Quantitative Determination of Apixaban: Method Comparison Studies Between Different Assays and Platforms

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Introduction and Aim

Apixaban is a direct oral anticoagulant (DOAC) that specifically inhibits Factor Xa (FXa). Although laboratory monitoring of DOACs is not required per se, there is a growing recognition of the need to test in specific clinical situations and patient populations. The quantitative determination of DOACs is possible with chromogenic anti-Xa assays originally developed for quantitative determination of heparin enoxaparin concentration using drug-specific calibrators.

The INNOVANCE Anti-Xa assay from Siemens Healthineers is an FXa-based chromogenic assay used with drug-specific standards and controls for the quantitative determination of heparin activity (unfractionated heparin [UFH] and low molecular weight heparin [LMWH]), as well as rivaroxaban*, edoxaban*, and apixaban concentration in human citrated plasma. Respective applications are available on various hemostasis systems.

This study evaluates the comparability of the INNOVANCE Anti-Xa assay to measure apixaban by employing apixaban-specific standards and controls with liquid chromatography–tandem mass spectrometry (LC-MS/MS) and other commercial methods. The INNOVANCE Anti-Xa assay comparability on different systems from Siemens Healthineers was also investigated.

Methods

Assays

Samples were obtained from patients receiving apixaban therapy or suspected to be under therapy with apixaban and tested with the following assays/devices:

- INNOVANCE Anti-Xa assay from Siemens Healthineers for the quantitative determination of apixaban (performed on the CS-2500, CS-5100, CN-3000*, and CN-6000* Systems)
- Liquid chromatography–tandem mass spectrometry (LC-MS/MS; Swiss BioQuant, Reinach, Switzerland)
- STA-Liquid Anti-Xa assay (Stago; Asnière-sur-Seine, France) for the quantitative determination of apixaban (performed on the STA Compact Max System, Stago)
- HemosIL Liquid Anti-Xa assay (Werfen; Barcelona, Spain) for the quantitative determination of apixaban (performed on the ACL TOP Family 50 Series analyzer, Werfen).

Measurements

Method comparison of INNOVANCE Anti-Xa assay versus LC-MS/MS
A method comparison study was performed using the INNOVANCE Anti-Xa assay on the CS-2500 System versus the LC-MS/MS method for measuring apixaban concentrations of 101 frozen samples (n = 47 native samples, n = 52 spiked samples, and n = 2 controls). The sample measurements with the INNOVANCE Anti-Xa assay were done at Siemens Healthineers, and with the LC-MS/MS method at Swiss BioQuant.

Method comparison of INNOVANCE Anti-Xa assay versus STA Liquid Anti-Xa assay
Using a different set of 107 frozen samples (n = 102 native samples from the intended use population and n = 5 spiked samples), a method comparison study was performed comparing the INNOVANCE Anti-Xa assay on the CS-2500 System to the STA-Liquid Anti-Xa assay on the STA Compact Max system at Siemens Healthineers.

Method comparison of INNOVANCE Anti-Xa assay versus HemosIL Liquid Anti-Xa assay
Method comparisons using samples from patients under therapy or suspected to be under therapy with apixaban (n = 306) were enrolled at three clinical sites. N = 18 samples were diluted or spiked to cover the measuring interval of the INNOVANCE Anti-Xa assay (20–700 ng/mL). Samples were measured fresh or after frozen storage at the enrollment sites using the INNOVANCE Anti-Xa assay on the CS-2500 System and the HemosIL Liquid Anti-Xa assay on the ACL TOP Family 50 Series analyzer.

Method comparison of INNOVANCE Anti-Xa assay on different Siemens Healthineers systems
The samples described above (n = 306) were also measured at Siemens Healthineers with the INNOVANCE Anti-Xa assay on CS-5100, CN-3000*, and CN-6000* systems. All samples were measured following frozen storage.

Statistical analysis

The method comparison studies were performed and evaluated by Passing-Bablok regression using webMC (method comparison) in accordance with CLSI EP09-A3, "Measurement Procedure Comparison and Bias Estimation." Correlation coefficient (r), intercept, slope and bias at apixaban concentrations of 30 ng/mL and 50 ng/mL were calculated for the entire measuring interval (20–700 ng/mL) as well as for the lower concentration range (20–100 ng/mL). We also performed the regression in the lower concentration range to better evaluate the data in that range and achieve a more precise and correct estimate for the predicted bias at 30 ng/mL and 50 ng/mL.

Results

The summarized statistical results from the regression analysis are listed in Table 1 and are graphically displayed in Figures 1–4.

Table 1. Method comparison results.

Method 2 (y-axis)		Method 1 (x-axis)		Evaluated interval (ng/mL)	n	Correlation Coefficient (r)	Slope	Intercept (ng/mL)	Deviation at:	
Assay	System	Assay	System						30 ng/mL	50 ng/mL
INNOVANCE Anti-Xa	CS-2500	n/a	LC-MS/MS	20–350 (Figure 1)	101	0.987	1.034	0.759	1.8	2.5
				20–100	65	0.969	0.982	3.651	3.1	2.7
INNOVANCE Anti-Xa	CS-2500	STA Liquid-Anti-Xa	STA Compact Max	20–400 (Figure 2)	107	0.975	1.081	−2.329	0.1	1.7
				20–100	41	0.943	1.029	−0.178	0.7	1.3
INNOVANCE Anti-Xa	CS-2500	HemosIL Liquid Anti-Xa	ACL TOP Family 50 Series	20–600 (Figure 3)	301	0.989	1.026	−8.590	−7.8	−7.3
				20–100	90	0.935	0.801	8.889	2.9	−1.1
INNOVANCE Anti-Xa	CS-5100	INNOVANCE Anti-Xa	CS-2500	20–600 (Figure 4)	305	0.999	1.007	0.169	0.4	0.5
				20–100	103	0.998	1.018	−0.554	0.0	0.3
INNOVANCE Anti-Xa	CN-3000*	INNOVANCE Anti-Xa	CS-2500	20–600 (Figure 4)	306	0.998	1.023	1.023	1.7	2.2
				20–100	104	0.997	1.053	−0.211	1.4	2.4
INNOVANCE Anti-Xa	CN-6000*	INNOVANCE Anti-Xa	CS-2500	20–600 (Figure 4)	305	0.998	1.022	−1.532	−0.9	−0.5
				20–100	103	0.998	1.018	−1.036	−0.5	−0.1

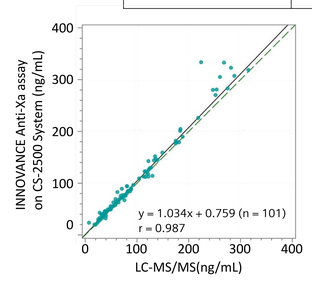


Figure 1. Passing-Bablok regression analysis for the INNOVANCE Anti-Xa assay on the CS-2500 System versus results obtained using the liquid chromatography–tandem mass spectrometry (LC-MS/MS).

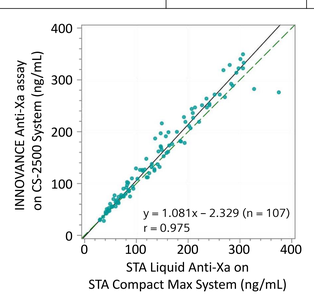


Figure 2. Passing-Bablok regression analysis for the INNOVANCE Anti-Xa assay on the CS-2500 System versus results obtained using the STA-Liquid Anti-Xa assay on the STA Compact Max System.

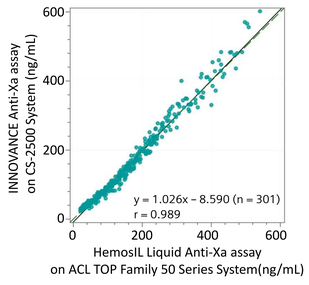
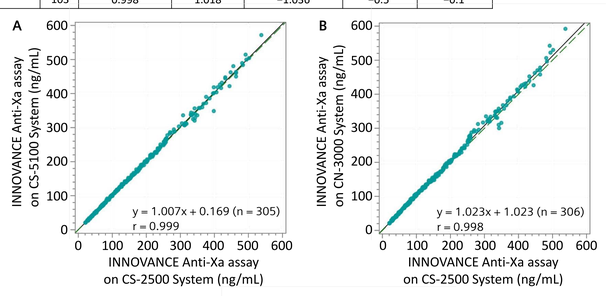


Figure 3. Passing-Bablok regression analysis for the INNOVANCE Anti-Xa assay on the CS-2500 System versus the HemosIL Liquid Anti-Xa assay on the ACL TOP Family 50 Series analyzer.



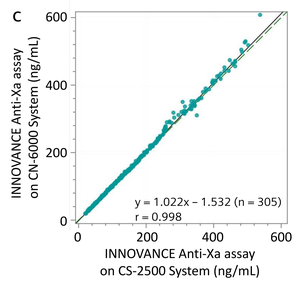


Figure 4. Passing-Bablok regression analysis for the INNOVANCE Anti-Xa assay performed on: A) the CS-2500 System versus the CS-5100 System, B) the CS-2500 System versus the CN-3000 System*, and C) the CS-2500 System versus the CN-6000 System*.

Conclusion

The method comparison studies demonstrate that the INNOVANCE Anti-Xa assay, in combination with the INNOVANCE Apixaban Standards and INNOVANCE Apixaban Controls on the CS-2500 System, compares very well with the LC-MS/MS method and with assays from Stago and Werfen, with correlation coefficients ≥ 0.975, intercepts ≤ 8.590 ng/mL, and slopes between 1.026 and 1.081.

The INNOVANCE Anti-Xa assay for Apixaban is available on various hemostasis systems from Siemens Healthineers. The method comparison study of the INNOVANCE Anti-Xa assay on the CS-2500 System versus the INNOVANCE Anti-Xa assay on the CS-5100 System, the CN-3000 System*, and the CN-6000 System* from Siemens Healthineers demonstrates an excellent agreement with correlation coefficients ≥ 0.998, intercepts ≤ 1.532 ng/mL, and slopes between 1.007 and 1.023.

In summary, the data demonstrate the accuracy and the comparability among systems for the INNOVANCE Anti-Xa assay in combination with INNOVANCE Apixaban Standards and INNOVANCE Apixaban Controls for the quantitative determination of apixaban in human-citrated plasma.

(*) Not available for sale in the U.S. The availability of products/features/applications varies by countries. Their future availability cannot be guaranteed.

LC-MS/MS refers to liquid chromatography–tandem mass spectrometry.

CS-2500 and CS-5100 Systems refer to Automated Blood Coagulation Analyzer CS-2500 and Automated Blood Coagulation Analyzer CS-5100, respectively.

CN-3000 and CN-6000 Systems refer to Automated Blood Coagulation Analyzer CN-3000 and Automated Blood Coagulation Analyzer CN-6000, respectively.

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