Neutralization of heparin interferences in DOACs' measurement with Anti-Xa chromogenic assays

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INTRODUCTION

Co-administration/switching of heparin and direct oral anti-factor Xa inhibitor (DiXaI) can occur in specific clinical situations. Overlap period due to the time needed for drug elimination can vary from patient to patient. DOAC measurement may be necessary in some critical conditions. Chromogenic anti-Xa kinetics assays are the most frequently used for testing these drugs. Since these assays are able to detect the activity of both DiXaI and heparins, the presence of heparin in the sample may influence the anti-Xa activity measurement of these DOAC, which may be over-estimated.

AIM

The aim of the study is to develop and to evaluate a specific sample dilution buffer, which neutralizes heparin interferences in the measurement of DOACs' anti-Xa activity in plasma using automated chromogenic assays.

METHOD

Anti-Xa assay and associated buffers

BIOPHEN™ Heparin LRT method is a kinetics chromogenic assay based on the inhibition of a constant and in excess amount of FXa, by heparin (or other anti-Xa substance) to be assayed, in presence of endogenous AT. The residual FXa hydrolyses a specific chromogenic substrate (SXa-11) releasing paranitroaniline (pNA). The amount of pNA released (measured by absorbance at 405 nm) is inversely proportional to the concentration of heparin (or other anti-Xa products) present in plasma. For this assay, plasma is diluted with physiological saline (reference buffer). To inhibit heparin interference, this buffer can be replaced by a special Tris-NaCl-EDTA dilution buffer (pH 7.85) containing an heparin blocker. To evaluate heparin inhibition in one stage chromogenic assay, specific DiXaIs calibrators and controls are used. Low range calibrations are tested on CS series, STA-R® series, ACL TOP® family and BCS® XP for measuring Anti-FXa activity of DiXaIs (Rivaroxaban, Apixaban, Edoxaban).

Plasma samples

Untreated or anticoagulated plasmas spiked with heparin at usual concentrations are used to evaluate precision, linearity and heparin neutralization.

RESULTS

Using low and standard DiXaIs' calibration curves (Rivaroxaban, Apixaban and Edoxaban), the dynamic range is from 10 to 600 ng/mL. Very good linearity and within-run performances are obtained with both diluents demonstrating the equivalence of both buffers for all DiXaIs' measurements and on all analyzers (0.4% to 7.2%) (Table 1).

Instruments	DOAC (Low Range)	Linearity		Within-run (CV %)	
		Physiological saline	Special buffer	Physiological saline	Special buffer
CS series	Rivaroxaban	R^2=0.998	R^2=0.988	CI=6.8% CII=2.6%	CI=7.2% CII=2.1%
	Apixaban	R^2=1.000	R^2=0.999	CI=1.3% CII=1.5%	CI=2.9% CII=1.1%
	Edoxaban	R^2=0.997	R^2=0.996	CI=0.4% CII=0.4%	CI=2.1% CII=0.8%
STA-R®	Rivaroxaban	R^2=0.998	R^2=0.998	CI=0.0% CII=0.7%	CI=0.0% CII=0.7%
	Apixaban	R^2=1.000	R^2=1.000	CI=2.9% CII=0.0%	CI=2.9% CII=2.9%
	Edoxaban	R^2=0.996	R^2=0.996	CI=6.6% CII=1.5%	CI=4.2% CII=1.3%
ACL Top®	Rivaroxaban	R^2=1.000	R^2=1.000	CI=3.4% CII=1.0%	CI=3.0% CII=2.6%
	Apixaban	R^2=0.998	R^2=0.999	CI=4.5% CII=0.4%	CI=2.0% CII=0.9%
	Edoxaban	R^2=0.999	R^2=0.999	CI=4.3% CII=1.0%	CI=2.6% CII=0.6%
BCS® XP	Rivaroxaban	R^2=1.000	R^2=1.000	CI=1.8% CII=0.4%	CI=0.4% CII=0.9%
	Apixaban	R^2=1.000	R^2=0.999	CI=2.4% CII=1.0%	CI=0.6% CII=1.8%
	Edoxaban	R^2=0.998	R^2=0.997	CI=4.1% CII=5.8%	CI=5.2% CII=3.5%

Table 1: Performances of BIOPHEN™ Heparin LRT combined with physiological saline or special dilution buffer, on various analyzers (CS-5100, CS-2x, STA-R® Max, ACL Top® 550 and BCX® XP), according to the DOAC (anti-Xa).

The anti-FXa activities are consistent between the two diluents in the absence of heparin (recovery >90%). Conversely, when heparin and DiXaIs are present in the same sample, heparin interferes with the measurement inducing an over-estimation proportional to the heparin concentration. Using the specific sample diluent, a much better compliance is observed with expected DiXaI concentrations, with no Heparin interference (Figure 1).

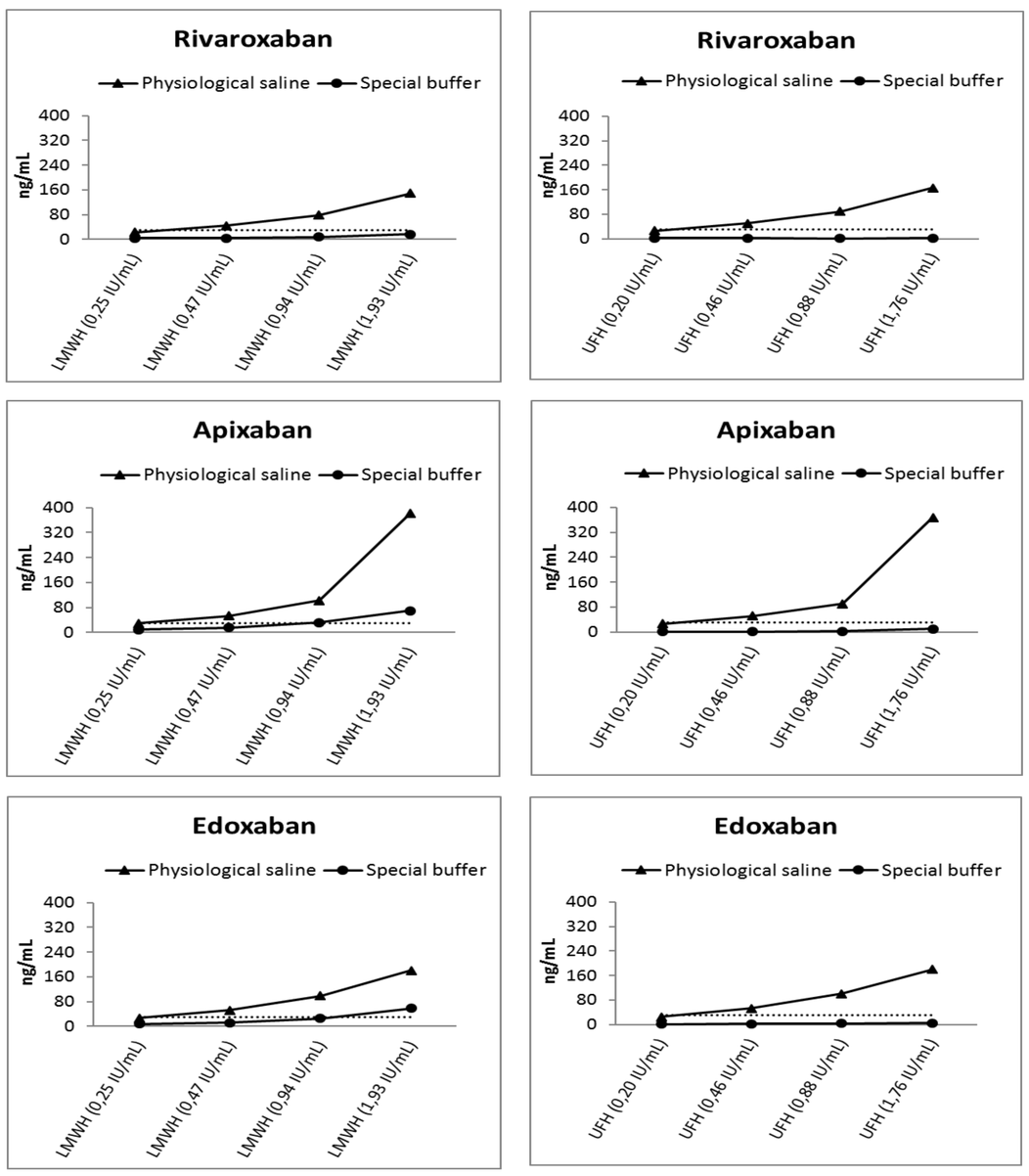

Figure 1: Inhibition of heparins' interference (LMWH / UFH) in DiXaIs' measurement with BIOPHEN™ Heparin LRT in combination with specific DiXaI calibrators and controls using a specific sample diluent, on CS series.

CONCLUSIONS

BIOPHEN™ Heparin LRT combined with this new specific diluent allows measuring DOACs' anti-Xa activity on any coagulation analyzer without heparin interference. In presence of heparin and DiXaI in tested plasmas, the falsely elevated measurement of Apixaban, Edoxaban, and Rivaroxaban concentrations can be suppressed using this new heparin neutralizing diluent.

REFERENCES

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