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PEARLS OF LABORATORY MEDICINE

II. Direct Oral Anticoagulants (DOACs): Laboratory Methods for Assessing Dabigatran

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Simplified Definition of Terms

Venous thromboembolism (VTE): clots within the deep venous system, also known as deep vein thrombosis (DVT) or pulmonary embolism (PE)

Pharmacokinetics (PK): drug concentration after administration

Pharmacodynamics (PD): the drug effect after administration

Peak levels: the maximum drug concentration after drug administration

Trough levels: the drug level just before the next drug dose

On-therapy range – reflecting the range of expected drug levels from lowest trough to highest peak for a given dose and indication

Dabigatran (Pradaxa)[1]

Dabigatran etexilate: Pradaxa (Boehringer Ingelheim) is prodrug that converts to active dabigatran which is an oral direct thrombin inhibitor, immediate acting

Binds free and bound thrombin (factor IIa)

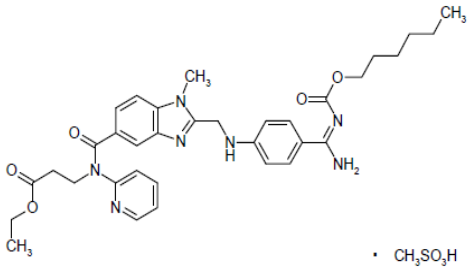
Bioavailability: ~ 3 – 7%

80% renally excreted

Peak concentration: 1.5 – 3.0 hrs after ingestion

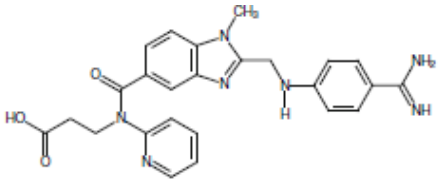
Half-life: ~13 hours

Dabigatran etexilate (BIBR 1048)



Hydrolysis

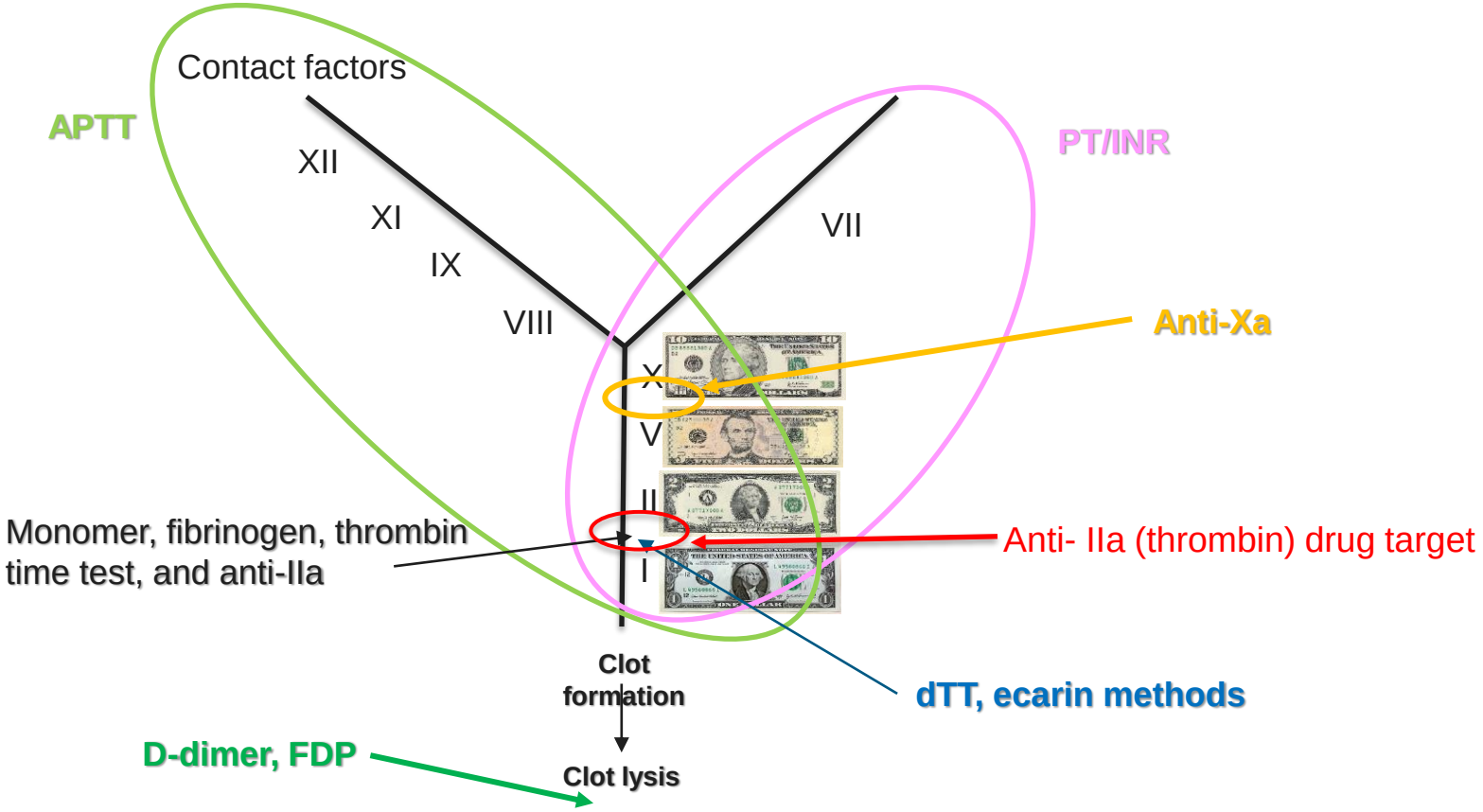
Dabigatran (BIBR 953)



Expected Dabigatran levels (PK)[2,3]

	Stroke prevention in NVAF	Treatment of DVT/PE	VTE Prophylaxis after knee/hip surgery
Dose	150mg bid	150mg bid	110mg 1st day, then 220mg qd
Mean Peak, ng/mL (25th – 75th percentile)	175 (117 – 275)	175 (117 – 275)	71 (35 – 162)
Mean Trough, ng/mL (25th – 75th percentile)	91 (61 – 143)	60 (39 – 95)	22 (13 – 35)

Targets for anticoagulation and laboratory testing



Dabigatran PD effect on PT and APTT[4]

Unreliable to quantify, monitor, or exclude drug presence

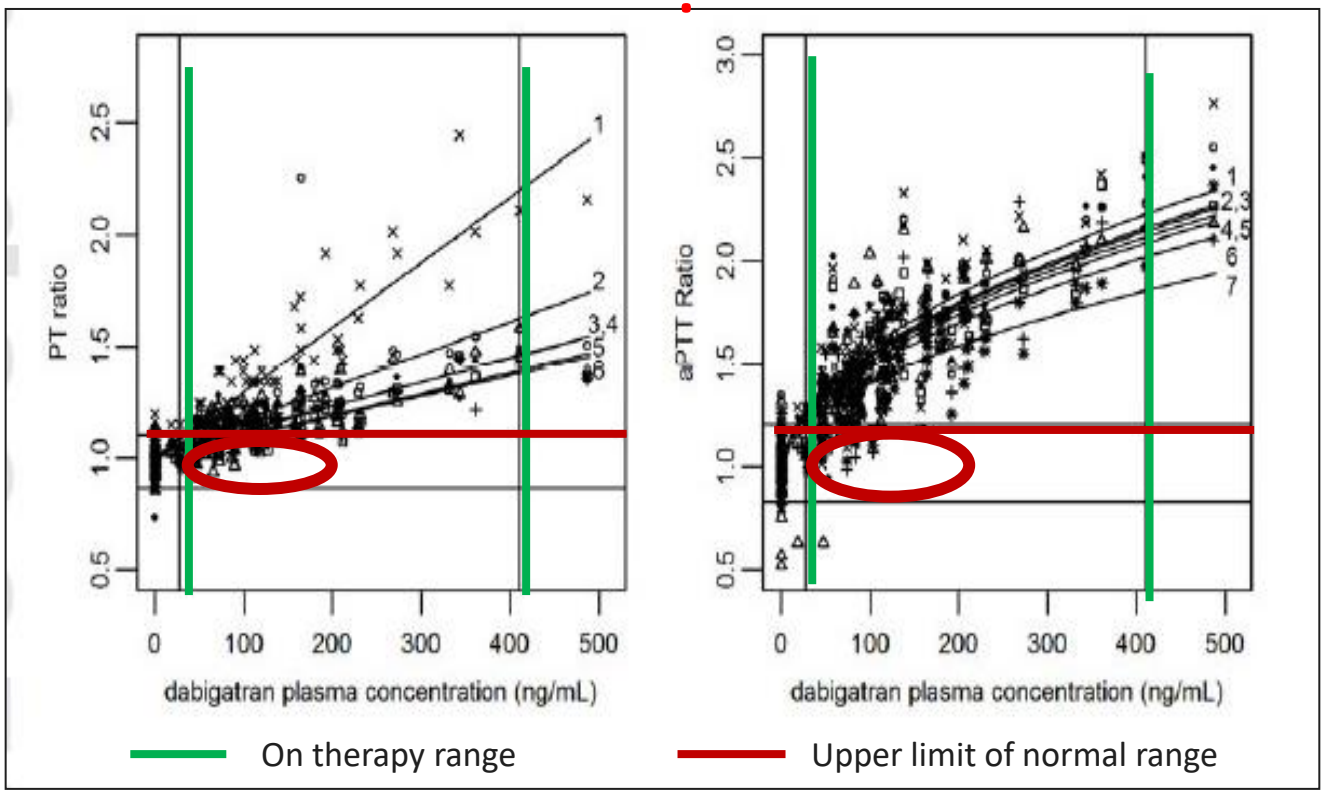
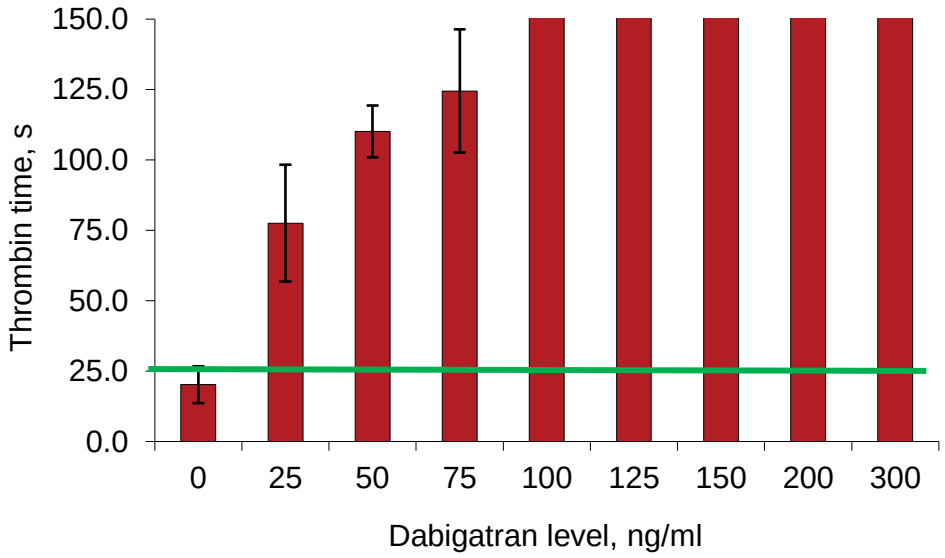


Figure modified from reference 4

Dabigatran – Thrombin Time (TT) Response[4,5]

Neat or slightly diluted plasma + Thrombin (~2NIH U/mL) → TT (s)

Modified from reference #5



Exquisitely sensitive to dabigatran presence:

- Not suitable for quantifying
- Normal TT essentially excludes dabigatran

Methods for Quantifying Dabigatran

PK: Mass spectometry[3,6,7]

Gold standard method[2]

LLOQ between 1-3ng/mL

Reportable range: 5 – 500ng/mL

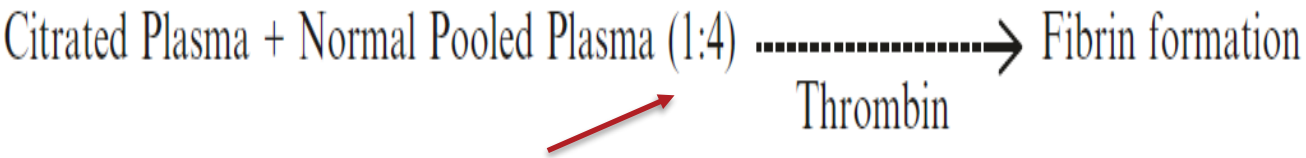
Within assay precision: <6%

Between assay precision: <10%

Requires internal standard

Report functional metabolites

PK: Dilute Thrombin Time[3,6,7]



1:2 for lower dabigatran levels
1:8 for higher dabigatran levels

LLOQ: <40ng/mL; with lower dilution <10ng/mL

Within run precision:<10%

Between run precision reported as high as 30%

Ecarin methods[3,6,7]

Ecarin, a metalloprotease from the venom of Echis carinatus (saw-scaled viper) converts prothrombin to meizothrombin

Meizothrombin is a potent thrombin intermediate that can be inhibited by dabigatran or other direct thrombin inhibitors (e.g. bivalirudin), but not by heparin

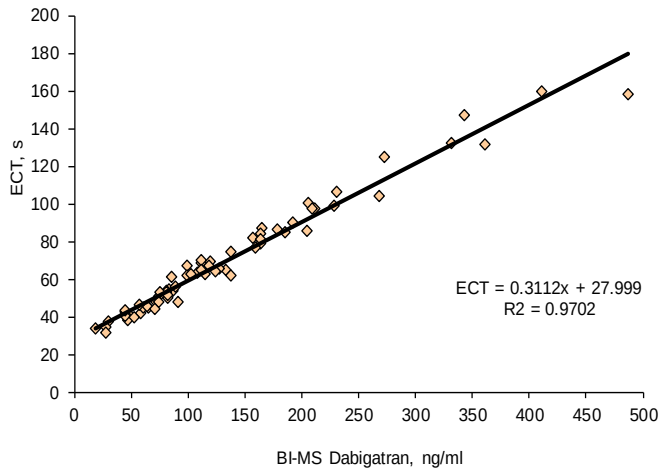
Classic ecarin method is clot based (ECT) and influenced (prolonged clotting time) by low factor II and fibrinogen levels
Chromogenic ecarin assay available (ECA)

Within run precision <5%; between run precision: 6 - 16%[3,6]
LLOQ: reported between 14 – 46ng/mL[3,6]

PK: Ecarin Clotting Time (ECT)[3,6,7]

DTI + prothrombin (from sample) + ecarin → [DTI-meizothrombin complex] + excess meizothrombin

[DTI-meizothrombin complex] + excess meizothrombin → Fibrinogen → Fibrin fromation (clot detection method)



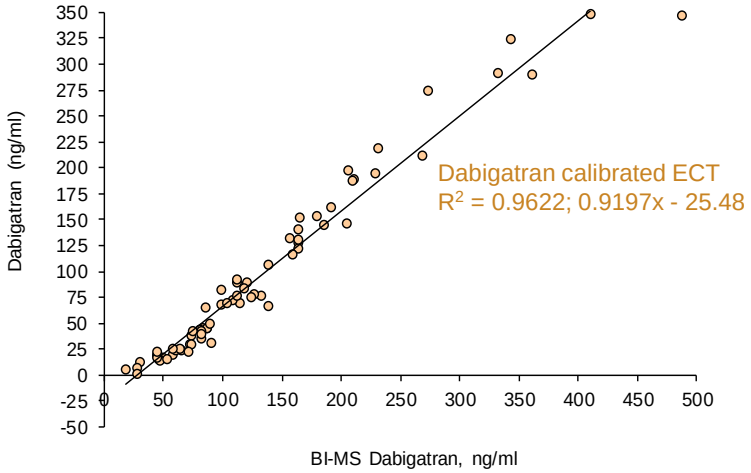


Figure modified from reference 7

PK: Chromogenic Ecarin Assay (ECA)[3,6,7]

DTI + exogenous prothrombin + ecarin → [DTI-meizothrombin complex] + excess meizothrombin

[DTI-meizothrombin complex] + excess meizothrombin + Substrate → Peptide + pNA (yellow color – absorbs at 405nm)

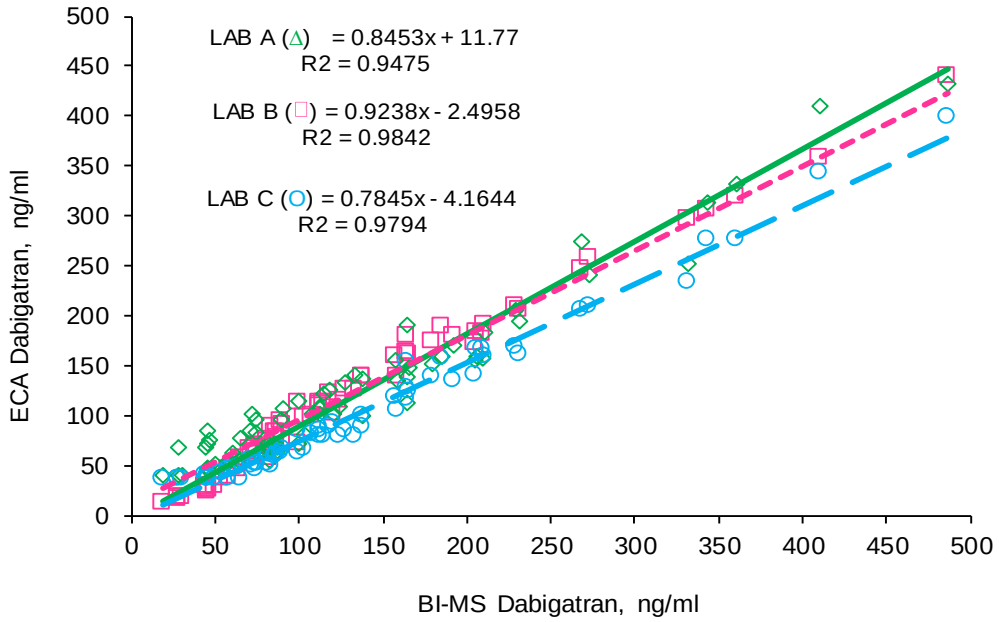
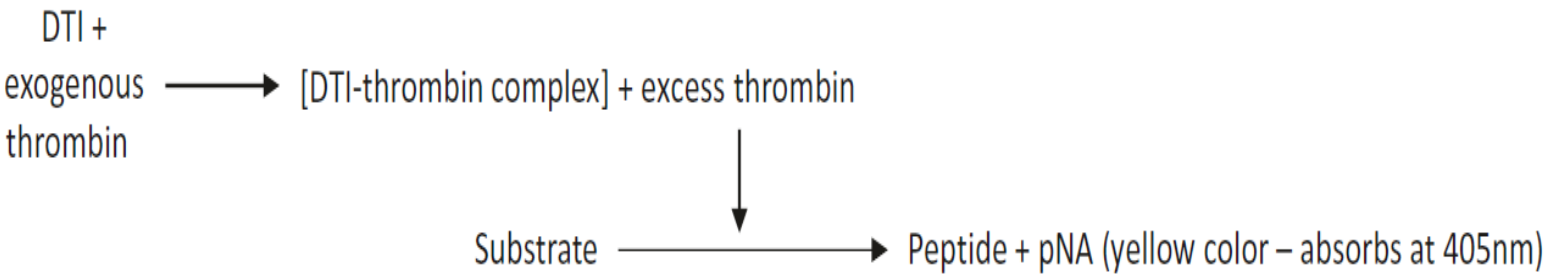


Figure modified from reference 7

PK: Chromogenic IIa Method[6,8-10]



Measurement range[8]:

Dabi low: 0 – 120ng/mL

Dabi high: 50 – 500ng/mL

Within run precision[8]: <2.2%

Between run precision[8]: <5.3%

Summary: Laboratory assessment of dabigatran anticoagulation

- Screening tests such as PT and APTT are insufficient for assessing dabigatran anticoagulation
- A normal thrombin time virtually excludes dabigatran presence
- Mass spectrometry methods are considered the gold standard for measuring dabigatran
- Alternative quantitative methods have been demonstrated to be equivalent to mass spectrometry including the drug calibrated dilute thrombin time, ecarin clotting time, ecarin chromogenic assays. More data is required for chromogenic anti-IIa methods
- Alternative methods for quantifying dabigatran can be adapted to open-system (programmable) automated coagulation analyzers
  - However, there are no FDA approved methods

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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenters completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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