Preliminary Performance Data of an Anti-Xa Assay for the Quantitative Determination of Edoxaban

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SIEMENS Healthineers

Introduction and Aim

Patients require therapeutic anticoagulation with either vitamin K antagonists, or direct oral anticoagulants (DOACs) for prevention of stroke in nonvalvular atrial fibrillation and for therapy in venous thromboembolism. Although laboratory monitoring of DOACs is not required, testing may be helpful for patient management in specific clinical situations and for particular patient populations. Chromogenic anti-Xa assays originally developed for the quantitative determination of heparins are proven valuable tools for the quantitative determination of Xa-directed DOACs (i.e., rivaroxaban, apixaban, edoxaban). In combination with drug-specific calibrators, they allow quantification over a wide concentration range.

The INNOVANCE® Anti-Xa* assay in combination with drug specific standards and controls is used for the quantitative determination of heparins (unfractionated heparin [UFH] and low molecular weight heparin [LMWH]), rivaroxaban, and apixaban in human citrated plasma. Respective applications for automated analyzers are based on a chromogenic assay principle. New applications employing edoxaban-containing standards and controls are under development for the quantitative determination of edoxaban. The aim of the studies was to assess the performance of these new edoxaban applications.

Methods

Materials

- INNOVANCE Anti-Xa assay kit: a factor Xa-based chromogenic assay kit by Siemens Healthineers to be used for the quantitative determination of heparin activity (unfractionated heparin (UFH) and low molecular weight heparin (LMWH)), rivaroxaban concentration, and apixaban concentration
- INNOVANCE Edoxaban Standards: a set of two edoxaban standards by Siemens Healthineers
- INNOVANCE Edoxaban Controls: a set of two edoxaban controls by Siemens Healthineers
- Standard Human Plasma: stabilized and lyophilized plasma by Siemens Healthineers
- Analyzer applications for the quantitative determination of edoxaban for the Sysmex® CS-2500 System, Sysmex CS-5100 System, and Sysmex CN-3000/6000 Systems*
  - Results obtained on the Sysmex CN-6000 System are valid for the Sysmex CN-3000 System and vice versa
  - Results obtained on the Sysmex CS-2500 System are valid for the Sysmex CS-5100 System and vice versa
- STA-Liquid Anti-Xa assay by Stago for the quantitative determination of edoxaban (STA Compact Max System)

Studies

Linearity: Studies were performed and evaluated in accordance with CLSI EP06-Ed2, Evaluation of Linearity of Quantitative Measurement Procedures. Linearity was determined using 11 samples spiked with increasing concentrations of edoxaban, covering a range from 10.5 to 378 ng/mL. Four replicates per concentration level were measured for each plasma sample. Measurement of samples was performed using three combinations of reagents and standards on the Sysmex CS-2500 System, and one combination on the Sysmex CN-6000 System. Results (means) were plotted versus expected values, and a weighted (1/VAR) linear regression was fitted. To determine the deviation of the assay results from linearity, the difference between expected and predicted values was calculated for each dilution sample concentration and checked for fulfillment of predefined criteria.

Lower Limit of quantitation (LLoQ): Studies were performed and evaluated in accordance with CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

For determination of the LLoQ, four replicate measurements were done on five samples with analyte concentrations of 16, 18, 20, 22, and 24 ng/mL (± 20% of the targeted value of 20 ng/mL), using two reagents lots and one standards lot over 3 days on one analyzer.

For each reagent the analytical total error for every sample was calculated according to the Westgard model:

$TE = |Bias| + 2*SD_i$ with $Bias = \bar{x} - AC$

The relative analytical total error was calculated as follows:

$TE\% = 100*TE/AC_i$

The reagent lot-specific LLoQ -value was the lowest mean value of the measured concentration, for which the calculated TE-value did not exceed the acceptable upper limit, and for which the TEs of all higher concentrations also did not exceed this limit. The maximum of the reagent specific values was the LLoQ for the method.

Dilution Recovery: Studies were performed and evaluated in accordance with CLSI EP34-ED1, Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking. Dilution recovery was evaluated by using five samples spiked with edoxaban concentrations above the upper limit of the analytical measuring interval (AMI: 20–350 ng/mL). Each sample was separated in two aliquots, and each aliquot was manually diluted 1:2 using Standard Human Plasma (SHP). Two single determinations were performed per diluted aliquot. This procedure was performed independently by two different operators, resulting in eight values per sample (2 dilutions × 2 replicates × 2 operators). The raw values obtained by measuring the diluted samples were evaluated using prior established reference curves. Per sample, the eight results were multiplied by the dilution factor of 2, and the means were calculated (measured concentration). The deviation (%) from the expected concentration was determined for each sample.

Precision: Precision studies were performed according to a 5 × 2 × 4 model. The 5 × 2 × 4 model is a modification of the 20 × 2 × 2 model which is described in CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures. On the Sysmex CS-2500 System and CN-6000 Systems, plasma pools containing edoxaban were measured over 5 days (two runs per day with each four determinations per run) using one system. Analysis of variance with two influencing nested factors (day/run) was performed and repeatability CV (%), between-run CV (%), within-day CV (%), between-day CV (%), and total CV (%) were calculated.

Method Comparison: Studies were performed and evaluated (Passing-Bablok) in accordance with CLSI EP09-A3, "Measurement Procedure Comparison and Bias Estimation", using frozen samples from patients under therapy or suspected to be under therapy with edoxaban and a maximum of 10% spiked samples. Frozen samples covered specific indications (e.g., prevention of stroke and systemic embolism, Deep-Vein-Thrombosis [DVT] and/or Pulmonary embolism [PE]) and specific subpopulations (e.g., ≥ 75 years, patients in need of invasive procedures, patients with renal insufficiency, patients at extremes of body weight, patients with bleeding or taking aspirin). The INNOVANCE Anti-Xa assay on the Sysmex CS-2500 System was compared to the STA-Liquid Anti-Xa assay on the STA Compact Max System. The INNOVANCE Anti-Xa assay on the Sysmex CS-5100 System and the INNOVANCE Anti-Xa assay on the Sysmex CN-3000 System were compared to the INNOVANCE Anti-Xa assay on the Sysmex CS-2500 System. Correlation coefficient (r), intercept, and slope were calculated. Evaluation of deviations at edoxaban concentrations of 30 ng/mL and 50 ng/mL were conducted using measuring intervals from 20–100 ng/mL.

Results

Linearity

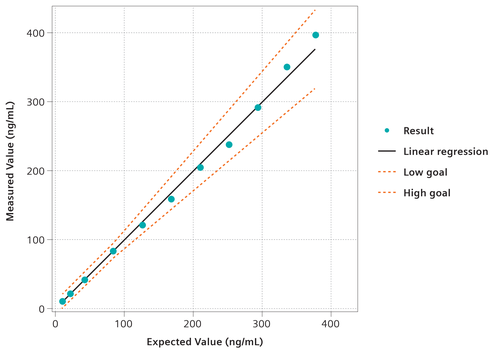

Figure 1. Linearity. Exemplary graphic evaluation of the Sysmex CS-2500 System and Reagents / Standards Lots 1 / 1.

Table 1. Linearity results.

System	Reagents / Standards Lots	Linear Regression Model with Std. Error of Regression			Linearity Range (ng/mL)
		Order	Coef	Coefficient Value	
Sysmex CS-2500	1 / 1	1st	Std. Error of Regression	7.93682	10.5–378.0
		1st	b0	0.00000	
		1st	b1	0.99837	
	2 / 2	1st	Std. Error of Regression	4.12330	10.5–378.0
		1st	b0	0.00000	
		1st	b1	0.95733	
	3 / 3	1st	Std. Error of Regression	12.68411	10.5–378.0
		1st	b0	0.00000	
		1st	b1	0.98057	
Sysmex CN-6000	3 / 2	1st	Std. Error of Regression	11.47482	10.5–378.0
		1st	b0	0.00000	
		1st	b1	0.97824	

For both analyzer types, linearity from 10.5 ng/mL to 378.0 ng/mL could be demonstrated.

LLoQ

Table 2. LLoQ results.

System	Reagents Lot	Standards Lot	Sample	Assigned Concentration (ng/mL)	Mean of Measured Concentration (ng/mL)	Absolute Value of Bias (ng/mL)	Standard Deviation (ng/mL)	Total Error (TE) (ng/mL)	LoQ Result (ng/mL)
Sysmex CS-2500	1	1	1	16	16.9	0.9	1.658	4.23	16.9
	2	1	1	16	15.9	0.1	1.443	2.97	
Sysmex CN-6000	1	1	1	16	12.1	3.9	0.928	5.77	14.0
	2	1	1	16	14.0	2.0	0.990	3.98	

For both analyzer types, a LLoQ of ≤ 20 ng/mL was shown.

Dilution Recovery

Table 3. Dilution recovery results.

System	Sample	Expected Concentration (ng/mL)	Measured Concentration (ng/mL)	Deviation (%)
Sysmex CS-2500	1	640	676.5	5.7
	2	580	622.3	7.3
	3	520	533.8	2.6
	4	460	471.0	2.4
	5	400	422.0	5.5
Sysmex CN-6000	1	640	667.3	4.3
	2	580	609.8	5.1
	3	520	530.3	2.0
	4	460	451.5	-1.8
	5	400	408.3	2.1

An extended measuring interval (EMI) from 350 to 700 ng/mL was confirmed for both analyzer types.

Precision

Table 4. Precision results.

System	Statistical Parameter	Plasma Pool 1	Plasma Pool 2	Plasma Pool 3	Plasma Pool 4	Plasma Pool 5
Sysmex CS-2500	n	40	40	40	40	40
	Mean (ng/mL)	31.5	46.9	99.8	212.5	332.4
	Repeatability CV (%)	3.90	3.06	2.04	1.53	2.04
	Between-run CV (%)	1.08	0.00	0.93	0.00	1.61
	Within-day CV (%)	4.04	3.06	2.24	1.53	2.60
	Between-day CV (%)	0.00	1.10	0.00	0.61	0.78
	Total CV (%)	4.04	3.25	2.24	1.64	2.71
Sysmex CN-6000	N	40	40	40	40	40
	Mean (ng/mL)	28.0	50.9	104.3	219.1	337.4
	Repeatability CV (%)	3.04	1.98	0.61	0.37	0.85
	Between-run CV (%)	2.00	1.38	0.12	0.19	0.00
	Within-day CV (%)	3.64	2.42	0.63	0.42	0.85
	Between-day CV (%)	3.06	0.00	0.57	0.37	0.41
	Total CV (%)	4.76	2.42	0.85	0.56	0.95

Precision data demonstrate a total CV of < 5 % for all samples using both analyzer types.

Method Comparison

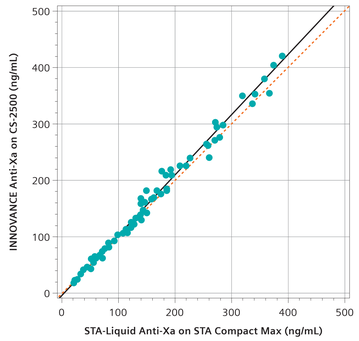

Figure 2. Method Comparison. Exemplary graphic evaluation of the INNOVANCE Anti-Xa assay on the Sysmex CS-2500 System versus the STA-Liquid Anti-Xa assay on the Compact Max System.

Table 5. Method comparison results.

Method 2 (y-axis)		Method 1 (x-axis)		n	Coefficient	Slope	Intercept	Deviation at 30 ng/mL (MI 20–100 ng/mL)	Deviation at 50 ng/mL (MI 20–100 ng/mL)
Assay	System	Assay	System		(r)		(ng/mL)		
INNOVANCE Anti-Xa	Sysmex CS-2500	STA Liquid-Anti-Xa	STA Compact Max	79	0.994	1.063	-3.063	0.5	1.5
INNOVANCE Anti-Xa	Sysmex CS-5100	INNOVANCE Anti-Xa	Sysmex CS-2500	74	0.993	0.984	0.355	-0.16	-0.82
INNOVANCE Anti-Xa	Sysmex CN-3000	INNOVANCE Anti-Xa	Sysmex CS-2500	74	0.994	1.000	0.500	-1.0	-1.0

Method comparison results demonstrate the suitability of the INNOVANCE Anti-Xa assay for the quantitative determination of edoxaban when using applications on the Sysmex CS-2500 System, the Sysmex CS-5100 System, and Sysmex CN-3000 System.

Conclusions

In combination with INNOVANCE Edoxaban Standards and INNOVANCE Edoxaban Controls, the INNOVANCE Anti-Xa assay demonstrates good performance on the Sysmex CS-2500, Sysmex CS-5100, and Sysmex CN-3000/6000 Systems regarding linearity, lower limit of quantitation, dilution recovery, precision, and method comparison. The method comparison study of the INNOVANCE Anti-Xa assay on the Sysmex CS-2500 System versus the STA Liquid Anti-Xa assay on the STA Compact Max System demonstrated a good agreement, indicating that INNOVANCE Anti-Xa assay can be used for the quantitative determination of edoxaban in human citrated plasma.

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** Not available for sale in the U.S.*

The products / features / applications mentioned here are not commercially available in all countries and are subject to local regulations. Their future availability cannot be guaranteed.

INNOVANCE Edoxaban Standards and INNOVANCE Edoxaban Controls are under development and not commercially available. Its future availability cannot be ensured.

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

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