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PEARLS OF LABORATORY MEDICINE

IV. Direct Oral Anticoagulants (DOACs): Impact & Interference of DOACs on Coagulation Testing

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DOACs: New Consideration

Dabigatran is an oral direct thrombin inhibitor that inhibits free and bound thrombin

Apixaban, betrixaban, edoxaban and rivaroxaban are oral direct anti-Xa drugs that inhibit both free and bound factor activated FX (FXa)

DOACs have varying performance characteristics when assessing pharmacodynamics or pharmacokinetics using global or specific assays.

As DOACs are target specific, these drugs may impact coagulation assays and likely lead to erroneous result interpretation and potential patient mismanagement



DOACs: Considerations

- Will clot-based methods be affected by DOACs
- Will non-clot-based methods be affected by DOACs
- Will collection of trough samples sufficiently mitigate any DOAC interference
- Are there alternative strategies for coagulation testing while on DOACs

Clot based methods for coagulation

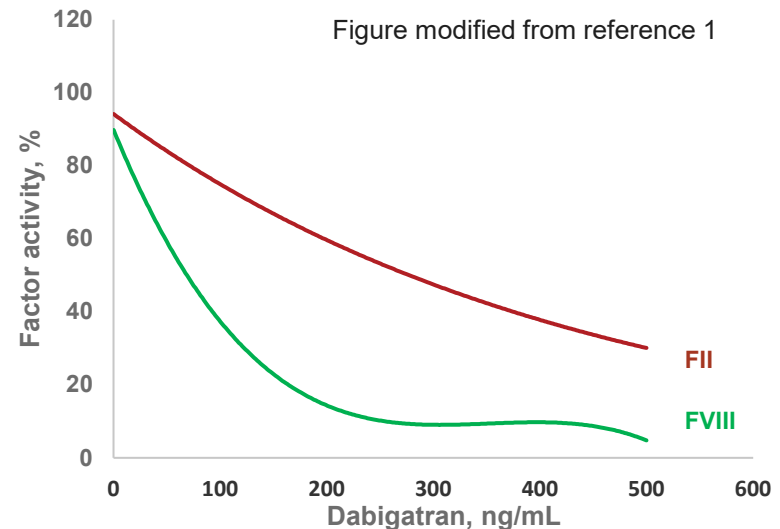
1. Screening tests
 1. PT/INR and APTT
 2. Mixing studies
2. Factor assays
 - Factors II, V, VII and X – PT based assays
 - Factors VIII, IX, XI, XII and contact factors – APTT based assays
 - Inhibitor assays
 - Fibrinogen
3. Thrombophilia related testing
 - Antithrombin activity
 - Lupus anticoagulant (different methods)
 - Protein C
 - Protein S
 - Activated Protein C Resistance
4. Others
 - Thrombin time, ecarin clotting time, reptilase clotting time



Factor Assays – One stage Assays¹

Dil Patient sample + Factor Deficient plasma $\xrightarrow{\text{PT or APTT}}$ Clot time

1. Clot time is inversely proportional to factor activity
2. A calibration curve will provide a relationship between factor activity and clot time
3. Factor activity is extrapolated from calibration curve based on obtained clot time from sample



Caution when measuring factor activity in DOAC treated patients as this may lead to misdiagnosis and mismanagement

This caution also applies to clot-based inhibitor studies (e.g. Bethesda assay)

Fibrinogen and Thrombin time²

Fibrinogen

Patient sample, diluted $\xrightarrow{\text{High conc Thrombin}}$ Clotting time

Thrombin time

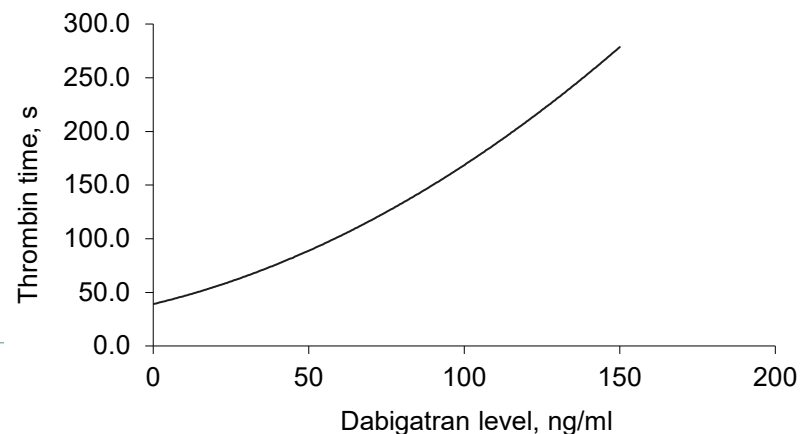
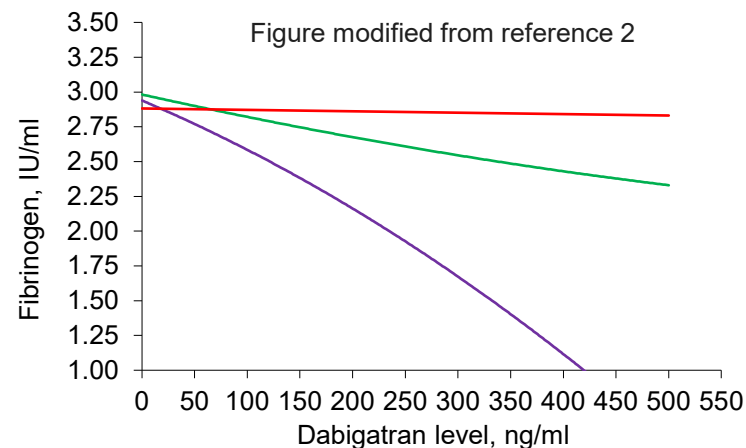
Patient sample, neat or diluted $\xrightarrow{\text{Low conc Thrombin}}$ Clotting time

As dabigatran inhibits thrombin, its presence will prolong clotting times

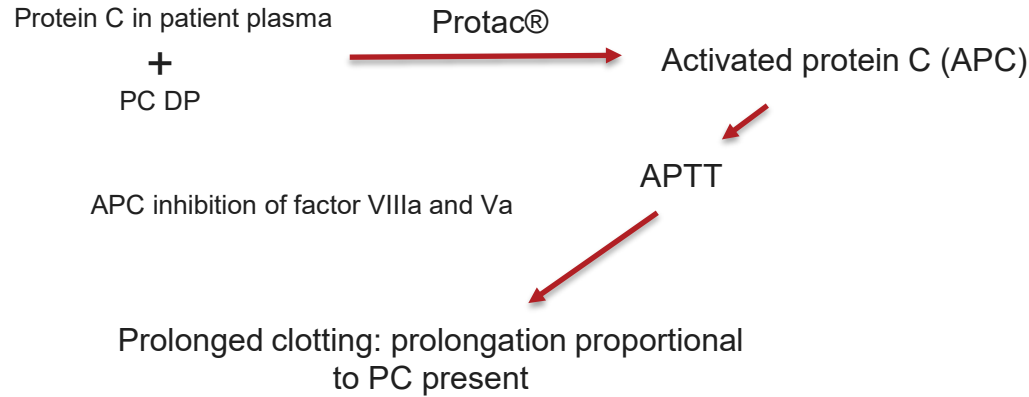
Anti-Xa DOACs do not affect either test

Clotting time inversely proportional to FBG level

Figure modified from reference 2

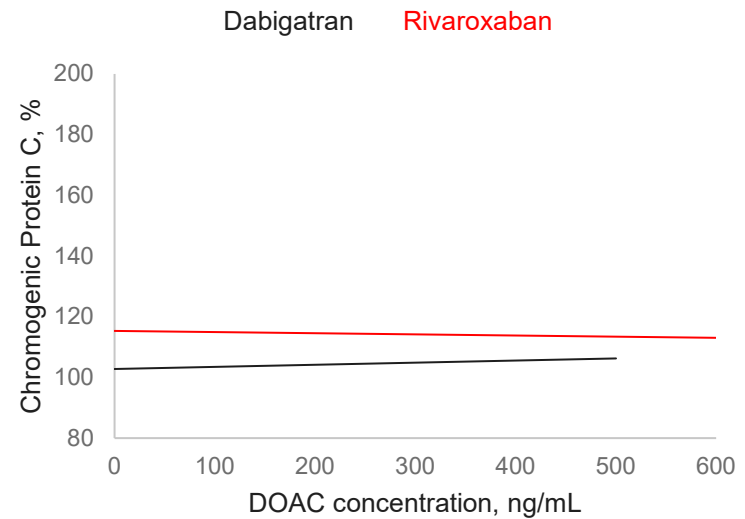
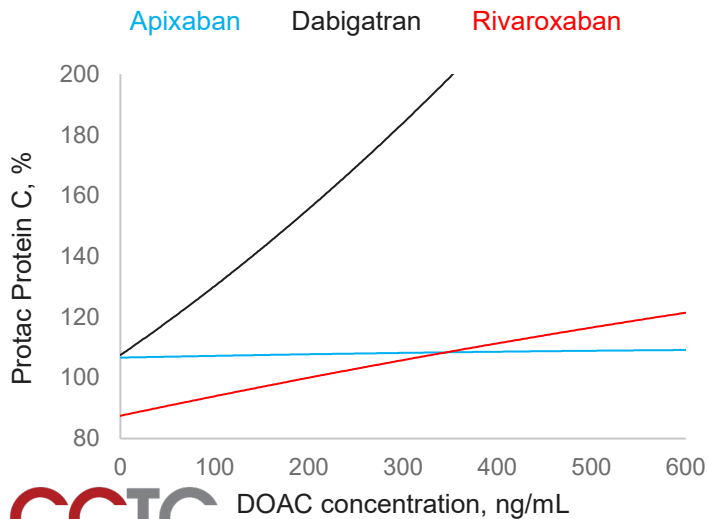


Protein C testing^{1,3}

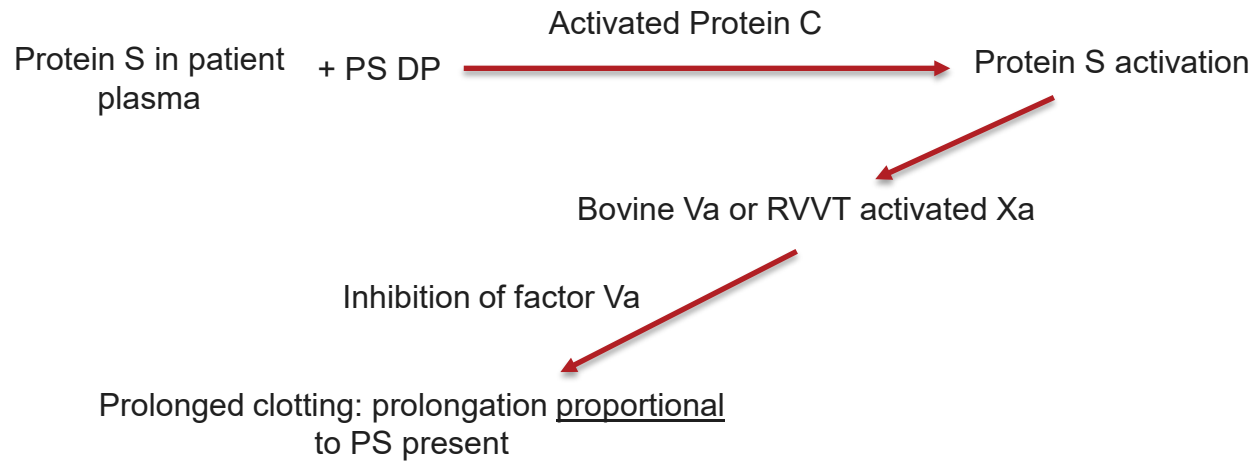


DOACs may prolong the APTT, which may cause false increase in Protein C

Alternatively, chromogenic protein C is not affected by DOACs

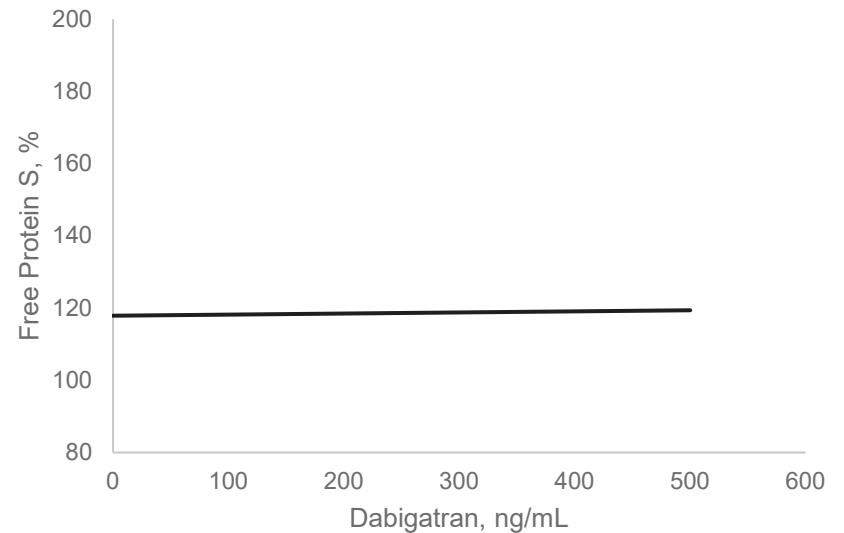
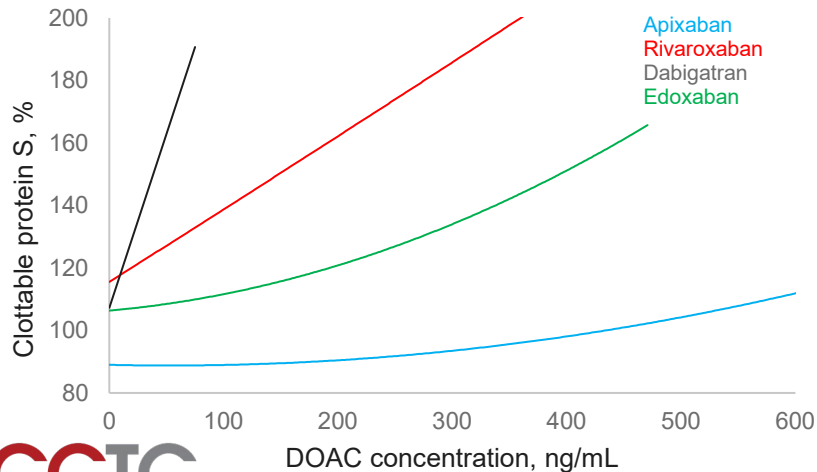


Protein S testing^{1,3}



DOACs – Increase clotting time, therefore falsely increasing PS result

Alternatively, free protein S by latex immunoassay is not affected by DOACs



Activated Protein C Resistance (APCR)^{1,3}

Patient sample $\xrightarrow{\text{APTT}}$ Clotting time, s

$$\text{APCR Ratio} = \frac{\text{APTT} + \text{APC}}{\text{APTT}}$$

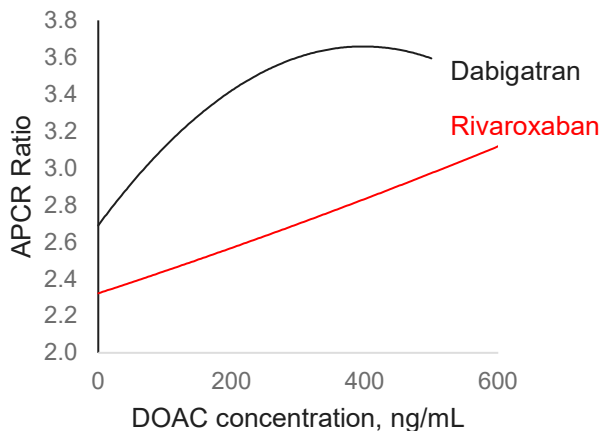
Patient sample $\xrightarrow{\text{APTT} + \text{APC}}$ Clotting time, s

Ratios >2.0 = negative APCR

DOACs – Increase clotting time may result in false negative APCR

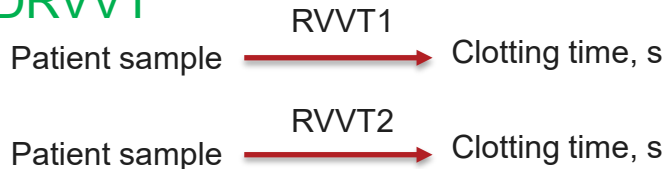
EQA reports have also indicated DOAC false positive APCR

Alternatively genetic testing could be performed, with caveat that unusual APCR mutations may be missed



Lupus Anticoagulant Testing^{1,3,4}

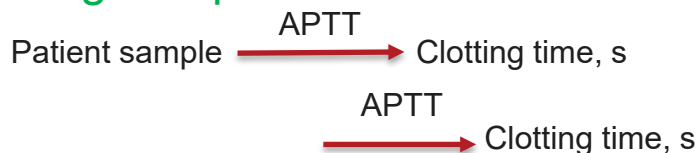
DRVVT



$$\frac{\text{RVVT1}}{\text{RVVT2}} > 1.2 = \text{LA}$$

DOACs – Increase clotting time, may falsely indicate LA

Hexagonal phase

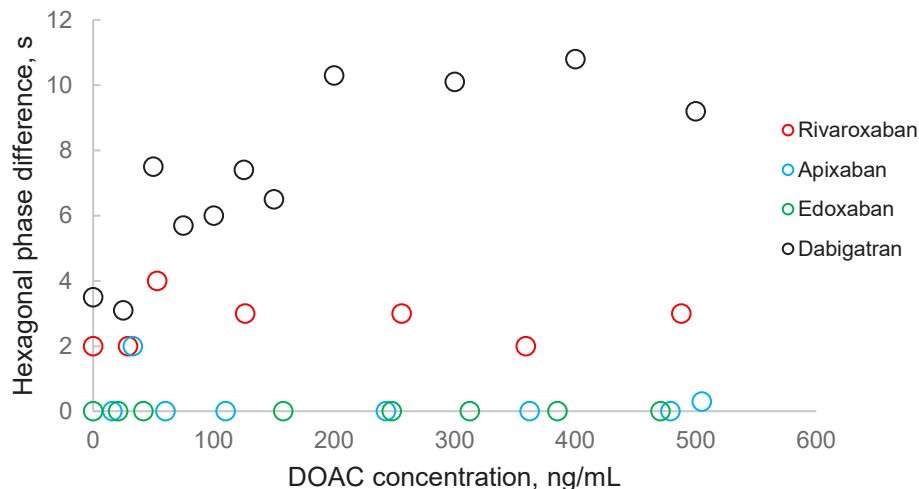
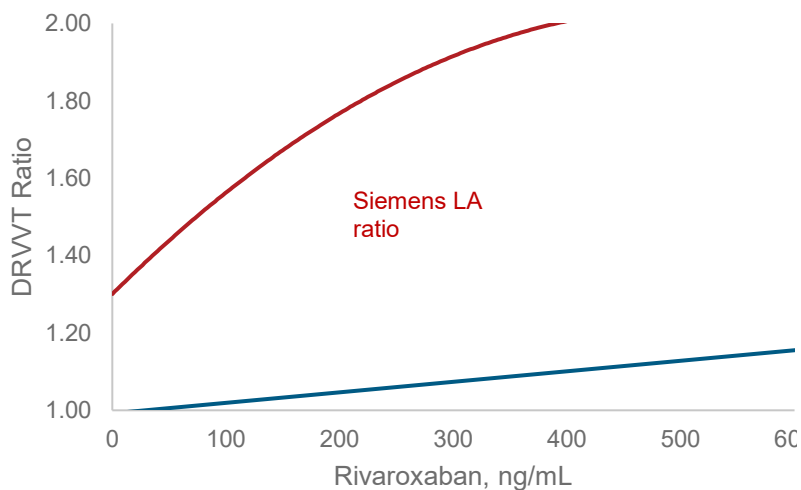


If PE APTT is >8s shorter than APTT

Anti-Xa DOACs do not affect hexagonal phase LA testing

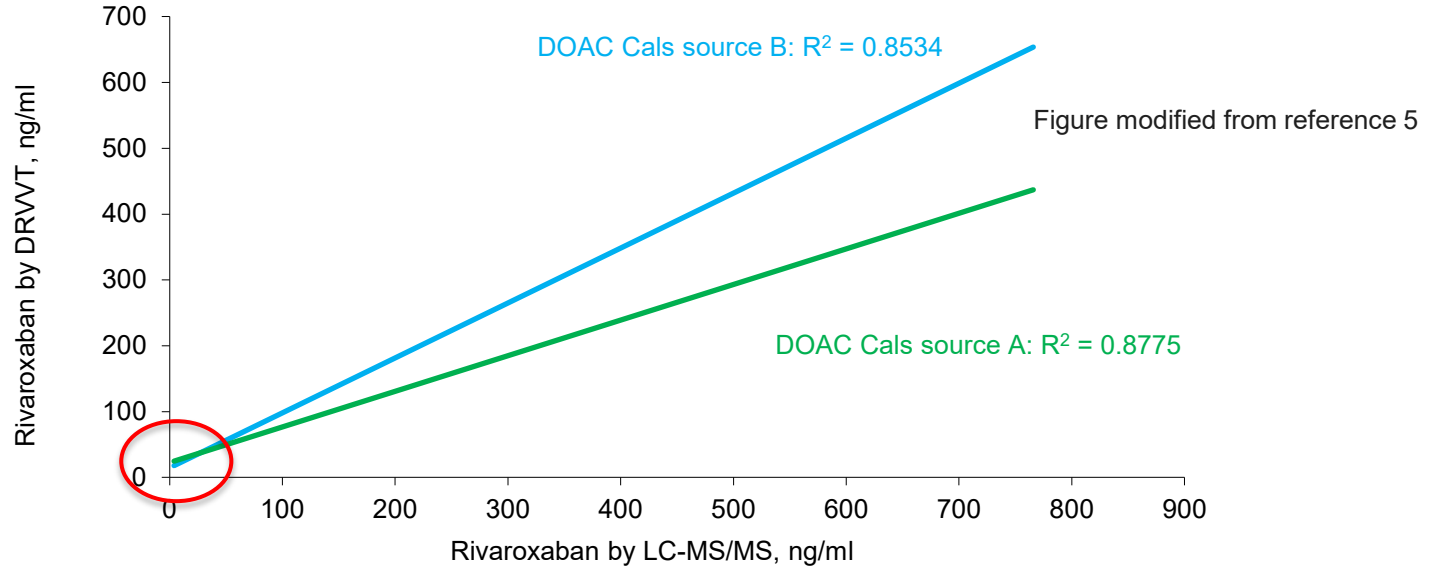
Patient sample + PE

Dabigatran false positive LA at higher concentrations



Advantage of Lupus Anticoagulant Testing Interference⁵

Because of linear dose dependent response for LA2 reagent to DOACs, it has been proposed as a method for DOAC quantitation



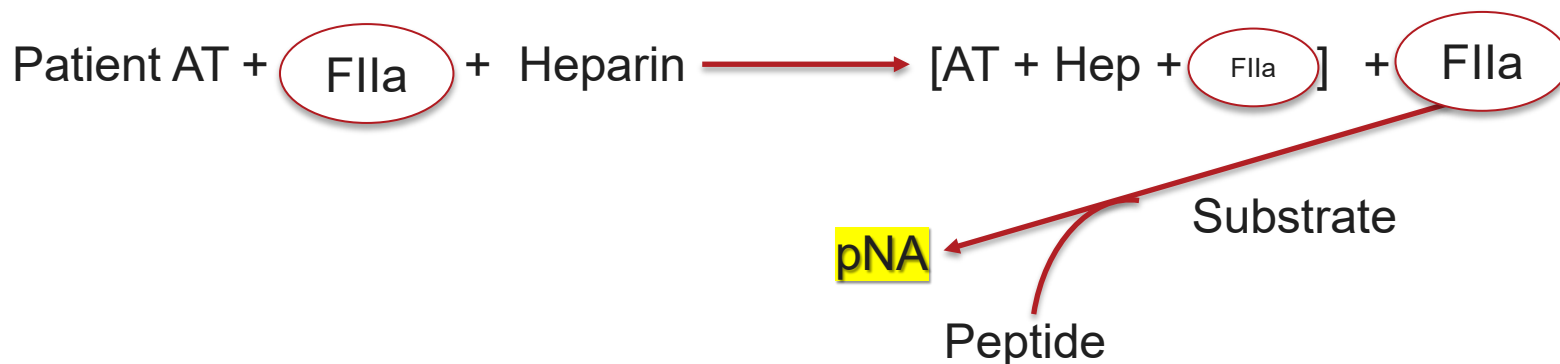
However, lower limit of quantitation may not be sufficiently adequate

Chromogenic methods

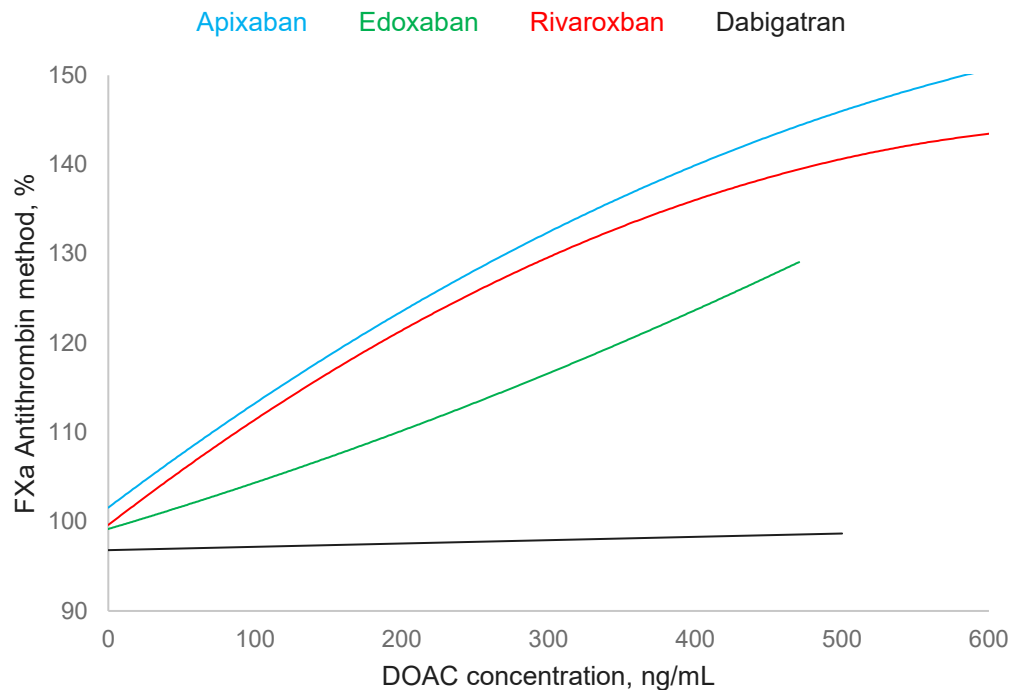
Methods typically are two stage assays

1. Sample mixed with activated factor
2. Addition of specific substrate

Example: Antithrombin activity



Representative DOAC interference on chromogenic assay^{1,3}



Antithrombin activity using factor Xa substrate



Common Chromogenic coagulation methods ^{6,7}

Test	Factor II or X dependent	DOAC affect
Factor VIII	Yes	Yes
Factor IX	Yes	Yes
Factor X	Yes	Anti-Xa DOAC
Antithrombin	Yes	Yes
Protein C	No	No
Plasminogen	No	No
Alpha-2-antiplasmin	No	No
Anti-Xa	Yes	Anti-Xa DOAC
Ecarin	Yes	Anti-IIa DOAC



Test methods not affected by DOACs⁶⁻⁸

Latex immunoassays: D-dimer, VWF, Protein S, HIT

ELISA methods: D-dimer, Protein C, Protein S, HIT

Agglutination methods: monomer, VWF:RCo

Platelet function testing (most)

Esoteric Tests that may be affected by DOACs

Thrombin based platelet aggregation

Thrombin activatable fibrinolysis inhibitor

May alter fibrinolysis assays



Are trough collections adequate to minimize DOAC interference?⁶

Trough levels yellow	Test Bias	Dabigatran (35 – 95ng/mL)	Apixaban (22 –177ng/mL)	Betrixaban (12ng/mL)	Edoxaban (10 – 39ng/mL)	Rivaroxaban (6 – 239ng/mL)
Antithrombin-IIa method	False ↑	~25	No effect	No effect	No effect	No effect
Antithrombin-Xa method	False ↑	No effect	~40 - 110	No effect	~120 - 270	~100- 130
Protein C-clot based	False ↑	~25	>750	Unknown	~280	~22
Protein C - chromogenic	--	No effect	No effect	No effect	No effect	No effect
Protein S – activity	False ↑	~25	~470	~30	~270	~220
Protein S - antigen	--	No effect	No effect	No effect	No effect	No effect
LA Screen	↑	~25	~80 – 110	~10	~20 – 35	~10-30
LA Confirm	↑	~25	~80	~10	~20	~20 – 30
LA Ratio	False ↑	~25	~200 - >750	~50	~30 - >500	~10 - >720
APCR	Biased Ratios#	~25 - 200	>740	Unknown	>300	>300

Method and reagent dependent



Minimizing DOAC interference?^{7,9,10}

1. Selecting tests that are not affected by DOACs
2. Use of ex-vivo DOAC neutralizing products:
 - DOAC-Stop
 - DOAC-Remove
 - Filters

Note of caution:

No ex-vivo neutralizing product is currently FDA-approved for use

Neutralizing products are not interchangeable

Some products have some degree of inducing coagulation

Variable plasma volume recovery



Summary: DOACs and interference with coagulation testing

- Clot-based methods are affected by DOACs **Mostly true**
- Non-clot-based methods are not affected by DOACs **Mostly true**
- Collection of trough samples will sufficiently mitigate any DOAC interference **Cautiously false**
- Alternative strategies for testing while on DOACs
Consider alternative methods or DOAC neutralizing techniques



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Disclosures/Potential Conflicts of Interest

Disclosures:

Dorothy Adcock has received honoraria from Siemens Healthcare Diagnostics.

Robert Gosselin has provided expert testimony for dabigatran and rivaroxaban testing, has received honoraria from Siemens Healthcare Diagnostics, Machaon Laboratories and Diagnostica Stago, and serves as a consultant for Diagnostic Grifols and UniQure, and advisory board member for BioMarin.

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