Measurement of Anti-FIIa activity of Bivalirudin in plasma with clotting and chromogenic assays

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INTRODUCTION

Bivalirudin is a direct reversible thrombin (FIIa) inhibitor indicated as an anticoagulant agent in some interventional cardiology procedures in replacement of heparin. It has a short plasma half-life of 25-30 minutes, is cleaved by thrombin and does not cause Heparin-induced thrombocytopenia. Clearance rate is decreased in patients with moderate and severe renal diseases with a prolonged plasma half-life, requiring dose adjustment in these patients. Monitoring Bivalirudin concentration in plasma is then useful.

AIM

The aim of this study is to evaluate performances of a new simple and rapid dedicated method for the measurement of anti-FIIa activity of Bivalirudin. Anti-FIIa clotting or chromogenic methods are used with Bivalirudin calibrators and controls, and adapted on different coagulation analyzers. Bivalirudin recovery was evaluated with both assays by testing various plasma samples from healthy volunteers or treated patients.

METHOD

Anti-FIIa assays:

BIOPHEN™ DTI is a chromogenic method based on the inhibition of a constant and in excess amount of thrombin. Hydrolysis of a thrombin specific chromogenic substrate (CS-01(81)) by residual thrombin, releases pNA. The amount of pNA released (measured at 405 nm) is proportional to the residual thrombin activity. There is an inverse linear relationship between the concentration of Bivalirudin and color development. HEMOCLOT™ Thrombin Inhibitors method is a clotting assay (diluted thrombin time). The diluted tested specimen is mixed with normal pooled human plasma. Clotting is then initiated by adding highly purified human thrombin, essentially in the α-form. Measured clotting time is related to Bivalirudin concentration in tested plasma. Clotting and chromogenic assays are used in combination with Bivalirudin calibrators and controls. A method with a calibration range from 0 to 5 µg/mL is developed on CS and STA-R® instruments for measuring anti-FIIa activity of Bivalirudin in patients' plasmas.

Plasma Samples:

Bivalirudin lyophilized calibrators and controls are evaluated for precision, linearity, dynamic range and stability. Citrated plasma samples covering the whole concentration measuring are used for comparison study, and are from healthy volunteers and patients treated with bivalirudin. Plasma samples were stored at −70 °C until use.

RESULTS

Both normal and abnormal controls demonstrated excellent inter-assay and intra-assay precisions for all analyzers. The coefficient of variation (CV%), is from 1.5 to 6.0% (Table 1).

Precision		Mean (µg/mL)	BIOPHEN™ DTI		HEMOCLOT™ TI	
			CS-5100 CV %	STA-R® Max CV %	CS-5100 CV %	STA-R® Max CV %
intra-series	N		40	3	5	40
	QC1	1.6	3.2 %	2.7%	2.1%	3.6 %
	QC2	4.1	3.2 %	2.6%	1.5%	2.1 %
inter-series	N		30	8	8	30
	QC1	1.6	2.4 %	6.0%	3.8%	3.3 %
	QC2	4.1	2.6 %	5.5%	4.3%	3.1 %

Table 1 : Performance of BIOPHEN™ DTI and HEMOCLOT™ Thrombin Inhibitors reagents on various analyzers; inter-series: n=30, 10 runs, 5 days or n=8, 4 runs, 4 days.

Using a calibration curve from 0 to 5 µg/mL, the dynamic range is from 0 to 19.5 µg/mL with appropriate redilution (Figure 1).

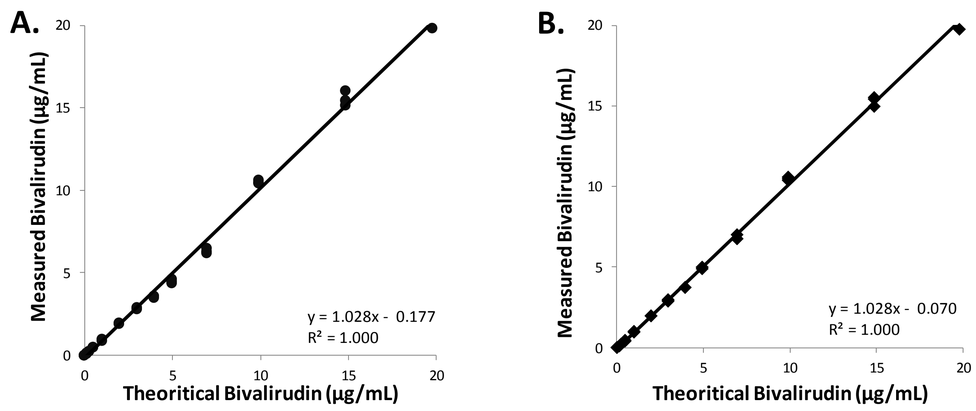

Figure 1: Evaluation of linearity for the BIOPHEN™ DTI - Bivalirudin (A) and HEMOCLOT™ Thrombin Inhibitors - Bivalirudin (B). Linear regression analysis for bivalirudin following preparation of dilution series with spiked plasmas.

After reconstitution, BIOPHEN™ Bivalirudin Calibrators and Controls are stable for 17H on board of the CS series and STA-R® Max instruments in opened vials. In closed vials stability is of 48H at room temperature (18-25° C) and 7 days at 2-8° C.

Specificity is verified using plasma without Bivalirudin (≤ LOQ*) with both methods and plasmas from patients treated with Bivalirudin.

*LOQ (BDTI/CS) ≤ 0.28 µg/mL - LOQ (HTI/STAR) ≤ 0.26 µg/mL

Interferences are summarized on table 2.

	Hemoglobin	Bilirubin	Intralipids	Heparins*	DOACs**
CS-5100 BIOPHEN™ DTI	125 mg/dL	15 mg/dL	500 mg/dL	2 UI/mL	400 ng/mL
STA-R® Max HEMOCLOT™ TI	500 mg/dL	60 mg/dL	1000 mg/dL		100 ng/mL (Apixaban)

Table 2: Interferences of BIOPHEN™ DTI and HEMOCLOT™ Thrombin Inhibitors methods for Bivalirudin on various analyzers using spiked lyophilized control plasmas. *Heparins tested are UFH and LMWH, **DOACs tested are Rivaroxaban, Apixaban and Edoxaban. HEMOCLOT™ Thrombin Inhibitors is sensitive to Heparins, Rivaroxaban and Edoxaban.

Correlation is determined using clinical samples, statistical analysis demonstrated an excellent correlation between clotting and chromogenic methods on CS-5100 (r = 0.983; p < 0.0001) and on STA-R® Max (r = 0.979; p = 0.0036). Results obtained are reliable and statistically equivalent for both methods (Figure 2).

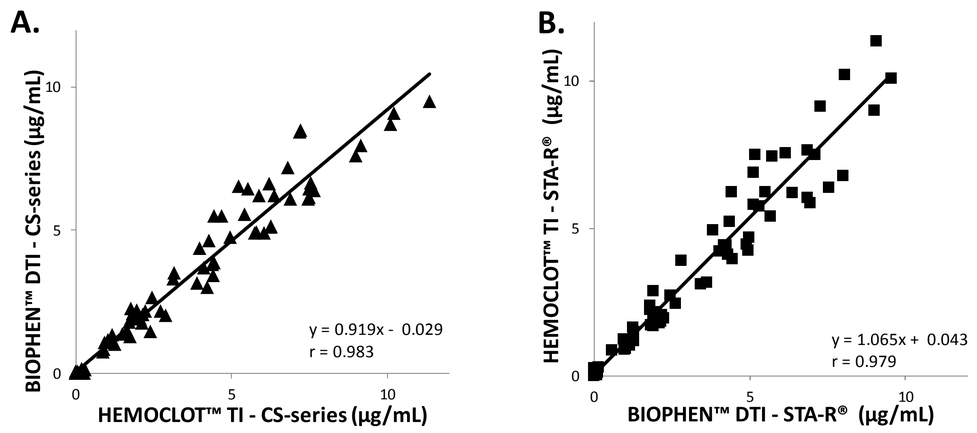

Figure 2: Correlation results of relevant samples tested in this study and shown by regression analysis, using both methods on CS-5100 and STA-R® Max. (A) Correlation of BIOPHEN™ DTI - Bivalirudin and HEMOCLOT™ Thrombin Inhibitors - Bivalirudin on CS-5100 (B) Correlation of BIOPHEN™ DTI - Bivalirudin and HEMOCLOT™ Thrombin Inhibitors - Bivalirudin on STA-R® Max. Statistical significance was defined with hypothesis test yielding a P-value < 0.05.

CONCLUSIONS

Anti-IIa clotting (HEMOCLOT™ Thrombin Inhibitors) and chromogenic (BIOPHEN™ DTI) assays, combined with dedicated calibrators and controls, allow measuring Bivalirudin anti-FIIa activity in citrated plasma, accurately and reliably, and can be used for drug dosage adjustment when required in some specific clinical situations. A good correlation is observed between both assays, which can be used on all current instruments.

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