

Measuring concentrations of Rivaroxaban, Apixaban, Edoxaban

Methods and Challenges

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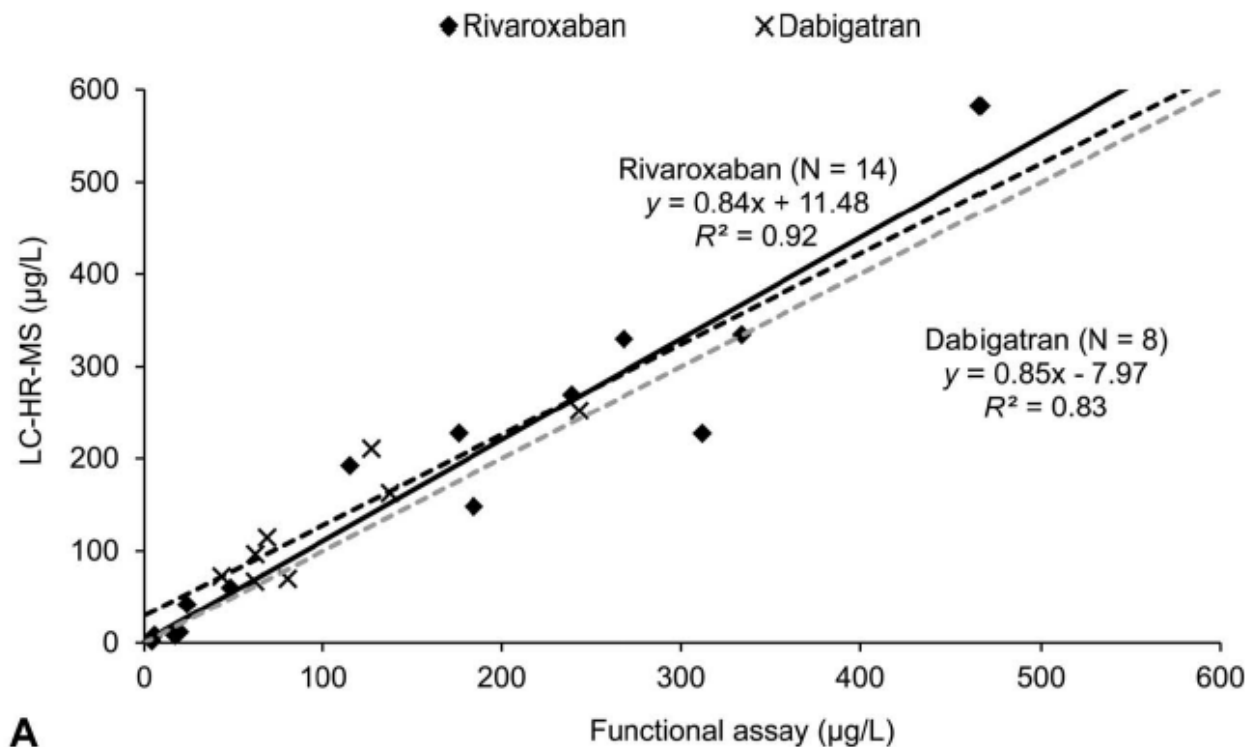
UK NEQAS Blood Coagulation

Disclosures/COI

- Speaker/advisory board/consultancy fees
 - Bayer (rivaroxaban)
 - Bristol Myers Squibb (apixaban)
 - Daiichi Sankyo (edoxaban)

Measurement of the Direct Oral Anticoagulants Apixaban, Dabigatran, Edoxaban, and Rivaroxaban in Human Plasma Using Turbulent Flow Liquid Chromatography With High-Resolution Mass Spectrometry

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Anti Xa – Stago

Anti IIa -
ecarin/chromogenic
/Hyphen calcs

Direct factor Xa inhibitors

Measurement of oral anti-Xa agents

Recommendation

- **Anti-Xa chromogenic assays should be used to determine plasma concentration of direct FXa inhibitors.**
- **Product-specific calibrator should be used and results should be expressed in mass concentration.**
- **LMWH reference standards should not be used as calibrators for direct FXa inhibitors.**
- **PT and APTT should not be used to measure the plasma concentration of Xa inhibitors.**

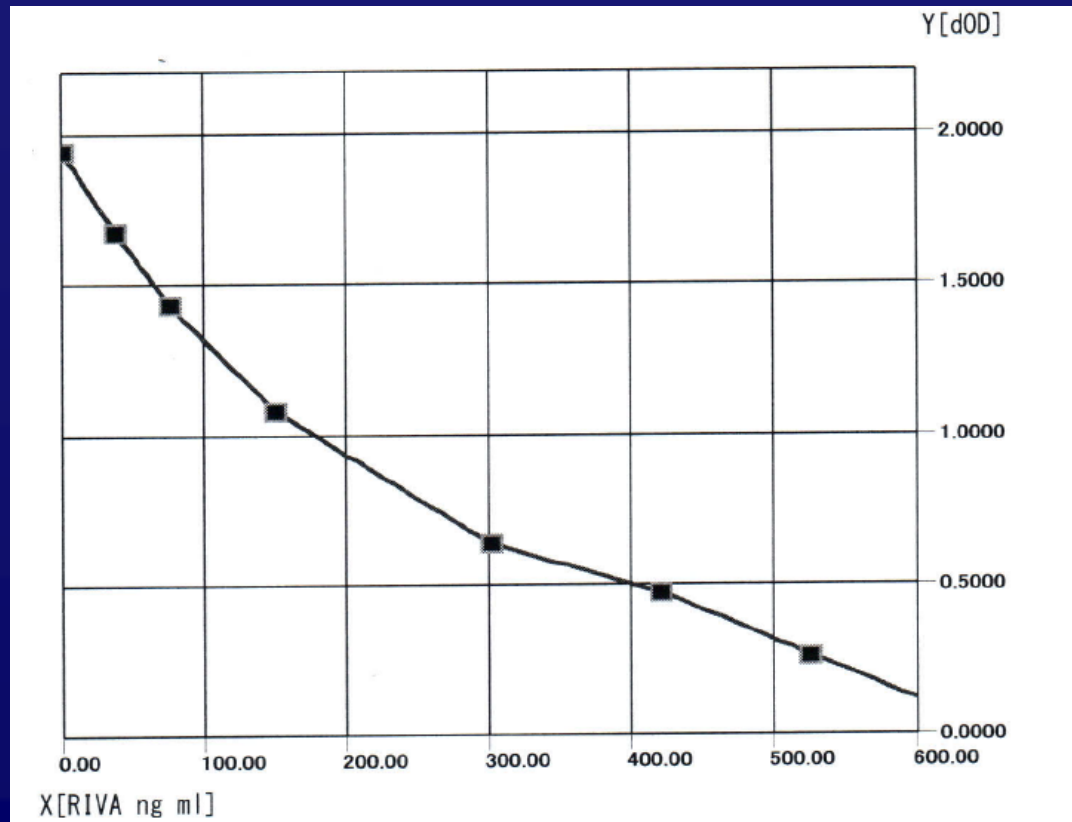
Anti Xa assay

- Xa is added to plasma sample
- Any Xa inhibitor present neutralises some of Xa
- Artificial substrate is added comprising several amino acids linked to a colourless molecule (pNA)
- Any residual Xa cleaves the bond and yellow colour develops

Anti Xa assay

- No drug, no inhibition of Xa – more colour
- More drug, more inhibition, less colour.
- Natural Xa inhibitors from test sample (AT, TFPI) usually no effect due to assay conditions
- **AT in reagents?**
- Assay calibrated by adding known concentrations of drug (commercial calibrators)
- Not specific for one drug - Heparin. LMWH inhibit via Antithrombin, any direct Xa inhibitor will be detected depending on reagents

Anti Xa assay for Rivaroxaban



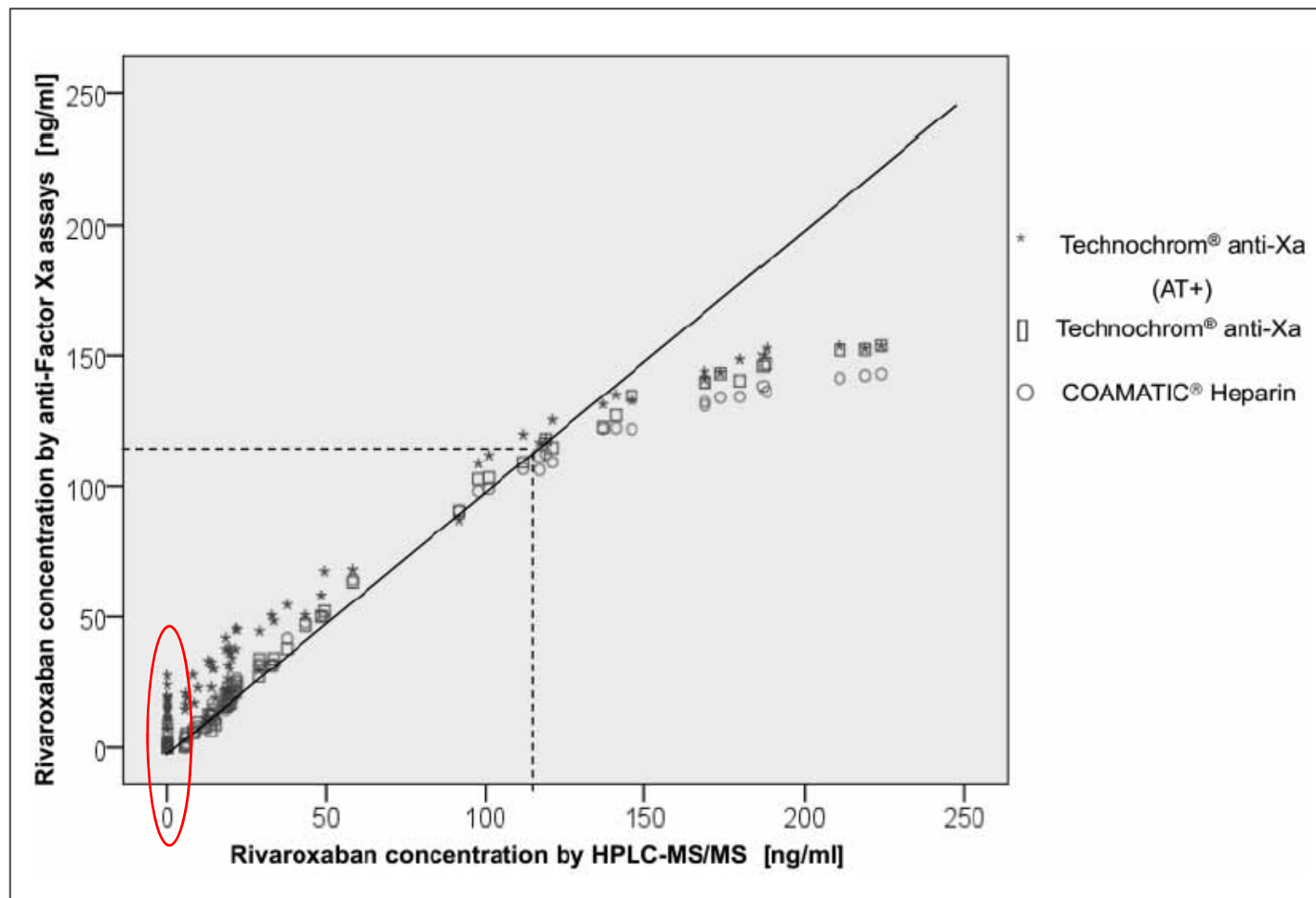
	RIVA ng ml	dOD
M	0.00	1.9400
M	37.50	1.6737
M	75.00	1.4310
M	150.00	1.0782
M	300.00	0.6353
M	420.00	0.4722
M	525.00	0.2639

Anti Xa assays for Rivaroxaban

(Mani et al 2012)

- Samples with <25 ng/ml require a low calibrator set (0,15, 60,100 ng/ml) for precise measurement
- Assay with added Antithrombin overestimated apparent rivaroxaban by 15-30 ng/ml

Anti Xa assays are unreliable below 25-30 ng/ml (Mani et al 2012, patients on Rivaroxaban)



Specific assays for DOAC

UK NEQAS - May 2014. Secondary care

	Apixaban	Dabigatran	Rivaroxaban
Chromogenic	43	13	123
Clotting	-	62	-
LC MS/MS	1	1	1
% of centres with an assay	7%	12%	20%

610 responses

Rivaroxaban assays in different centres

(Oct/Nov 2014)

55 centres

Anti Xa assays

Calibrators: Hyphen 26; Stago 10; Technoclone 6

Sample	Median (ng/ml)	Range (ng/ml)	CV
1	8*	0 - 102	186%
2	37	13 - 80	38%
3	140	94 - 473	34%

* Sample contained no rivaroxaban but only 7 centres recognised a lower limit of quantification by reporting as “less than”

Countries

- 19 UK
- 14 Italy
- France, Belgium, Germany, Republic of Ireland, Israel

Rivaroxaban Anti Xa assays with different calibrators

(Oct/Nov 2014)

Mass spec	Hyphen (26) median	Stago (10) median	Technolcone (5) median
<2.0 ng/ml*	6 ng/ml*	15 ng/ml*	0 ng/ml*
37 ng/ml	38 ng/ml	38 ng/ml	28 ng/ml
141 ng/ml	143 ng/ml	130 ng/ml	145 ng/ml

*sample 1 contained no rivaroxaban

Apixaban assays in different centres

(Oct/Nov 2014)

24 centres (55 for rivaroxaban, 49 for dabigatran)

Anti Xa assays

Calibrators: Hyphen 6; Stago 9; Technoclone 5

Sample	Median (ng/ml)	Range (ng/ml)	CV
1 (<1)	4.0	0 - 60	168%
2 (52)	45	21 - 69	22%
3 (193)	179	131 - 221	11%

(Tandem mass spec results in brackets)

Sample 1 contained no apixaban, 8 centres reported 0 and 3 centres recognised a lower limit of quantification by reporting as “less than”

Apixaban Anti Xa assays with different calibrators

(Oct/Nov 2014)

Mass spec	Hyphen (6) ng/ml	Stago (9) ng/ml	Technolcone (5) ng/ml
<1	0 ,0, 1, 8 <27,<30	0,5,7,7,9,12,20,30 <20	0,0,0,4,10
52	43 (38-48)	46 (38-69)	40 (25-58)
193	166 (146-184)	182 (156-213)	185 (153-210)

Sample 1 contained no apixaban

Development of a New Automated Assay for Measurement of the Oral Direct Factor Xa Inhibitor Edoxaban Plasma Concentration

Tristan Hervé¹, Sandra Beaufile², Ling He³, Jarema Kochan³, François Depasse¹

Diagnostica Stago, 3, allée Thérèse, 92600 Asnières sur Seine, France - ²Diagnostica Stago R&D, 125, avenue Louis Roche, 92230 Gennevilliers, France - ³Daiichi Sankyo Inc., 399 Thornall Street, Edison, NJ 08837, USA

- STA-Liquid Anti Xa
- Specific Calibrators and controls
- CV 3-7% between assay
- LLOD - 15 ng/ml
- LLOQ – 20 ng/ml
- ULOQ – 150 ng/ml or 450 with sample re-dilution

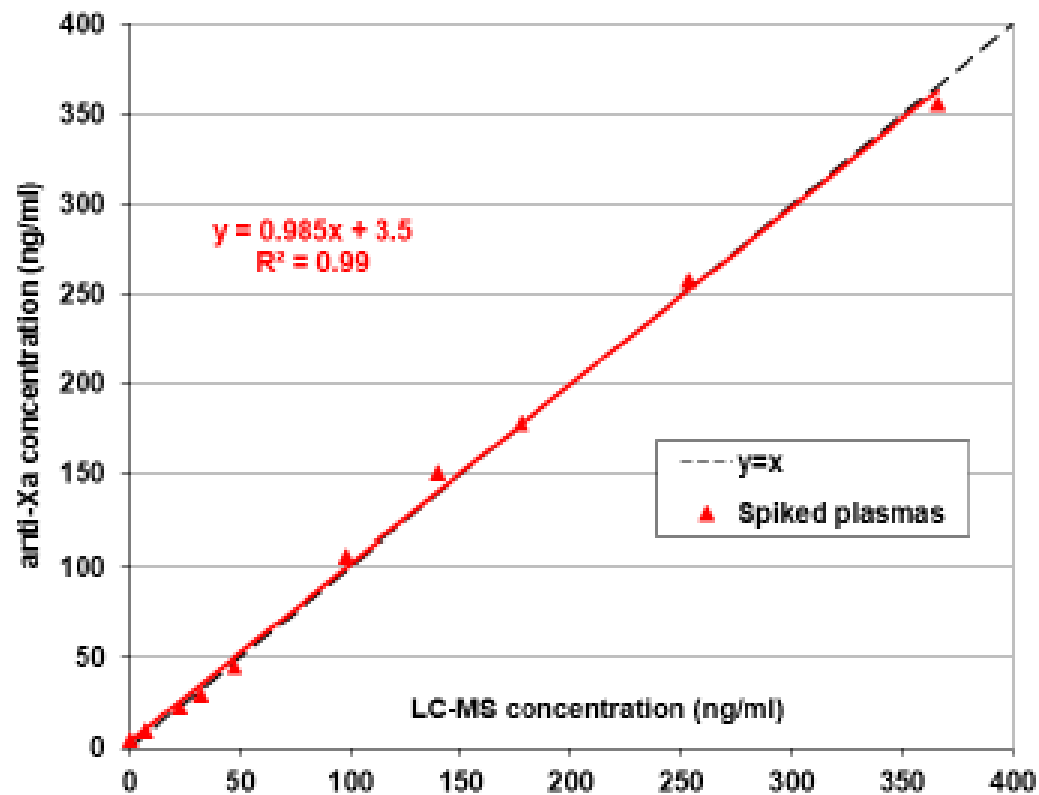
- NOT YET LAUNCHED

Development of a New Automated Assay for Measurement of the Oral Direct Factor Xa Inhibitor Edoxaban Plasma Concentration

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Anti-Xa vs LC-MS comparison for spiked plasmas:



Apixaban/Eliquis SPC

- PT INR APTT are affected. Changes are small at the expected therapeutic dose and subject to a high degree of variability.
- Calibrated quantitative anti Xa may be useful in exceptional circumstances
- Detailed table of expected anti Xa activity – Max, Min, 5th-95th percentiles in ng/ml and IU/ml of NVAF stroke prevention and for treatment and prevention of VTE

Edoxaban/Lixiana SPC

- Edoxaban prolongs clotting tests such as PT APTT
- Changes expected at the therapeutic dose are small, subject to a high degree of variability and not useful for monitoring
- Concomitant VKA and edoxaban – concomitant therapy can increase INR post Lixiana by up to 46%
- Pharmacodynamic effects measured by Anti Xa are predictable and correlate with dose and concentration of edoxaban
- Calibrated quantitative anti Xa may be useful in exceptional circumstances
- Detailed table of anti Xa activity by creatinine clearance

Rivaroxaban/Xarelto SPC

- PT APTT Heptest are affected by rivaroxaban
- Dose dependent effect on PT with Neoplastin. Other reagents provide different results. PT done in seconds not INR.
- Conversion between warfarin and Riva - anti Xa PiCT Heptest can be used to test for effects of Riva as these are not affected by warfarin
- Calibrated quantitative anti Xa assay may be useful in exceptional circumstances
- 20 mg dose - Geometric mean (90% prediction interval) 2-4 hr post dose 215 µg/ml (22-535) and 24 hr post dose- 32 µg/ml (6-239)

What is needed?

SPC

- More information/data/references to PT APTT and Anti Xa results with different reagents
- Anti Xa levels in more detail where lacking
- Statements that normal PT and/or APTT don't exclude presence of therapeutic levels

What is needed?

Other needs

- Wider availability of anti Xa assays – more centres, 24/7
- More Proficiency testing
- POC tests for emergency depts/ thrombolysis etc?
- International Standards and International Units - each product ? Single preparation?
- Commercial available CE marked Edoxaban anti Xa assays
- Published data on stability of drugs in blood samples