0.0%	0.0%	0.0%
TE	0.5%	1.1%	1.6%	0.9%	3.0%	2.6%	2.1%	7.2%
R^2	1.000							

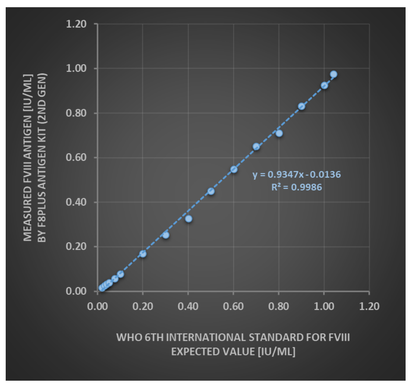
Figure 1



FVIII Antigen Recovery of Plasma Samples by VisuLize Factor VIII Antigen (1st Gen) and VisuLize F8PLUS Antigen (2nd Gen) Kits.

Antigen levels in plasma samples containing various levels of FVIII were determined by both VisuLize Antigen Kits from one production lot each according to kit instruction. VisuLize F8PLUS Antigen Kit slightly under-estimated FVIII compared to VisuLize Factor VIII Antigen Kit, however recoveries of FVIII antigen in plasma samples were generally in an agreement by the two kits (N = 182).

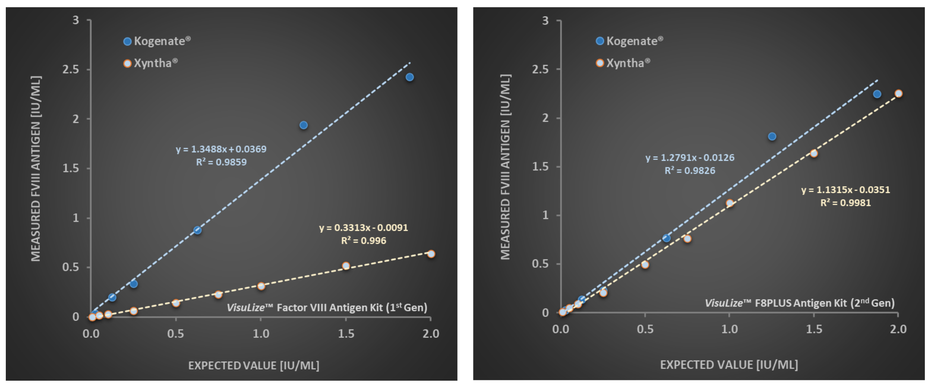
Figure 2



FVIII Antigen Recovery of WHO 6th International Standard (for Factor VIII) by VisuLize F8PLUS Antigen Kit.

WHO FVIII Standard was diluted to various FVIII antigen levels (between 0.02 IU/mL to 1.04 IU/mL) using kit sample diluent and FVIII antigen levels in these samples were then determined by VisuLize Factor F8PLUS Antigen Kits from three independent validation lots according to kit instruction. Average antigen recovery of FVIII was within ±20% of expected antigen value.

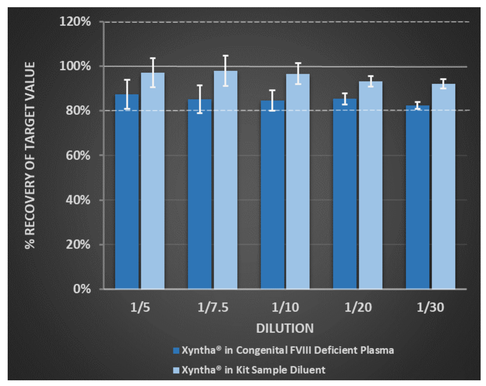
Figure 3



Recovery of Kogenate® (full-length rFVIII) and Xyntha® (BDD-rFVIII) by VisuLize Factor VIII Antigen (1st Generation) and the New VisuLize F8PLUS Antigen (2nd Generation) Kits.

Both FVIII products were reconstituted and spiked into congenital FVIII-deficient plasma to various activity levels according to labeled activity potency. FVIII antigen levels were then determined by both VisuLize Antigen Kits using the kit plasma calibrator.

Figure 4



Recovery of Diluted Xyntha® (BDD-rFVIII) by VisuLize F8PLUS Antigen Kit.

Xyntha® was reconstituted and spiked into congenital FVIII-deficient plasma to activity level of 5.0 IU/mL according to labeled activity potency. This spiked sample was further diluted 5 to 30 times using either congenital FVIII-deficient plasma or kit sample diluent and Xyntha® antigen levels were then determined by VisuLize F8PLUS Antigen Kits from three independent lots according to kit instruction. Average antigen recovery of Xyntha® was within ±20% of expected activity value (MEAN ± SE is shown).