Improved specificity and performances of a novel photometric automated assay for Factor XIII activity

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INTRODUCTION

Factor XIII (FXIII) protransglutaminase circulates in plasma as A_2B_2 tetramer, the A subunit being the functional form. When activated by thrombin and calcium to FXIIIa, it acts in the last step of the coagulation cascade and contributes to Fibrin crosslinking and clot stiffness. Measurement of FXIII is of high usefulness in many contexts (congenital or acquired FXIII deficiencies, low FXIII with bleeding complications in trauma or surgery, FXIII autoantibodies, monitoring FXIII substitutive therapy…). BIOPHEN™ Factor XIII is a new automated chromogenic assay for the rapid testing of FXIII concentration in citrated human plasma, measured through FXIIIa transglutaminase activity, and usable on any analyzer with 340nm wavelength.

AIM

The aim of this study is to evaluate performances of the new chromogenic FXIII assay adapted on CS coagulation analyzers and to compare tests results with those obtained with the reference method.

METHOD

FXIII Assays

BIOPHEN™ Factor XIII is a chromogenic method for the quantitative measurement of FXIII activity. FXIII, in the tested sample, is converted into FXIIIa by thrombin in presence of calcium. Soluble fibrin, also generated by the action of thrombin, accelerates the reaction while an anti polymerization peptide avoids clot formation. FXIIIa transglutaminase activity between a synthetic peptide substrate and glycine ethyl ester (GEE) leads to the formation of ammonia (NH4+). Ammonia is then assayed through the reaction of glutamate dehydrogenase (GLDH) converting NADPH into NADP+, in presence of ammonia and alpha ketoglutarate. The conversion of NADPH into NADP+ can be detected at 340 nm, and the slope of the absorbance decrease at 340nm is directly proportional to the concentration of FXIII in the tested sample.
Comparison study is performed with Berichrom FXIII (Siemens Healthineers, Marburg, Germany) on CS-2500 (Sysmex, Kobe, Japan).

Plasma Samples

Commercial lyophilized normal and pathological plasmas are used to evaluate precision, linearity, dynamic range and stability. Citrated plasma samples covering the whole concentration measuring range and used for comparison study are from healthy volunteers, patient with deficiency (< 5%) and samples with low FXIII levels.

RESULTS

The test showed good performance characteristics with low intra-assay coefficients of variation (2.7% to 4.9%) and inter-assay coefficients of variation (1.5% to 1.9%) on CS-series (Table 1).

	Mean (FXIII %)	CV% intra-series	CV% inter-series
		CS-5100	
QC1	102	2.7%	1.5%
QC2	29	4.9%	1.9%

Table 1: Performance of BIOPHEN™ Factor XIII on CS-5100 using lyophilized control plasmas, intra-series (n=40) and inter-series (n=30, 10 runs, 5 days).

Using a calibration curve from 0 to 150%, the dynamic range is from 5 to 300% with automated redilution (Figure 1) with a very good recovery at very low FXIII levels (0 to 10%).

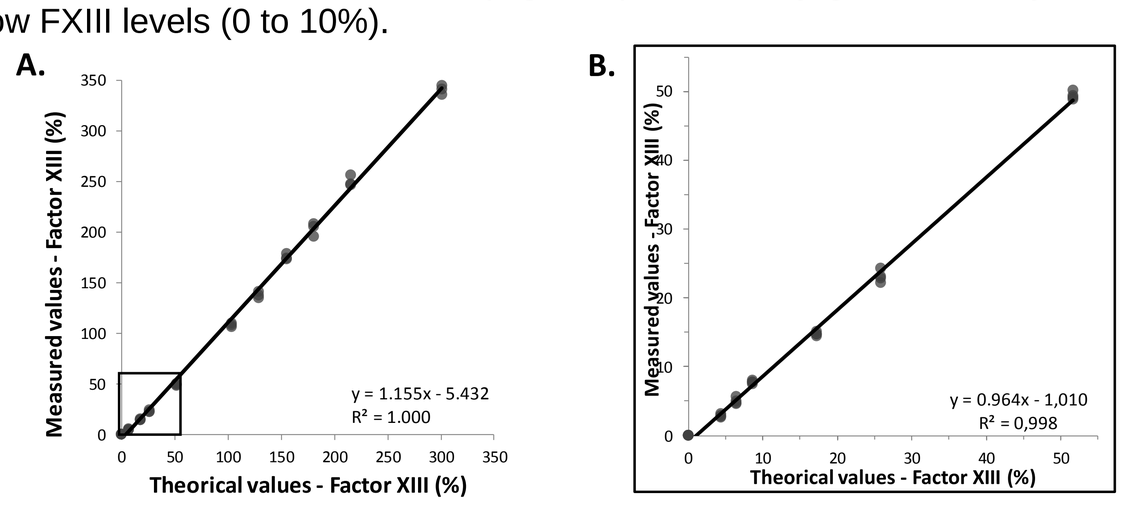

Figure 1: Linear regression analysis for Factor XIII following preparation of dilution series with spiked plasmas. Linearity for the BIOPHEN™ Factor XIII reagent (A). Linearity of low range (B).

Specificity is verified with FXIII deficient plasma (<0.1%) and reference interval in normal plasmas is from 60 to 146% (n=120). Interference study is performed using 2 levels of controls (Table 2).

	Hemoglobin	Bilirubin	Intralipids	Heparins*	DOACs**	Fibrinogen	Ammonium
No interference up to	250 mg/dL	60 mg/dL	250 mg/dL	2 UI/mL	400 ng/mL	6 g/L	0.5mM

Table 2: Interferences in BIOPHEN™ Factor XIII on CS-series using spiked lyophilized plasmas. *Heparins tested are UFH and LMWH, ** DOACs tested are Dabigatran, Rivaroxaban, Apixaban and Edoxaban.

Extended stability is measured: 5 days on-board, 1 week at 2 – 8° C, 48 hours at room temperature and 2 months frozen.

Good correlation with predicate devices on CS-2500 (r=0.989, p < 0.001), and between analyzers (r = 0,997, p < 0.0001). Very low concentrations are correctly measured (Figure 2).

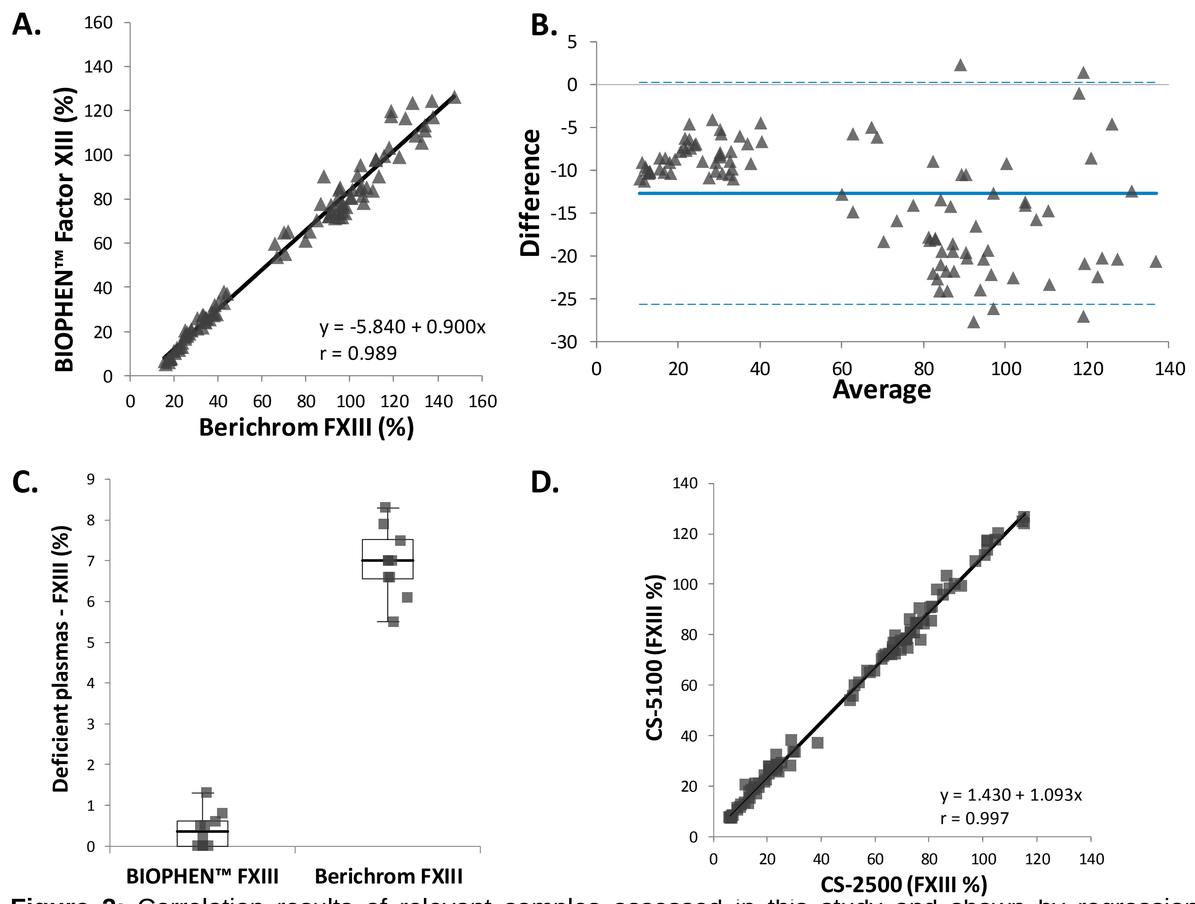

Figure 2: Correlation results of relevant samples assessed in this study and shown by regression analysis, Bland and Altman difference plot and Compare Pairs (deficient plasmas), using reference method or analyzer. (A) Correlation and (B) Bland and Altman difference plot of BIOPHEN™ Factor XIII and Berichrom FXIII (n = 102). (C) Compare Pairs using 10 individuals deficient plasmas (FXIII < 5%). (D) Correlation of BIOPHEN™ Factor XIII on CS-2500 and CS-5100 (n = 98). Statistical significance was defined by a hypothesis test yielding a P-value of less than 0.05.

Analysis done by Bland and Altman difference plot showed good agreement between both reagents. A slight measurement difference, especially in low and very low range measurement, is observed (Figure 2B and 2C). This difference may be due to the higher blank value and higher LOQ value of the reference reagent.

CONCLUSIONS

BIOPHEN Factor XIII is a simple, automated (usable on all analyzers with 340nm wavelength), highly stable, and reliable method for measurement of FXIII activity in citrated human plasma.

It is accurate and precise, with low CVs, well correlated to existing methods, and offers an extended dynamic range, with high on-board stability.

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