CPC049 Anti-Factor Xa chromogenic assay for measuring rivaroxaban plasma concentrations using calibrators and controls: results of a multicentre field trial

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Introduction

- Rivaroxaban is an oral, direct Factor Xa inhibitor[1] that has been approved for the prevention and treatment of thromboembolic disorders
- Unlike some traditional anticoagulants, routine coagulation monitoring is not required with rivaroxaban[2] owing to its predictable pharmacokinetics and pharmacodynamics[3–5]
- In clinical practice, measurement of rivaroxaban exposure may be useful in some circumstances (e.g. prior to urgent surgery)
- Studies have indicated that routine clotting assays are not suitable for the quantitative measurement of rivaroxaban exposure, and anti-Factor Xa chromogenic assays have been identified as potential assays for the measurement of rivaroxaban plasma concentrations[6,7]

Objective

- To evaluate the suitability of anti-Factor Xa chromogenic assays for the measurement of rivaroxaban plasma concentrations (ng/ml) using rivaroxaban calibrators and controls, and to assess the inter-laboratory precision of the measurement

Methods

- Twenty-four centres in Europe and North America were provided with:
  - A set of rivaroxaban calibrators containing 0, 41, 209 and 422 ng/ml rivaroxaban
  - A set of pooled human plasma controls containing 20, 199 and 662 ng/ml rivaroxaban. The concentrations of rivaroxaban in the pooled human plasma controls were unknown to the participating laboratories
- The evaluation was carried out over 10 days by each laboratory using local anti-Factor Xa reagents (Table 1) as well as the centrally provided reagent, a modified STA® Rotachrom® assay
- Day-to-day precision and accuracy were evaluated by producing a calibration curve each day and by testing in duplicate three pooled human plasma controls
- The control plasma sample containing the highest concentration of rivaroxaban was diluted with calibrator containing 0 ng/ml rivaroxaban (1:3 dilution) and re-tested if the measured level was above the highest concentration limit of the calibration curve
- The rivaroxaban concentrations in the three control plasma samples were calculated from linear calibration curves generated using rivaroxaban calibrators by each participating laboratory

Results

- Inter-laboratory precision of the measurements (Table 2):
  - Local anti-Factor Xa reagents: the mean rivaroxaban concentrations (measured/actual values) were: 17/20, 205/199 and 668/662 (diluted sample) ng/ml, and the coefficient of variation (CV) was 37.0%, 13.7% and 14.1%, respectively
  - Modified STA® Rotachrom® method: the mean rivaroxaban concentrations (measured/actual values) were 18/20, 199/199 and 656/662 (diluted sample) ng/ml, and the CV was 19.1%, 10.9% and 10.0%, respectively
- Intra-laboratory precision of the measurements (Table 2):
  - Local anti-Factor Xa reagents: the CV was 27.7% (20 ng/ml), 4.0% (199 ng/ml) and 5.2% (662 ng/ml; diluted sample), respectively
  - Modified STA® Rotachrom® method: the CV was 17.2% (20 ng/ml), 5.1% (199 ng/ml) and 5.8% (662 ng/ml; diluted sample), respectively
- Median rivaroxaban concentrations in all control plasma samples are shown in Figure 1 (using local reagents) and Figure 2 (using the modified STA® Rotachrom® test set-up) for each participating laboratory

Table 1. Local anti-Factor Xa reagents and instruments used by the participating laboratories

Routine reagent			Diagnostica Stago			Instrumentation Laboratory		Siemens	Chromo-genix	Hyphen BioMed	
			STA® Rotachrom® Heparin	Stachrom® Heparin	STA® Staclot® Heparin	HemosIL® Heparin	HemosIL® Liquid Heparin	Berichrom® Heparin	Coamatic® Heparin	Biophen Heparin	Total
Two-step method (T) or Competition method (C)			C	T	T	T	T	T	C	T	
Routine instrument	Diagnostica Stago	STA-R	4	1						3	8
		STA Compact®		1	1						2
	Trinity Biotech	AMAX 190+		1							1
	Instrumentation Laboratory	ACL 9000				1					1
		ACL TOP®				3	1		1		5
		ACL Elite® Pro							1		1
	Siemens	BCS®						1		1	2
	Sysmex	CA-1500							1		1
Total			4	3	1	4	1	1	3	4	21

Table 2. Inter- and intra-laboratory precision of rivaroxaban plasma concentration measurements with anti-Factor Xa chromogenic assays using rivaroxaban calibrators and control plasma samples

Human plasma controls – theoretical values (ng/ml)	N	20	199	662	662 (diluted*)
Inter-laboratory precision					
Local anti-Factor Xa reagents (ng/ml)					
Mean ± SD	20	17±6.4	205±28.2	576±106.5	668±94.4
Mean CV (%)		37.0	13.7	18.5	14.1
STA® Rotachrom® (ng/ml)					
Mean ± SD	23	18±3.4	199±21.7	541±12.7	656±65.8
Mean CV(%)		19.1	10.9	2.3	10.0
Intra-laboratory precision					
Local anti-Factor Xa reagents (ng/ml)					
Mean CV (%)	20	27.7	4.0	2.4	5.2
Two-step methods: mean CV (%)	13	30.3	4.2	2.5	5.3
Competition methods: mean CV (%)	7	22.8	3.6	2.3	5.1
STA® Rotachrom® (ng/ml)					
Mean CV (%)	23	17.2	5.1	2.5	5.8

N=20 for the local reagents (a clot-based assay, STA® Staclot® Heparin was used by one laboratory, which was excluded from these analyses); N=23 for the modified STA® Rotachrom® test set-up.
*Samples were diluted 1:3 with calibrator (containing 0 ng/ml rivaroxaban).
CV, coefficient of variation; SD, standard deviation.

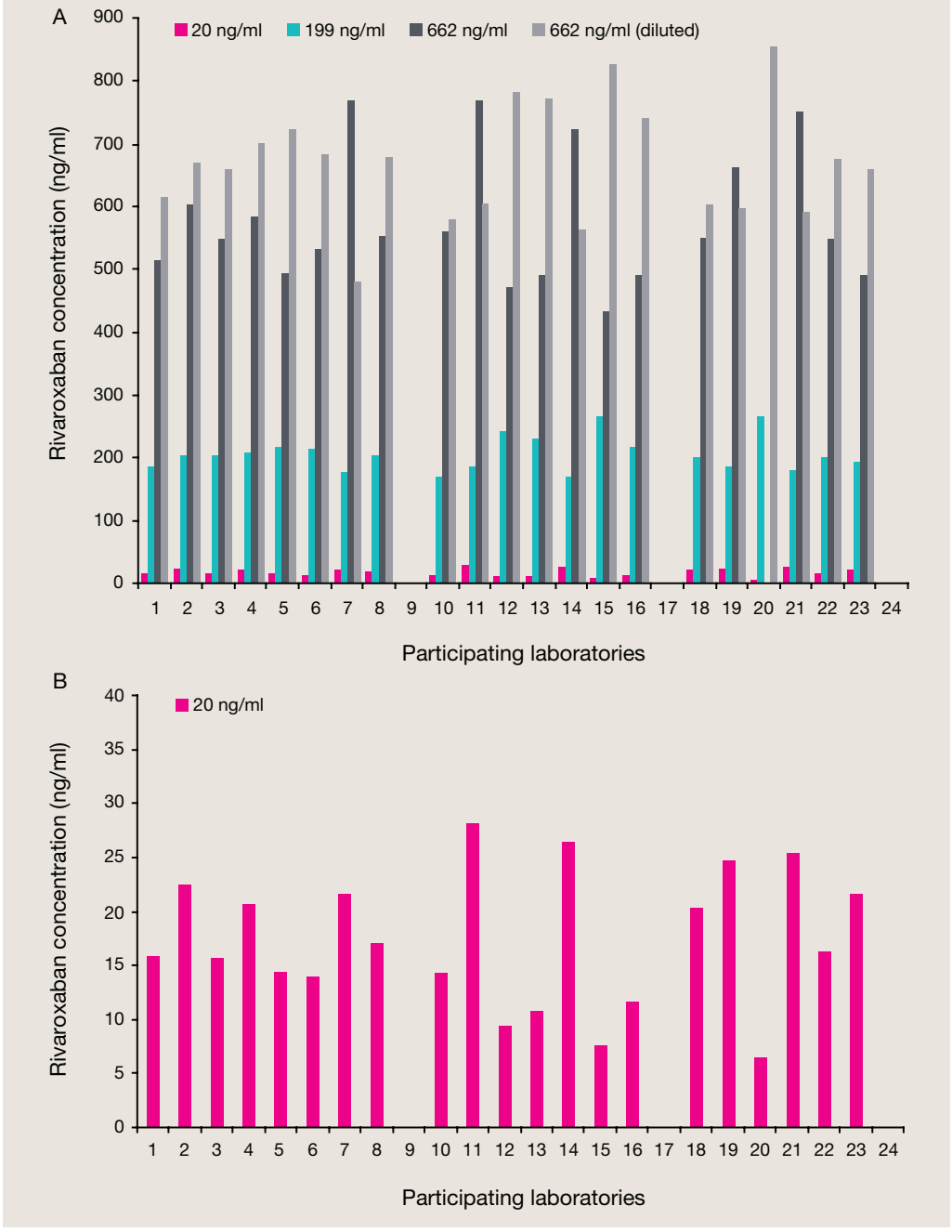

Figure 1. Rivaroxaban concentrations in control plasma samples measured using local anti-Factor Xa reagents. A) Measured values for plasma samples containing 20 ng/ml, 199 ng/ml, 662 ng/ml and diluted 662 ng/ml rivaroxaban, respectively; B) Measured values for the plasma sample containing 20 ng/ml rivaroxaban. Results are presented as median values from each site (N=21; including one laboratory that used the STA® Staclot® Heparin assay).

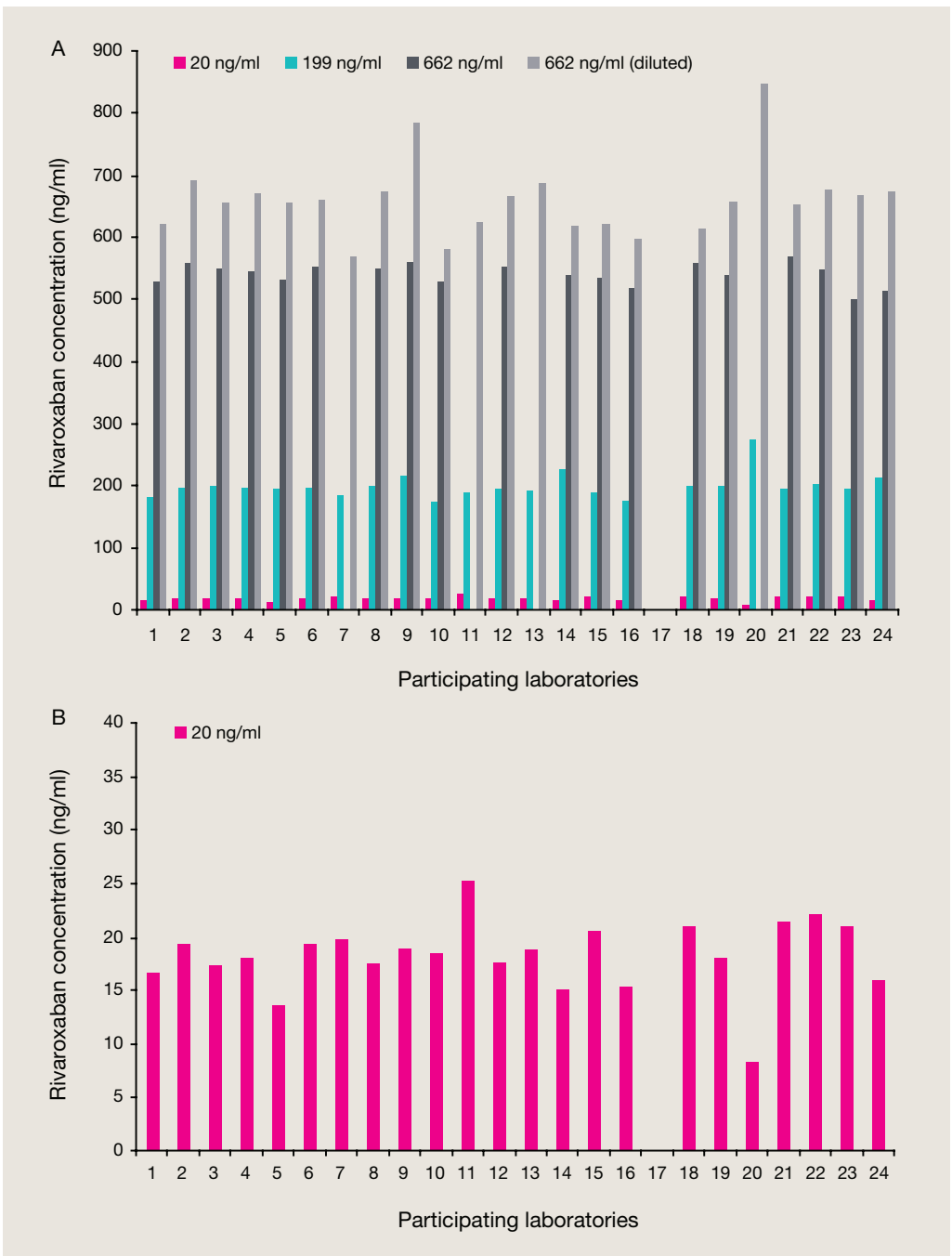

Figure 2. Rivaroxaban concentrations in control plasma samples measured using centrally provided reagents (STA® Rotachrom®). A) Measured values for plasma samples containing 20 ng/ml, 199 ng/ml, 662 ng/ml and diluted 662 ng/ml rivaroxaban, respectively; B) Measured values for the plasma sample containing 20 ng/ml rivaroxaban. Results are presented as median values from each site (N=23).

Conclusions

- The anti-Factor Xa chromogenic method can be used to assess rivaroxaban exposure (expressed in ng/ml), and the method is suitable for measuring a wide range of rivaroxaban plasma concentrations (approximately 20–660 ng/ml), with the use of rivaroxaban calibrators and controls
- Low rivaroxaban concentrations can be measured with acceptable inter-laboratory precision with the use of a modified anti-Factor Xa method (the modified STA® Rotachrom® test set-up)
- Further validation of these methods would be helpful in clinical settings

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Disclosure of conflict of interest
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