MEASUREMENT OF ANTICOAGULANT EFFECTS OF NEW ORAL ANTICOAGULANTS - PRACTICAL ASPECTS

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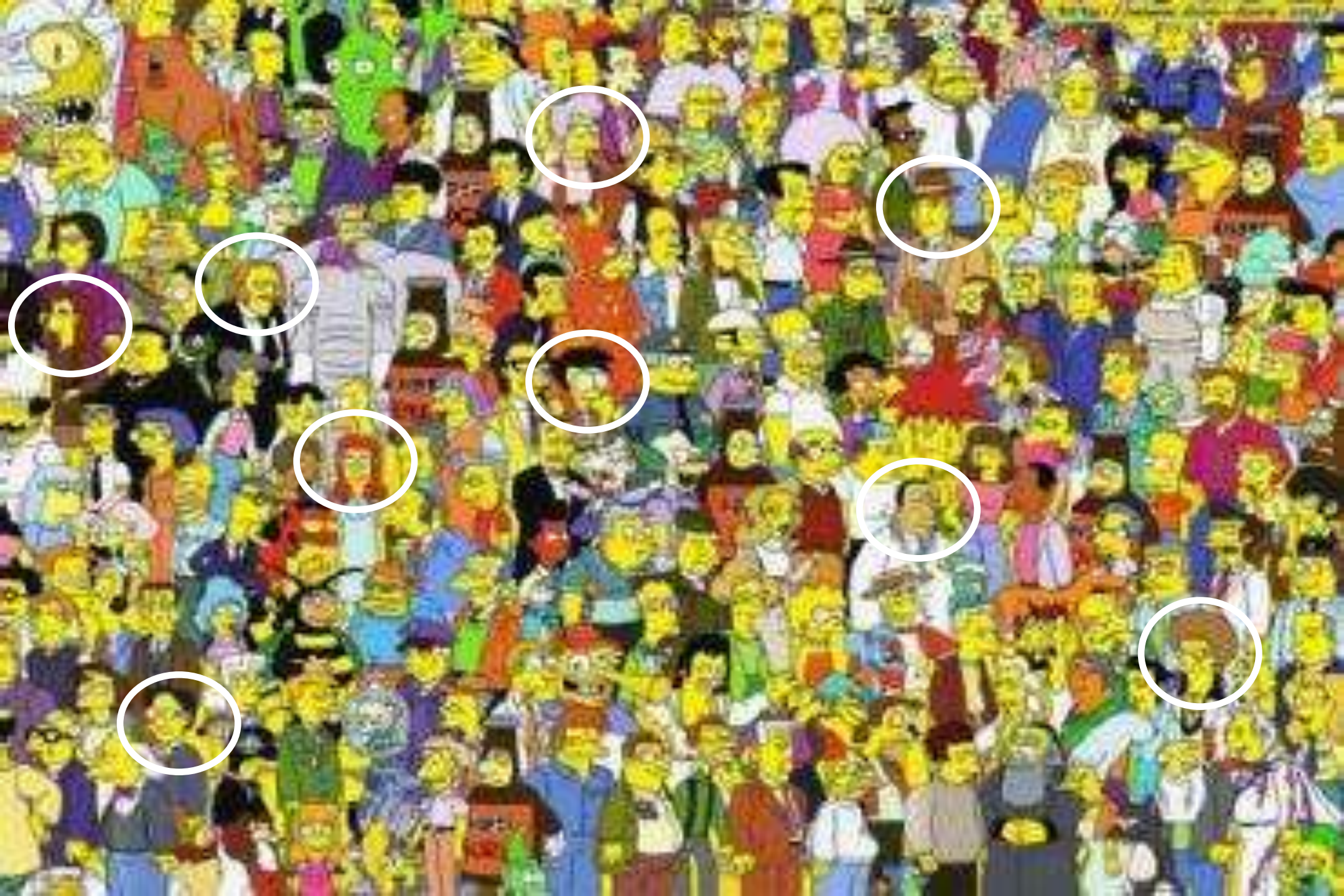
WARFARIN ERA

Warfarin monopoly on oral anticoagulant therapy, as well as that of other vitamin K antagonists derived from coumarins is over.

Eby C. Clin Chem 2013; 59: 732-4.



Warfarin monopoly on oral anticoagulant therapy, as well as that of other vitamin K antagonists derived from coumarins is over.

WARFARIN ERA

Within a few years it is likely that NOACs (DOACs) will be the dominant chronic anticoagulation therapy.

Eby C. IJLH 2013; 35: 262-8.

POSTWARFARIN ERA

Clinical Chemistry 59:5
732–734 (2013)

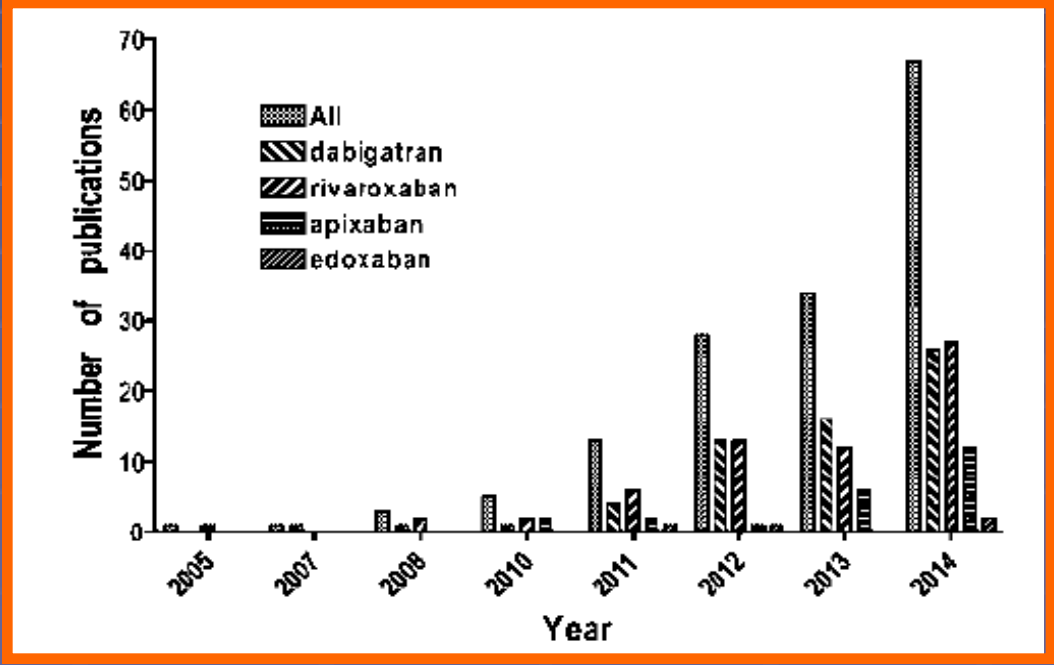
Editorials

Warfarin Replacements: Good for Patients, Challenging for Laboratories

Charles S. Eby[1*]

- 1109 articles

(Medline search using terms NOAC, DOAC, TSOAC, apixaban, dabigatran, rivaroxaban, edoxaban AND laboratory, monitoring, measurement or test)



Favaloro E, Lippi G. Semin Thromb Hemost 2015; 41: 208-27.

Laboratory testing in the postwarfarin era - what do we need to know?

Journal of Thrombosis and Haemostasis, **11**: 756–760 DOI: 10.1111/jth.12149

OFFICIAL COMMUNICATION OF THE SSC

Measuring oral direct inhibitors of thrombin and factor Xa: a recommendation from the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis

J Thromb Haemost 2013; **11**: 756–60.

T. BAGLIN,* A. HILLARP,† A. TRIPODI,‡ I. ELALAMY,§ H. BULLER¶ and W. AGENO**

Journal of Thrombosis and Haemostasis, **12**: 801–804 DOI: 10.1111/jth.12547

RECOMMENDATIONS AND GUIDELINES

Report of the Subcommittee on Control of Anticoagulation on the determination of the anticoagulant effects of apixaban: communication from the SSC of the ISTH

J. HARENBERG,* S. DU,* C. WEISS,† R. KRÄMER,‡ D. HOPPENSTEADT,§ and J. WALENGA§, FOR THE WORKING PARTY: METHODS TO DETERMINE APIXABAN OF THE SUBCOMMITTEE ON CONTROL OF ANTICOAGULATION OF THE INTERNATIONAL SOCIETY OF THROMBOSIS AND HAEMOSTASIS

**Medical Faculty Mannheim, Department of Clinical Pharmacology, Ruprecht-Karls-University Heidelberg, Mannheim; †Medical Faculty Mannheim, Medical Statistics and Biomathematics, Ruprecht-Karls-University Heidelberg, Mannheim; ‡Inorganic Chemistry Institute, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany; and §Loyola University Chicago, Maywood, IL, USA*

J Throm Haemost 2014; 12: 801-4.

Recommendation from the SSC (2013) on Control of Anticoagulation for measuring NOACs

ASSAYS

SEMIQUANTITATIVE

- for the estimation of the relative intensity of anticoagulation
- indicators of supratherapeutic, therapeutic or subtherapeutic anticoagulation status

- **Assays should not be used for the quantitative measurement of NOAC concentrations.**

QUANTITATIVE

- for the quantitative measurement of NOAC concentrations in plasma

QUANTITATIVE ASSAYS

DIRECT

LC tandem mass spectrometry

- gold standard – provide accurate measure of a wide range of NOAC concentrations in plasma samples (especially for low concentrations)

INDIRECT

- specific coagulation assays based on methods that are closely related to their anticoagulant targets and mechanism of action

standard calibration curve has to be generated with drug specific calibrators

SEMIQUANTITATIVE ASSAYS

THROMBIN TIME (TT)

- TT is very sensitive to the presence of dabigatran - suitable as a screening test for the exclusion of supratherapeutic dabigatran concentrations in plasma samples.

Dabigatran concentration (µg/L)	0	30	155	382	720
TT (s) BC Thrombin	21,6	127,5	>150	>150	>150
TT (ratio)	1,17	6,89	>8,11	>8,11	>8,11

N TT indicator of very low dabigatran concentration or no presence.

DILUTE THROMBIN TIME (dTT)

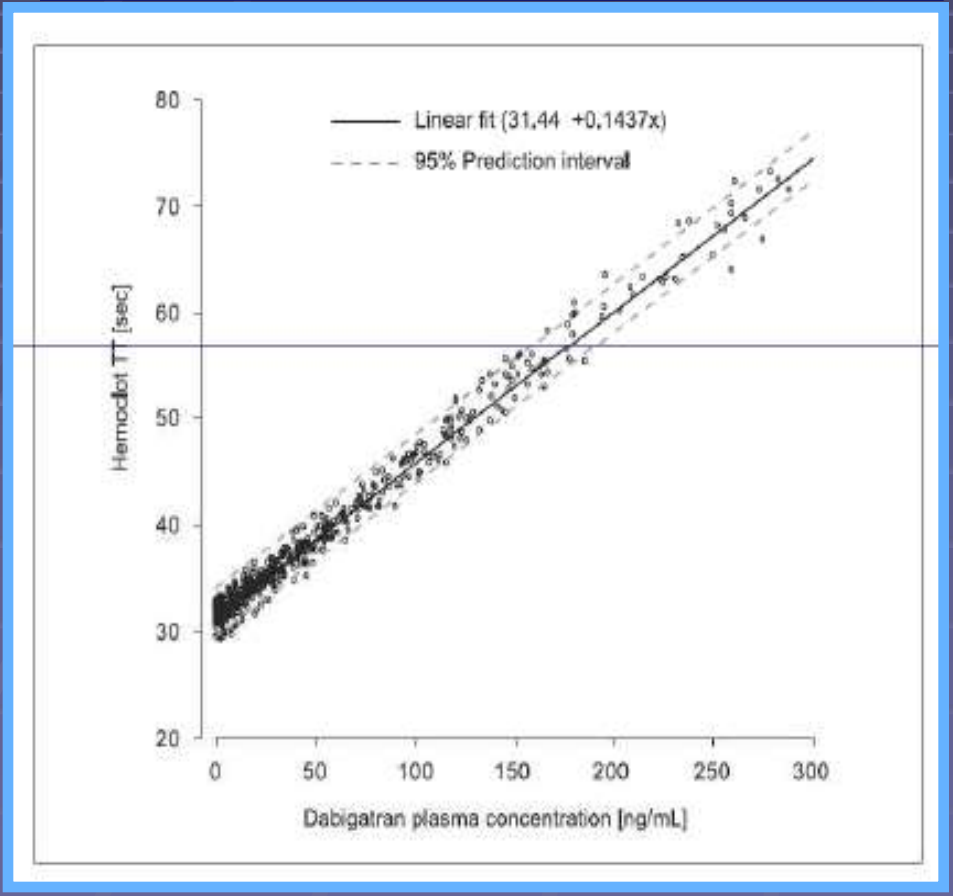
- The strong sensitivity of TT towards dabigatran led to the development of a CALIBRATED dTT using dabigatran standards to calculate dabigatran concentrations.
- Plasma samples are prediluted 1/8 or 1/20 in normal plasma before performing the assay.
- There is a linear relationship between dTT and dabigatran concentration in a wide range of concemtrations (a little bit not sensitive for low concentrations <50 ug/L).

QUANTITATIVE ASSAY

- high correlation of dTT with dabigatran plasma concentrations measured by LC-MS/MS (Douxfils 2014)

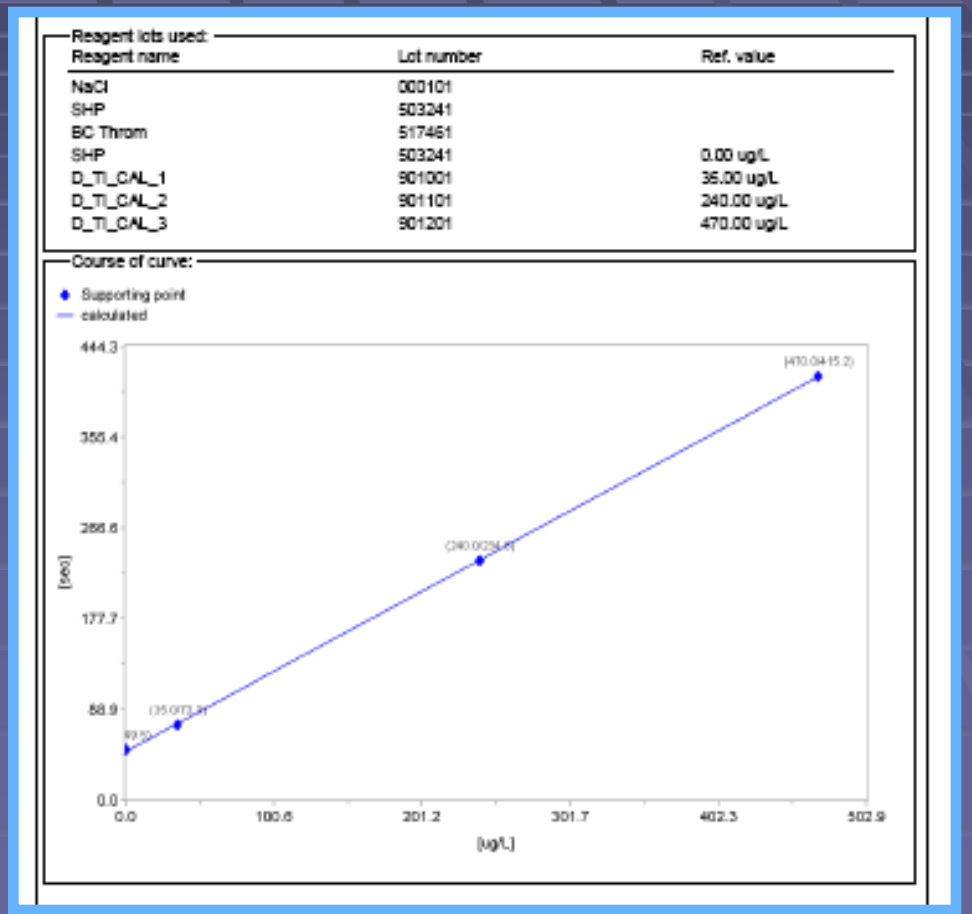
DILUTE THROMBIN TIME (dTT)

Commercially available kit HEMOCLOT ASSAY (HTI)



Ryn JV et al ,Thromb Haemost 2010; 103: 1116–1127

In-house method



BC Thrombin

Anticoagulant effects of NOACs on PT and APTT

The anticoagulant effect of each NOAC on PT and APTT has been demonstrated in a number of studies.

- In general a more pronounced influence has been observed for:
  - thrombin inhibitors on APTT
  - FXa inhibitors on PT
- PT and APTT expressed in s and ratios show a dose-dependent prolongation (increase) with increasing NOAC concentrations.

However, remarkable between-reagent variability has been observed.

PT and APTT reagent sensitivity to Dabigatran

PT reagents	APTT reagents
Thromborel S	Actin FSL
Recombiplastine 2G	Actin FS
STA Neoplastin Cl plus	Synthasil
Innovin	STA Cephascreen
STA Neoplastine R	STA PTT-A
	Pathromtin SL

Least sensitive

Most sensitive

PT reagent sensitivity

RIVAROXABAN	APIXABAN
Innovin	Innovin
Thromborel S	Thromborel S
STA Neoplastin Cl plus	STA Neoplastine R
Recombiplastine 2G	STA Neoplastin Cl plus
STA Neoplastine R	Recombiplastine 2G

Least sensitive

Most sensitive

APTT reagent sensitivity

RIVAROXABAN	APIXABAN
Synthasil	Actin FS
STA Cephascreen	Actin FSL
Actin FSL	STA Cephascreen
APTT-SP	Synthasil
Actin FS	APTT-SP
Pathromtin SL	Pathromtin SL

Least sensitive

Most sensitive

Determination of local PT and APTT reagents sensitivities to NOAC

IN-VITRO

using NOAC specific calibrators

(commercially available from several suppliers)

EX-VIVO

using plasma samples from patients receiving a specific NOAC

(NOAC concentrations determined with the appropriate quantitative assay)

J Thromb Haemost 2013; **11**: 756–60.

In-vitro determination of local PT and APTT sensitivity to NOACs

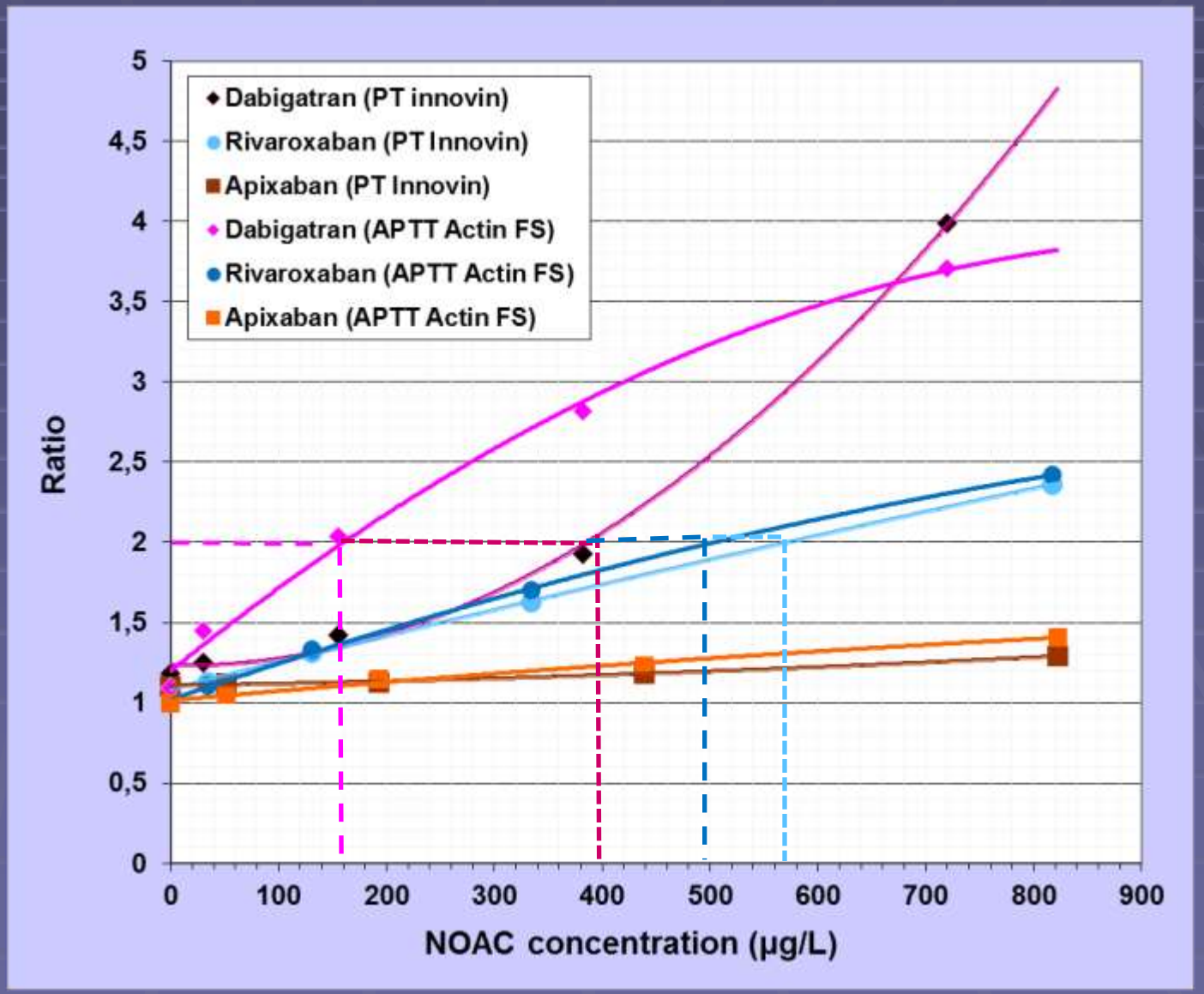
1. PT and APTT performed in pooled normal plasma samples spiked with increasing NOAC concentrations corresponding to through, peak and supratherapeutic levels:
   - Dabigatran concentrations 0, 30, 150, 382 and 720 µg/L
     (Neqas supplementary exercise, April 2012)
   - Rivaroxaban concentrations 0, 29, 121, 346 and 734 µg/L
   - Apixaban concentrations 0, 33, 164, 398 and 737 µg/L
     (Neqas supplementary exercise, May 2014)

2. PT and APTT performed using commercial Rivaroxaban calibrators (STA-Rivaroxaban Calibrator, Stago, France) with concentrations 0, 100, 254 and 494 µg/L

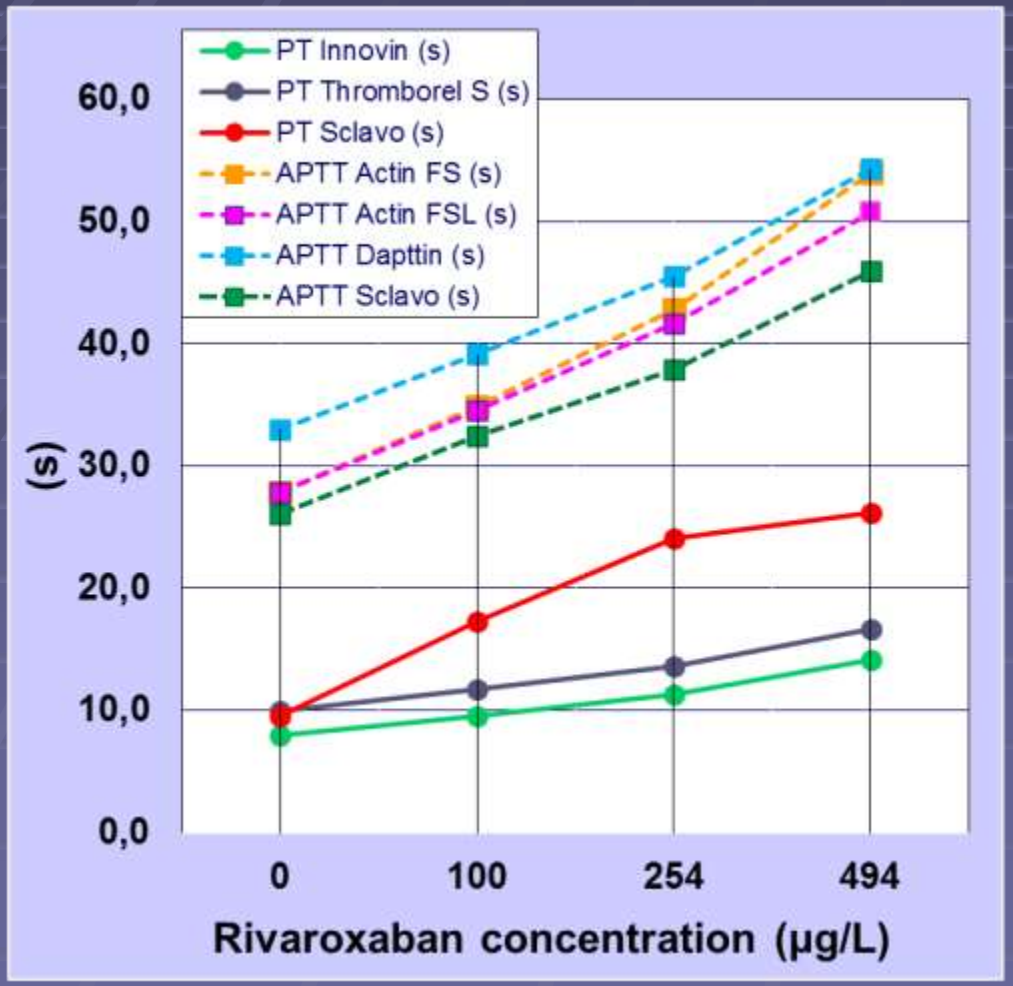
Local PT and APTT sensitivity to NOACs

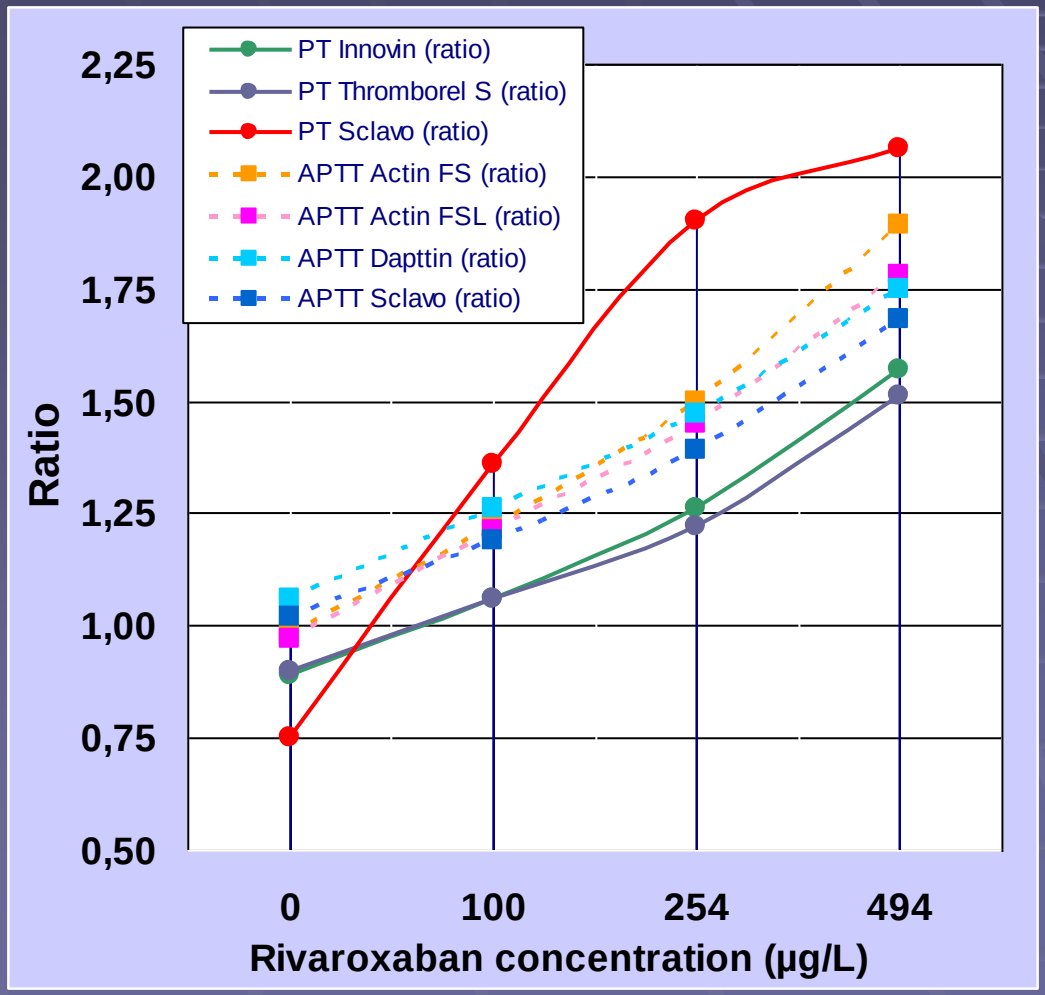
Neqas supplementary exercise, April 2012

Neqas supplementary exercise, May 2014



Local PT and APTT sensitivity to Rivaroxaban determined using Rivaroxaban calibrators





Ex-vivo effect of rivaroxaban

PATIENTS:

21 patient with nonvalvular atrial fibrilation (creatinine clearence >50 mL/min) receiving 20 mg of Rivaroxaban once daily

SAMPLES:

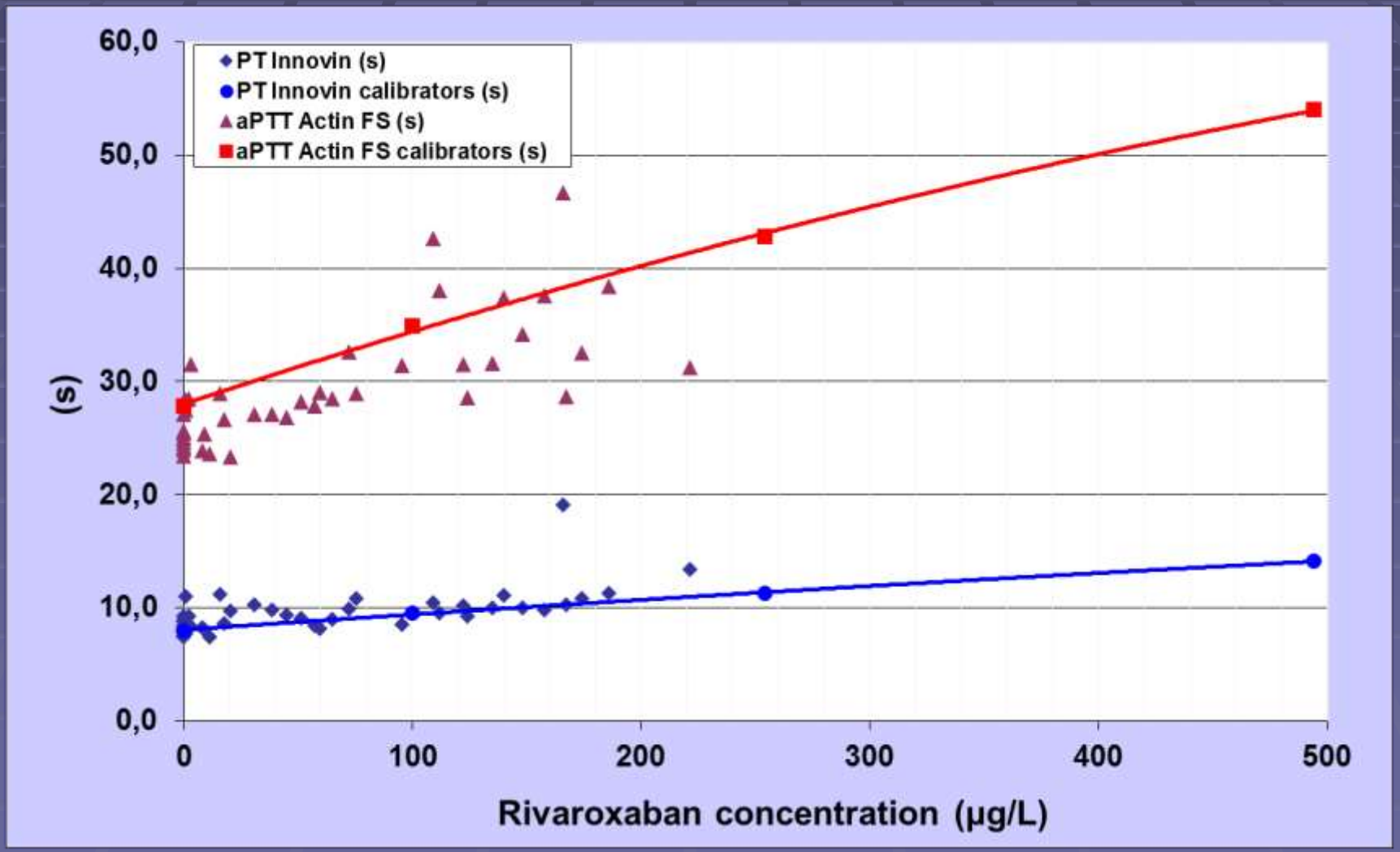
For each patient blood samples were taken:

- before the next dose (through concentration)
- 3h after drug administration (peak concentration)

METHODS:

PT (Innovin), APTT (Actin FS) and anti-Xa assay (Berichrom Heparin calibrated with STA-Rivaroxaban Calibrators)

Real-world variability in rivaroxaban levels in patients with atrial fibrilation



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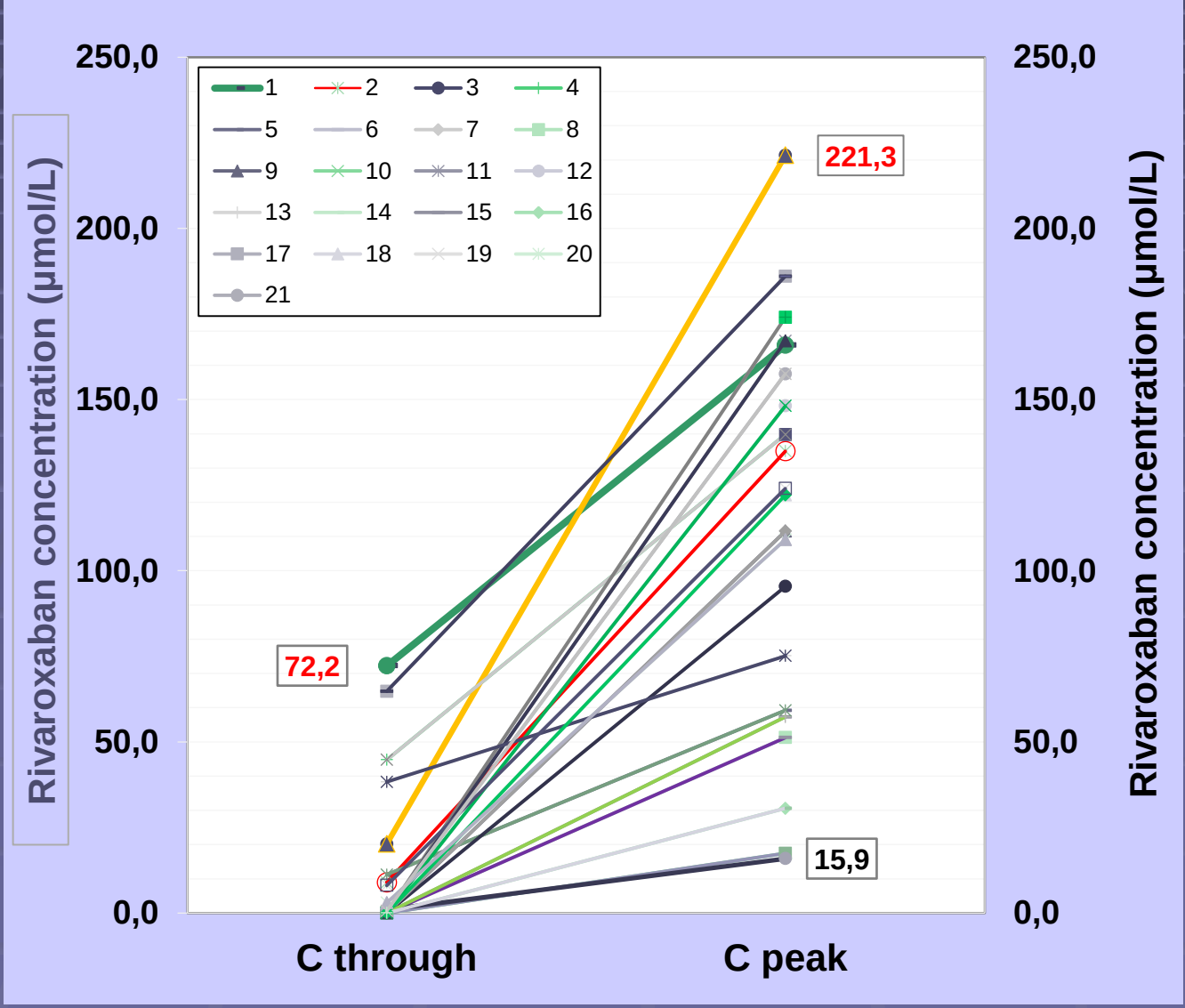

Journal of Thrombosis and Haemostasis, 11 (Suppl. 1): 122–128 DOI: 10.1111/jth.12227

INVITED REVIEW

The role of the laboratory in treatment with new oral anticoagulants

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Department of Haematology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Trust, Cambridge, UK

Table 1 General effects of thrombin and factor Xa inhibition by drugs such as dabigatran and rivaroxaban (and expected with other FXa inhibitors)

Test	Thrombin inhibition	Clinical utility	FXa inhibition	Clinical utility
PT	++	No	+++	Qualitative
INR	Not applicable	No	Not applicable	No
POC PT	Not applicable	No	++	Uncertain
APTT	+++	Qualitative	+	Uncertain
ACT	++	No	+	No

- **Attempts to adapt the INR system for specific NOAC**

The International Normalized Ratio calibrated for rivaroxaban has the potential to normalize prothrombin time results for rivaroxaban-treated patients: results of an *in vitro* study

A. TRIPODI,* V. CHANTARANGKUL,* C. GUINET† and M. M. SAMAMA†‡
**Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, IRCCS Cà Granda Ospedale Maggiore Policlinico Foundation and Università degli Studi di Milano, Milan, Italy; †Biomnis Laboratory, Ivry-sur-Seine; and ‡Hotel Dieu University Hospital, Paris, France*

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Table 1 International sensitivity index (ISI) calibration

		ISIrivaroxaban (CV% of the slope)	
Thromboplastin	ISIvka	Recombiplastin as arbitrary standard	Neoplastin-Plus as arbitrary standard
Recombiplastin	1.00	1.000	0.987 (1.0)
Thromborel S	1.07	1.382 (1.8)	1.361 (1.8)
Neoplastin-Plus	1.17	1.014 (1.0)	1.00
Neoplastin	1.75	1.196 (1.0)	1.177 (0.8)
Triniclot	1.22	1.224 (1.6)	1.208 (1.6)
Innovin	0.93	1.712 (1.4)	1.691 (1.6)

CV, coefficient of variation of the slope. ISIvka and ISIrivaroxaban refer to the ISIs valid for vitamin K antagonists and rivaroxaban, respectively.

$$INR_{rivaroxaban} = (PT_{patient}/PT_{normal})^{ISIrivaroxaban}$$

Measurement of NOACs in other media

The NOACs can be assesesed in alternate (nonplasma) samples.

- urine – measurement of dabigatran and rivaroxaban (qualitative assesment)

  (Harenberg J et al. Semin Thromb Hemost 2013; 39: 66-71.

  Harenberg J et al. Thromb J 2013; 11: article 15.)

- serum - measurement of rivaroxaban and apixaban

  (Harenberg J et al. Eur J Clin Invest 2014; 44: 743-52.

  Harenberg J et al. Semin Thromb Hemost 2014; 40: 129-34.)

Perspectives (the future)

- Indirect quantitative assays seem to be unreliable for the measurement of low plasma concentrations – there is a need to develop specific coagulation assays that are accurate for these low concentrations.
  - separate calibration curves in the low range ?
- There is a need for the development of a global coagulation test which is sensitive to all NOACs and for which a cut-off associated with bleeding or thrombotic can be provided.
  - dRVVT seems to be promising
- POCT including alternate samples such as urine and serum is under development – providing additional oportunities when plasma samples are unavailable.

Conclusions

- Up to now no biological assay can be recommended for the assesment of all NOACs.
- Each coagulation laboratory should be familiar with the sensitivity of its own PT and APTT reagents to each NOAC.
- A normal thrombin time suggests very low (not clinically relevant) level of dabigatran.
- For apixaban both PT and APTT are insensitive and patients may have normal coagulation times despite therapeutic concentrations, and anti-Xa assays should be used to determine plasma concentrations.
- As there are no therapeutic intervals set, threshold values of NOAC concentrations that might still be assumed as safe must be defined.

Postwarfarin era and laboratory responsabilities

Coagulation laboratory experts have a major role in ensuring patients receive appropriate testing and accurate interpretation of results, and must protect patients from inaccurate test results due to NOAC interference by educating clinicians and providing interpretive comments warning of possible confounding of results due to the presence of NOACs.

Eby C. IJLH 2013; 35: 262-8.

The laboratory needs to know the drug being used in order to perform appropriate testing and apply the appropriate calibration curve.