

# Assessment of Factor Xa Chromogenic Assays for Measuring the Pharmacodynamics of Rivaroxaban – an Oral, Direct Factor Xa Inhibitor

Elisabeth Perzborn<sup>1</sup>, Michaela Harwardt<sup>1</sup>, Meyer Michel Samama<sup>2,3</sup>

<sup>1</sup>Bayer Schering Pharma AG, Wuppertal, Germany; <sup>2</sup>Hôtel-Dieu University Hospital, Paris, France; <sup>3</sup>Biomnis Laboratories R&D, Ivry sur Seine, France

## Introduction

- ◆ Several assays measuring the amidolytic activity of Factor Xa on a chromogenic substrate are commercially available for the quantitative determination of unfractionated or low molecular weight heparins
- ◆ Rivaroxaban is a highly selective, reversible, direct Factor Xa inhibitor, unlike the heparins, which are indirect inhibitors and catalyze the inhibition of Factor Xa by antithrombin<sup>1,2</sup>
- ◆ Although rivaroxaban can be used without the need for routine coagulation monitoring or titration, a quantitative determination of rivaroxaban might be valuable in some instances, for example in case of significant overdose or to measure compliance

## Objective

- ◆ To evaluate commercially available Factor Xa chromogenic assays – based on the inhibition of a constant amount of Factor Xa – for the quantitative determination of rivaroxaban in human plasma

## Methods

- ◆ Blood from six healthy volunteers was collected into tubes containing 1/10 volume of 3.2% trisodium citrate. Platelet-poor plasma was obtained by immediate centrifugation at 1000 g for 10 minutes at 4°C. Platelet-poor plasma was pooled and stored at -70°C and thawed before use at 27°C
- ◆ Pooled plasma was spiked with increasing concentrations of rivaroxaban (0.15, 30, 60, 125, 250, 500, and 1,000 ng/mL; v/v=1/100) dissolved in dimethyl sulfoxide (DMSO)
- ◆ Assays were performed in platelet-poor plasma in microtiter plates, according to the manufacturers' instructions
  - Absorbance was measured at 405 nm using a Spectra Rainbow Thermo Reader (Tecan, Crailsheim, Germany), with the exception of the Rotachrom® Heparin assay (STA Compact®; Diagnostica Stago, Asnières, France)
  - Each assay was performed twice with duplicate samples

- ◆ Different types of assays (either one-stage or two-stage assays) were compared, including an excess of Factor Xa and antithrombin, or including an excess of Factor Xa without additional antithrombin
  - Assays including excess Factor Xa and antithrombin: Biophen® Heparin Anti-Xa (2 stages; Hyphen BioMed, Neuville-sur-Oise, France); Coatest® Heparin (Chromogenix Instrumentation Laboratory, Milan, Italy); Berichrom® Heparin (Siemens Healthcare Diagnostics, Deerfield, IL, USA); Stachrom® Heparin (Diagnostica Stago, Asnières, France); Actichrome® Heparin (anti-fXa; American Diagnostica, Stamford, CT, USA)
  - Assays including excess Factor Xa and no antithrombin: Biophen® Heparin (Hyphen BioMed); Coamatic® Heparin and Coatest® LMW Heparin (Chromogenix Instrumentation Laboratory, Milan, Italy); Rotachrom® Heparin (Diagnostica Stago, Asnières, France); Liquid Heparin assay (not commercially available; courtesy of Instrumentation Laboratory)

- Values were calculated using GraphPad Prism®, version 4.02 (GraphPad Software, La Jolla, CA, USA)
- The IC<sub>50</sub> values of rivaroxaban are given as mean ± standard deviation from four different measurements in ng/mL calculated from the concentration response curves
- Low molecular weight heparin calibrators were measured according to the manufacturers' instructions, and the IC<sub>50</sub> values of rivaroxaban were also calculated from these calibration curves, and expressed in IU/mL

- ◆ The half-maximal inhibitory concentration (IC<sub>50</sub>) represents the concentration of an inhibitor that is required to reduce the effect of the control by 50%

- Values were calculated using GraphPad Prism®, version 4.02 (GraphPad Software, La Jolla, CA, USA)
- The IC<sub>50</sub> values of rivaroxaban are given as mean ± standard deviation from four different measurements in ng/mL calculated from the concentration response curves
- Low molecular weight heparin calibrators were measured according to the manufacturers' instructions, and the IC<sub>50</sub> values of rivaroxaban were also calculated from these calibration curves, and expressed in IU/mL

## Results

- ◆ Rivaroxaban concentration-dependently reduced Factor Xa activity (Figures 1 and 2)
- ◆ The assays largely differed in their sensitivity for the detection of rivaroxaban (Figures 1 and 2; Table 1)
  - IC<sub>50</sub> values were in the range of 21–405 ng/mL
- ◆ The presence of additional antithrombin had no obvious effect on the sensitivity of the assays (Figures 1 and 2)
- ◆ Using low molecular weight heparin calibrator curves, the calculated IC<sub>50</sub> values given in IU/mL varied considerably between assays (Table 1)

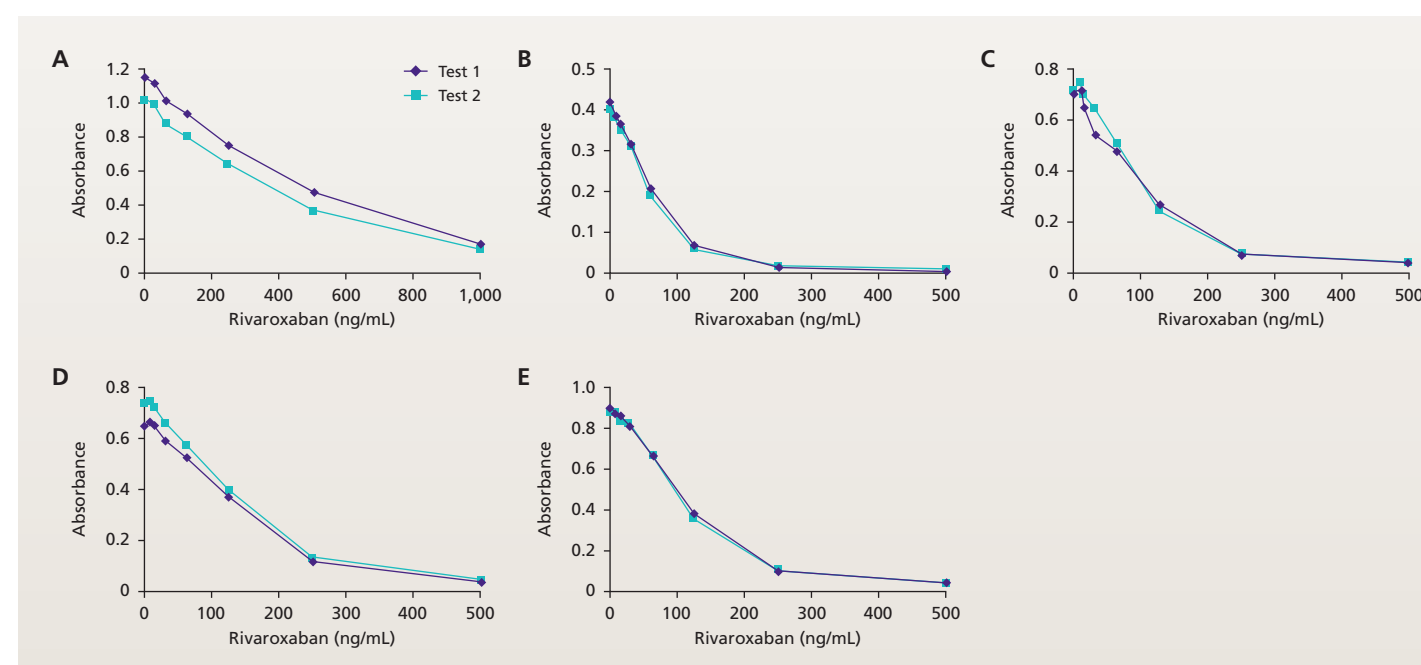


Figure 1. Influence of rivaroxaban on different Factor Xa assays (with additional antithrombin). (A) Biophen Heparin Anti-Xa (2 stages); (B) Coatest Heparin; (C) Berichrom Heparin; (D) Stachrom Heparin; and (E) Actichrome Heparin (anti-fXa).

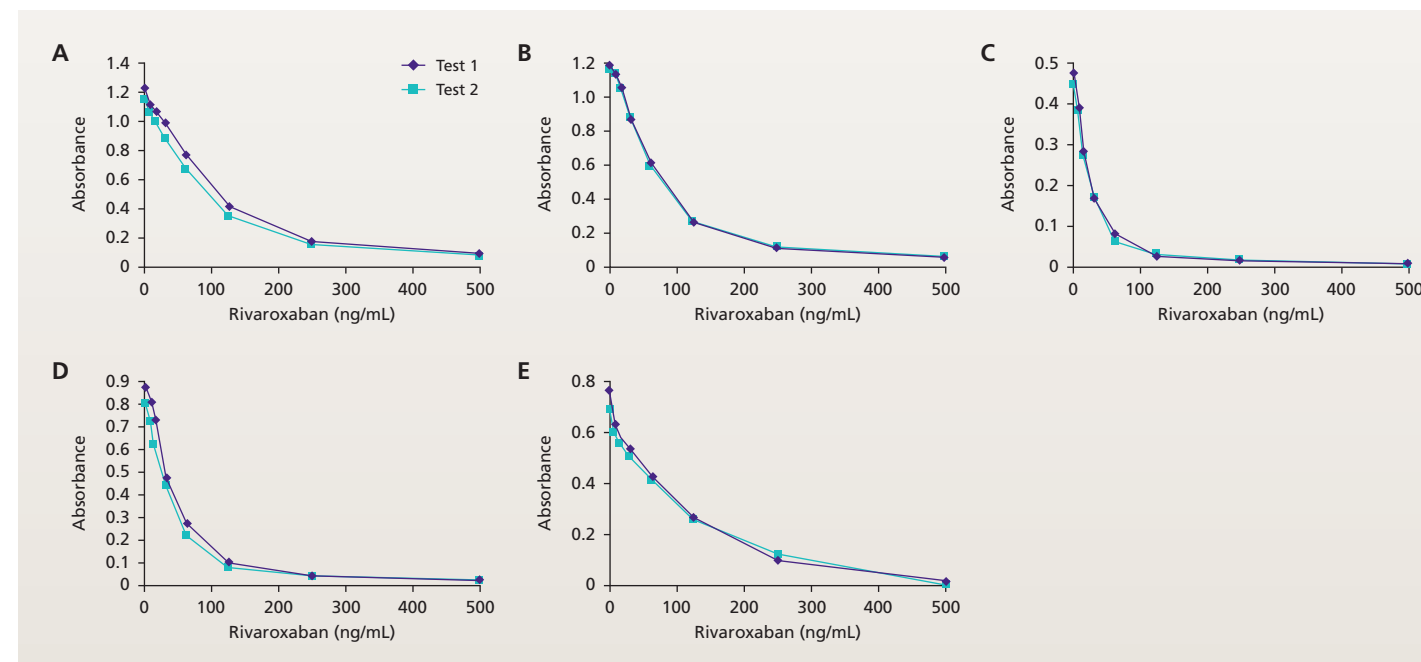


Figure 2. Influence of rivaroxaban on different Factor Xa assays (without additional antithrombin). (A) Biophen Heparin; (B) Coamatic Heparin; (C) Coatest LMW Heparin; (D) Rotachrom Heparin; and (E) Liquid Heparin assay.

## Conclusions

- ◆ Currently available Factor Xa chromogenic assays could be useful to measure rivaroxaban concentrations in human plasma
- ◆ Depending on the assay, rivaroxaban can be detected either at low or at high plasma levels, using rivaroxaban as a calibrator
  - Low molecular weight heparin calibrator curves cannot be used due to large variations between assays
- ◆ The plasma concentrations of rivaroxaban can be expressed in ng/mL, using rivaroxaban-spiked plasma probes as a calibrator

Table 1. IC<sub>50</sub> values of rivaroxaban in the different assays tested

	IC <sub>50</sub>	
	Rivaroxaban (ng/mL)*	Low molecular weight heparin (IU/mL) <sup>†</sup>
<b>Assays with additional antithrombin</b>		
Biophen Heparin Anti-Xa (2 stages)	405±20	1.2
Coatest Heparin	56±2	0.4
Berichrom Heparin	87±6	1.1
Stachrom Heparin	131±7	1.0
Actichrome Heparin (anti-fXa)	107±1	0.6
<b>Assays without additional antithrombin</b>		
Biophen Heparin	78±6	>1.5
Coamatic Heparin	60±2	>0.8
Coatest LMW Heparin	21±1	0.4
Rotachrom Heparin	35±2	0.7
Liquid Heparin assay	80±6	>2.0

\*Mean values ± standard deviation (n=4).

<sup>†</sup>Mean values.

IC<sub>50</sub>, half-maximal inhibitory concentration.

## References

- Perzborn E et al. *J Thromb Haemost* 2005;3:514–521.
- Hirsh J et al. *Chest* 2008;133(Suppl. 6):1415–1595.

## Disclosure of conflict of interest

This study was supported by Bayer Schering Pharma AG and Johnson & Johnson Pharmaceutical Research & Development, L.L.C. The data contained within this poster do not support or recommend the use of rivaroxaban in indications or countries in which it is not licensed.

Presented at the International Society on Thrombosis and Haemostasis (ISTH) XXII Congress, Boston, MA, USA; July 11–16, 2009