11th ECAT participants' meeting

INTERFERENCE OF DOACS IN COAGULATION TESTS

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QUALIblood, Namur, Belgium

QUALIblood
Your expert in blood testing

NTHC
Namur Thrombosis & Hemostasis Center

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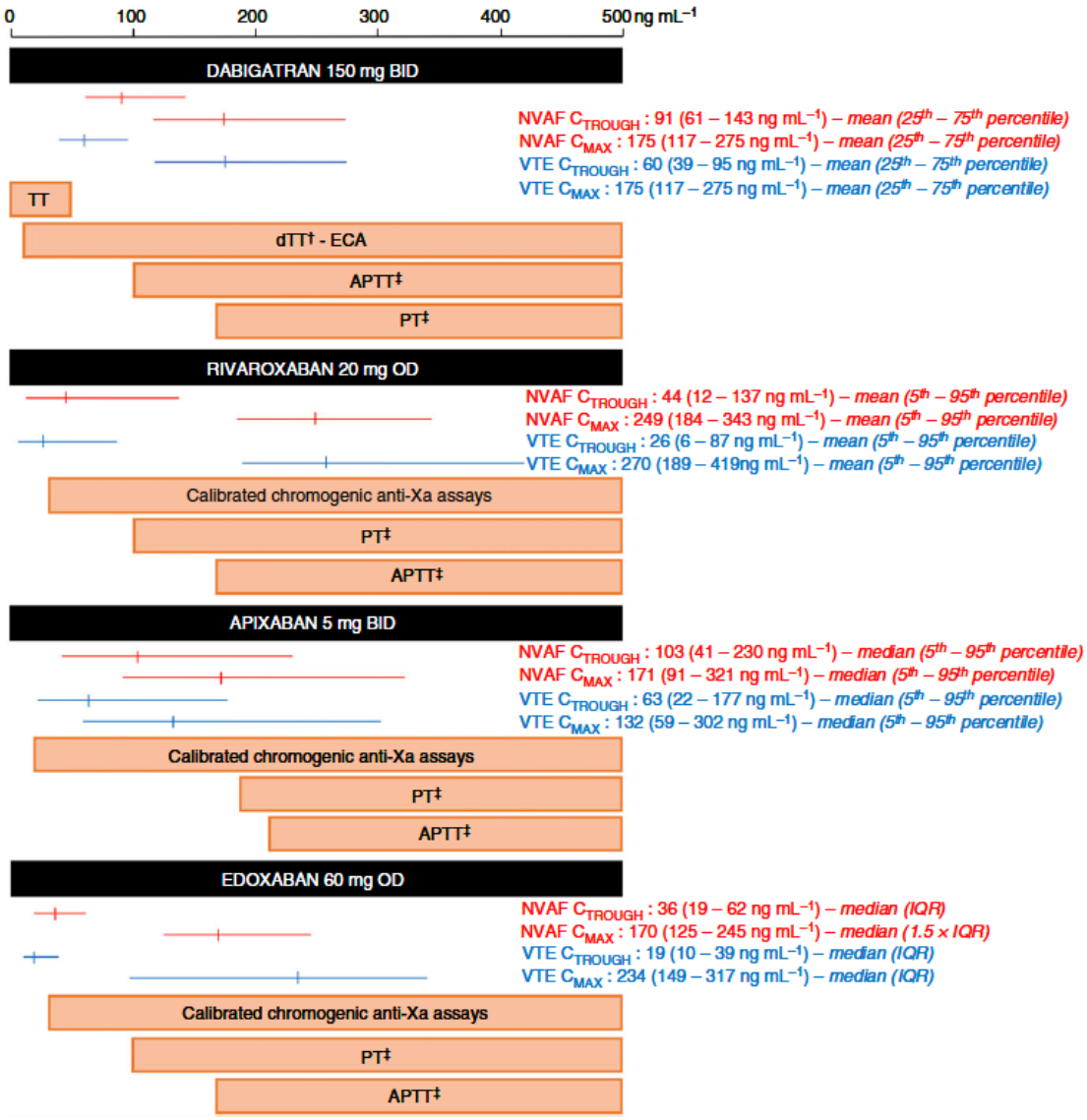
CONFLICTS OF INTEREST

- Advisory board
  - Bayer Healthcare
  - Roche
- Speaker fees
  - Bayer Healthcare
  - Daiichi Sankyo
  - Stago Diagnostica
  - Roche Diagnostics
- Research Grant
  - Bayer Healthcare
- Travel Grant
  - Bayer Healthcare
  - Boehringer Ingelheim
  - Stago Diagnostica
- Founder and CEO
  - QUALIblood

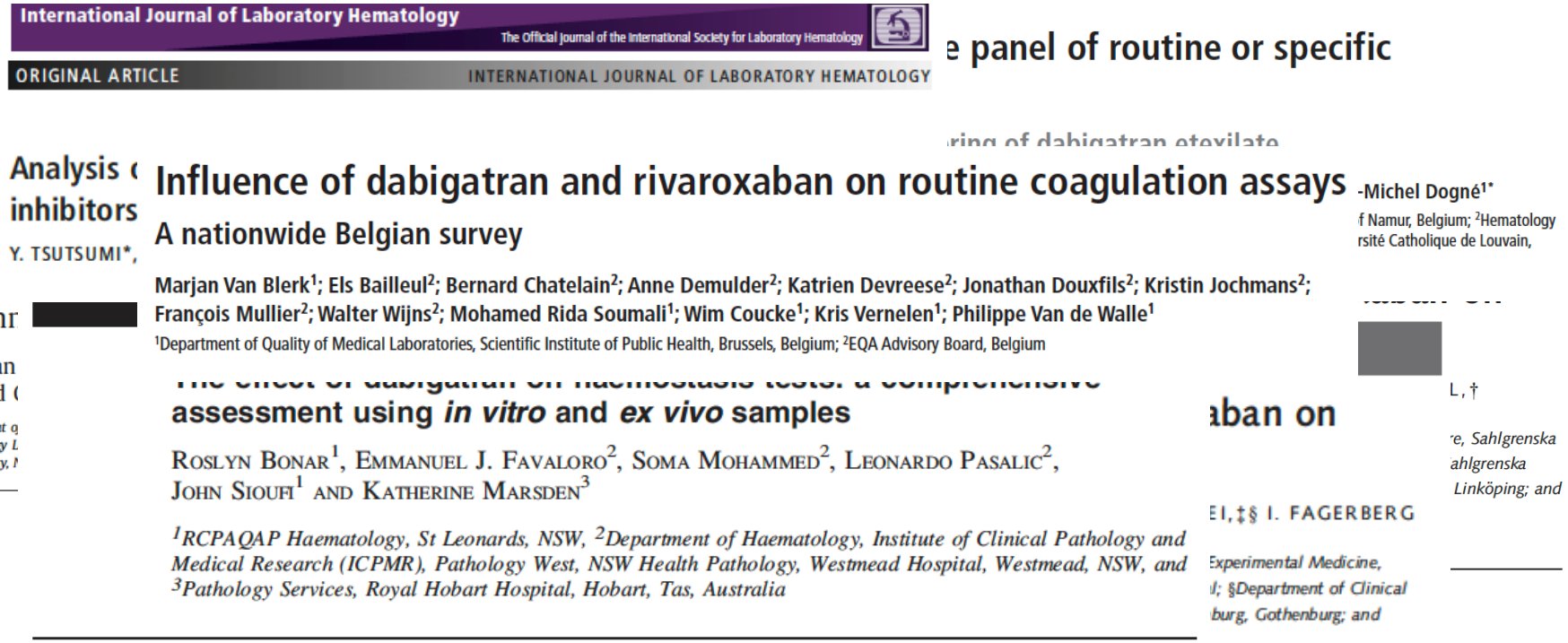
EXPECTED PLASMA CONCENTRATION



Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. Journal of thrombosis and haemostasis : JTH 2018; 16(2): 209-19.

INFLUENCE OF DOACS ON COAGULATION TESTING

Since the era of the DOACs, a lot of publication reported the influence of DOAC in several coagulation testing



International Journal of Laboratory Hematology

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ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Influence of dabigatran and rivaroxaban on routine coagulation assays

A nationwide Belgian survey

Marjan Van Blerk[1]; Els Bailleul[2]; Bernard Chatelain[2]; Anne Demulder[2]; Katrien Devreese[2]; Jonathan Douxfils[2]; Kristin Jochmans[2]; François Mullier[2]; Walter Wijns[2]; Mohamed Rida Soumali[1]; Wim Coucke[1]; Kris Vernelen[1]; Philippe Van de Walle[1]

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assessment using *in vitro* and *ex vivo* samples

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To cite this article: van Os GMA, de Laat B, Kamphuisen PW, Meijers JCM, de Groot PhG. Detection of lupus anticoagulant in the presence of rivaroxaban using Taipan snake venom time. *J Thromb Haemost* 2011; **9**: 1657–9.

INFLUENCE OF DOACS ON COAGULATION TESTING

But the recommendations are not so easy to draw since the interference depends on:

1. **The anticoagulant agent**
2. **The plasma level of the anticoagulant**
3. **The sensitivity of the test**

Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. Journal of thrombosis and haemostasis : JTH 2018; 16(2): 209-19.

INFLUENCE OF DOAC ON COAGULATION TESTING

First reports mentioned the impact of rivaroxaban and dabigatran on routine coagulation testing such PT and aPTT

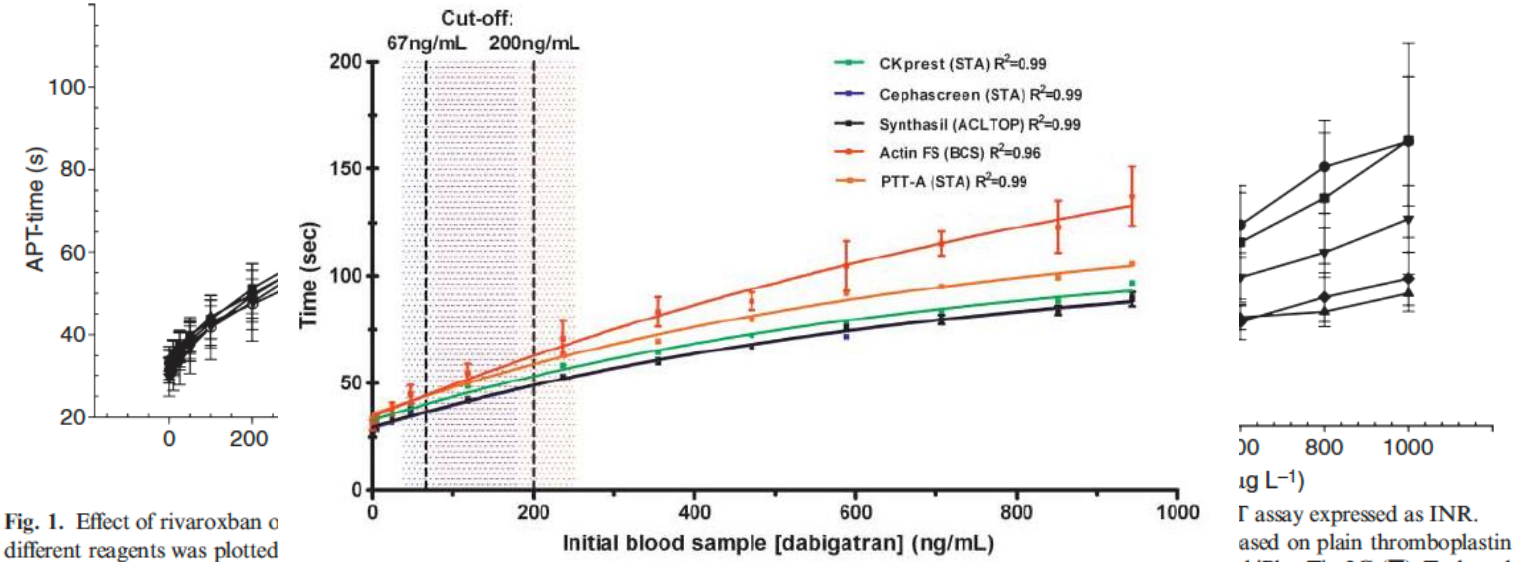


Fig. 1. Effect of rivaroxban o
different reagents was plotted
plasma. Triniclot aPTT HS (○), Actin FSL (●), PTT-Automate (■), APTT-SP (▲) and DG-APTT (▼). Results are the mean of 10 different healthy donors ± SD.

Γ assay expressed as INR.
ased on plain thromboplastin
reagents. STA-Neoplastine (●), RecombiPlastTin 2G (▼), Technoplastin HIS (■), Thromborel S (◆), and Dade Innovin (▲). (B) Analysis of four Owren-type PT assays, based on rabbit thromboplastin reagents: SPA+ (●), Nycotest PT (▼), Owren's PT (■), and Simple Simon (◆). Results are shown as the mean INR of 10 different healthy donors ± SD.

Hillarp A, Baghaei F, Fagerberg Blixter I, et al. Effects of the oral, direct factor Xa inhibitor rivaroxaban on commonly used coagulation assays. Journal of thrombosis and haemostasis : JTH 2011; 9(1): 133-9.

Douxfils J, Mullier F, Robert S, et al. Impact of dabigatran on a large panel of routine or specific coagulation assays. Laboratory recommendations for monitoring of dabigatran etexilate. Thromb Haemost 2012; 107(5): 985-97.

INFLUENCE OF DOAC ON COAGULATION TESTING

Similar results were also obtained with the other DOACs, namely, apixaban, edoxaban and betrixaban

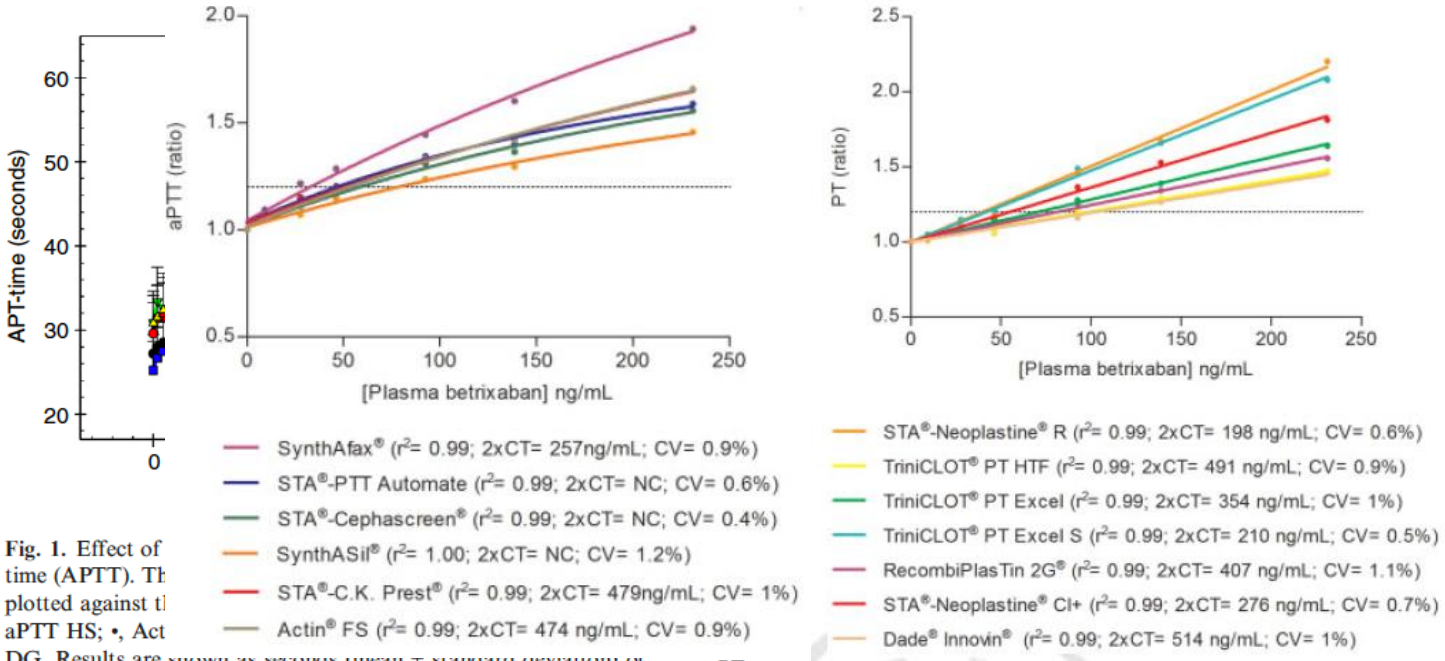


Fig. 1. Effect of
time (APTT). Th
plotted against t
aPTT HS; •, Act
DG. Results are shown as seconds (mean + standard deviation) of
nine different healthy donors.

PT assays, based on rabbit thromboplastin reagents: ○, SPA+; ▼,
Nycotest PT; •, PT Owren. Results are shown as INR (mean + standard deviation) of 10 different healthy donors.

Hillarp A, Gustafsson KM, Faxalv L, et al. Effects of the oral, direct factor Xa inhibitor apixaban on routine coagulation assays and anti-FXa assays. Journal of thrombosis and haemostasis : JTH 2014; 12(9): 1545-53.

Douxfils J, Chatelain B, Chatelain C, et al. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost 2016; 115(2): 368-81.

Siriez R, Evrard J, Dogne JM, et al. Betrixaban: Impact on Routine and Specific Coagulation Assays-A Practical Laboratory Guide. Thromb Haemost 2018; 118(7): 1203-14.

INFLUENCE OF DOAC ON COAGULATION TESTING

However, beside these screening tests, more specialized assays that are based on the same principle, i.e. clotting detection, may be impacted…

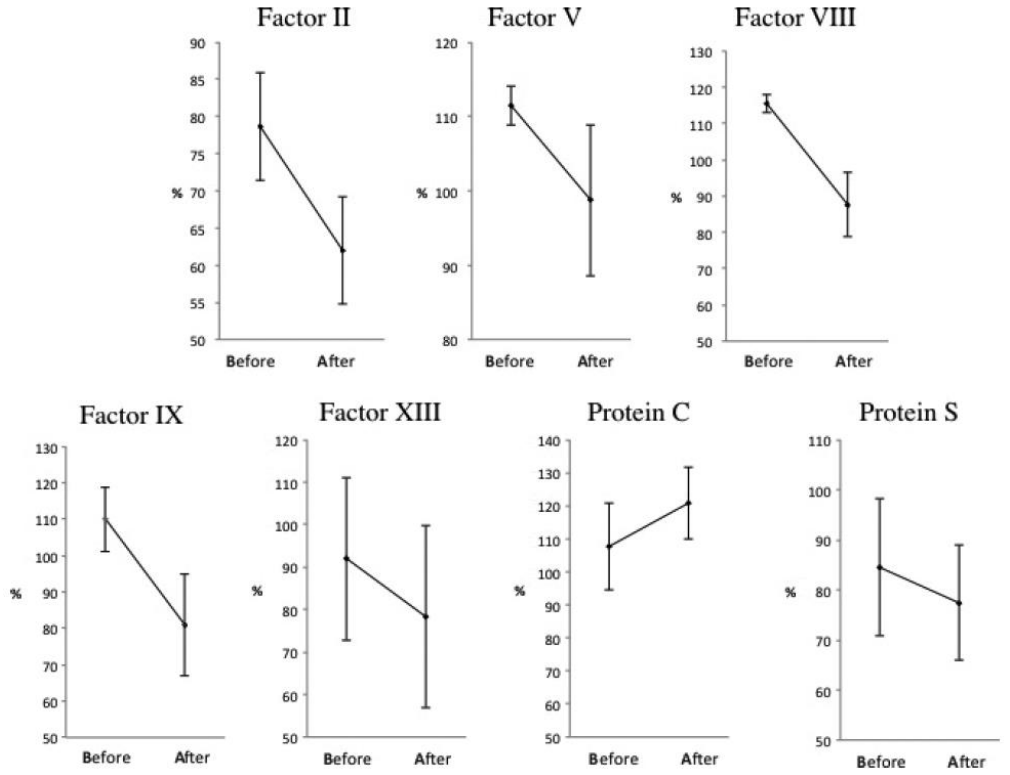


Figure 1. Factors II, V, VIII, IX, XIII, and protein S were decreased after administration of dabigatran. Only protein C was increased after administration of dabigatran.

Tsutsumi Y, Shimono J, Ohhigashi H, et al. Analysis of the influence of dabigatran on coagulation factors and inhibitors. International journal of laboratory hematology 2015; 37(2): 225-30.

INFLUENCE OF DOAC ON COAGULATION TESTING

Which may lead to misinterpretation of the drug effect by the physician

"… dabigatran affects several coagulation factors and the emergence of the VIII factor inhibitor mediates APTT prolongation. These results suggest that secondary factor VIII deficiency occurred after the dabigatran administration, especially in prolonged APTT patients. Therefore, FEIBA or recombinant factor VIIa may be useful as bypassing drugs for factor VIII deficiency during life-threatening bleeding events associated with prolonged APTT caused by dabigatran administration."

Thus, this is the role of the laboratory to guide the physicians in the interpretation of these observation since these results are spurious

DOACs may lead to misdiagnosis and improper clinical decisions and we need to know and document the magnitude of these interferences

Tsutsumi Y, Shimono J, Ohhigashi H, et al. Analysis of the influence of dabigatran on coagulation factors and inhibitors. International journal of laboratory hematology 2015; 37(2): 225-30.
Comment in: Gosselin RC, Adcock DM. Comment: Analysis of the influence of dabigatran on coagulation factors and inhibitors. International journal of laboratory hematology 2016; 38(1): e4.
Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. Journal of thrombosis and haemostasis : JTH 2018; 16(2): 209-19.

LUPUS ANTICOAGULANT DETERMINATION

Antiphospholipids antibodies are heterogeneous and no single test is sensitive to all LA, so a combination of two PL-dependent clotting assays is required – the dRVVT and the aPTTL

Table 1A
Percentage of pathological spiked samples in DRVVT normalized ratio. The absolute number of pathological samples and the total number of samples are given in brackets.

Spiked samples							
DOAC	Concentration [ng/mL]	Pathologic samples [%]					
		DRVVT(IL) screen	DRVVT(STA) screen	DRVVT(IL) confirm	DRVVT(STA) confirm	DRVVT(IL) normalized ratio	DRVVT(STA) normalized ratio
Apixaban	0	0 (0/10)	0 (0/10)	30 (3/10)	0 (0/10)	0 (0/10)	0 (0/10)
Apixaban	10	0 (0/10)	10 (1/10)	10 (1/10)	0 (0/10)	0 (0/10)	0 (0/10)
Apixaban	30	14.2 (1/7)	40 (4/10)	42.9 (3/7)	0 (0/10)	0 (0/7)	0 (0/10)
Apixaban	50	22.2 (2/9)	70 (7/10)	77.8 (7/9)	40 (4/10)	0 (0/9)	0 (0/10)
Apixaban	100	80 (8/10)	90 (9/10)	90 (9/10)	100 (10/10)	0 (0/10)	0 (0/10)
Dabigatran	0	22.2 (2/9)	10 (1/10)	0 (0/9)	0 (0/10)	0 (0/9)	0 (0/10)
Dabigatran	10	0 (0/10)	70 (7/10)	0 (0/10)	20 (2/10)	0 (0/10)	10 (1/10)
Dabigatran	30	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	20 (2/10)	30 (3/10)
Dabigatran	50	100 (10/10)	100 (10/10)	100 (10/10)	100 (9/9)	20 (2/10)	44.4 (4/9)
Dabigatran	100	100 (10/10)	100 (9/9)	100 (10/10)	100 (7/7)	20 (2/10)	71.4 (5/7)
Rivaroxaban	0	10 (1/10)	10 (1/10)	10 (1/10)	0 (0/10)	0 (0/10)	0 (0/10)
Rivaroxaban	10	50 (5/10)	30 (3/10)	10 (1/10)	20 (2/10)	10 (1/10)	20 (2/10)
Rivaroxaban	30	90 (9/10)	90 (9/10)	50 (5/10)	20 (2/10)	40 (4/10)	50 (5/10)
Rivaroxaban	50	100 (10/10)	100 (10/10)	80 (8/10)	70 (7/10)	50 (5/10)	60 (6/10)
Rivaroxaban	100	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	70 (7/10)	100 (10/10)

Flieder T, Weiser M, Eller T, et al. Interference of DOACs in different DRVVT assays for diagnosis of lupus anticoagulants. Thrombosis research 2018; 165: 101-6.
Devreese KMJ, Ortel TL, Pengo V, et al. Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH. Journal of thrombosis and haemostasis : JTH 2018; 16(4): 809-13.

LUPUS ANTICOAGULANT DETERMINATION

Antiphospholipids antibodies are heterogeneous and no single test is sensitive to all LA, so a combination of two PL-dependent clotting assays is required – the dRVVT and the aPTTL

Table 1B
Percentage of pathological patient samples in DRVVT normalized ratio. The absolute number of pathological samples and the total number of samples are given in brackets.

Patient samples							
DOAC	Concentration [ng/mL]	Pathologic samples [%]					
		DRVVT(IL) screen	DRVVT(STA) screen	DRVVT(IL) confirm	DRVVT(STA) confirm	DRVVT(IL) normalized ratio	DRVVT(STA) normalized ratio
Apixaban	0–20	20.7 (6/29)	43 (12/30)	3.4 (1/29)	30 (9/30)	10.3 (3/29)	10 (3/30)
Apixaban	20–40	57.1 (4/7)	62.5 (5/8)	14.7 (1/7)	75 (6/8)	14.7 (1/7)	12.5 (1/8)
Apixaban	40–120	60 (6/10)	80 (8/10)	70 (7/10)	90 (9/10)	10 (1/10)	0 (0/10)
Apixaban	> 120	80 (12/15)	93.3 (14/15)	93.3 (14/15)	100 (15/15)	6.7 (1/15)	13.3 (2/15)
Dabigatran	0–20	0 (0/7)	0 (0/7)	0 (0/7)	0 (0/7)	0 (0/7)	0 (0/7)
Dabigatran	20–40	100 (3/3)	100 (3/3)	100 (3/3)	100 (3/3)	0 (0/3)	0 (0/3)
Dabigatran	40–120	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	0 (0/5)	0 (0/5)
Dabigatran	> 120	100 (3/3)	100 (3/3)	100 (3/3)	100 (3/3)	0 (0/3)	0 (0/3)
Rivaroxaban	0–20	38.2 (13/34)	41.2 (14/34)	14.7 (5/34)	17.6 (6/34)	26.4 (9/34)	20.6 (7/34)
Rivaroxaban	20–40	87.5 (14/16)	93.8 (15/16)	12.5 (2/16)	50 (8/16)	56.3 (9/16)	31.3 (5/16)
Rivaroxaban	40–120	100 (10/10)	90.9 (10/11)	70 (7/10)	81.8 (9/11)	50 (5/10)	36.4 (4/11)
Rivaroxaban	> 120	100 (12/12)	100 (12/12)	100 (12/12)	100 (12/12)	100 (12/12)	91.7 (11/12)

Flieder T, Weiser M, Eller T, et al. Interference of DOACs in different DRVVT assays for diagnosis of lupus anticoagulants. Thrombosis research 2018; 165: 101-6.
Devreese KMJ, Ortel TL, Pengo V, et al. Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH. Journal of thrombosis and haemostasis : JTH 2018; 16(4): 809-13.

LUPUS ANTICOAGULANT DETERMINATION

Antiphospholipids antibodies are heterogeneous and no single test is sensitive to all LA, so a combination of two PL-dependent clotting assays is required – the dRVVT and the aPTTL

- **Dependence of the drug (apixaban < dabigatran < rivaroxaban)**
  confirmed by other studies in-vivo and ex-vivo

- **Dependence of the reagents**
  difference of sensitivity between the screen and the confirm but also between manufacturers

- **Depending on plasma levels**
  LA testing are more influenced at peak than trough

Flieder T, Weiser M, Eller T, et al. Interference of DOACs in different DRVVT assays for diagnosis of lupus anticoagulants. Thrombosis research 2018; 165: 101-6.
Hillarp A, Gustafsson KM, Faxalv L, et al. Effects of the oral, direct factor Xa inhibitor apixaban on routine coagulation assays and anti-FXa assays. Journal of thrombosis and haemostasis : JTH 2014; 12(9): 1545-53.
Antovic A, Norberg EM, Berndtsson M, et al. Effects of direct oral anticoagulants on lupus anticoagulant assays in a real-life setting. Thromb Haemost 2017; 117(9): 1700-4.
Ratzinger F, Lang M, Belik S, et al. Lupus-anticoagulant testing at NOAC trough levels. Thromb Haemost 2016; 116(2): 235-40.

ACTIVATED PROTEIN C RESISTANCE

In clinical laboratories, the first approach to detect the presence of FVL is usually a functional assay for APCr

- **Original APCr assay are based on aPTT in presence or absence of APC but they are not accurate and influenced by acute phase reaction, pregnancy and HRS/COC**

- **2nd generation of assay included mixture with FV-deficient human plasma but can be influenced by DTI and DFXaI**

- **Other tests have been developed using RVV (*Daboia russelli*) or noscarin (*Notechis scutatus*)**

Gessoni G, Valverde S, Valle L, et al. Effect of dabigatran on a prothrombinase-based assay for detecting activated protein C resistance: an ex vivo and in vitro study in normal subjects and factor V Leiden carriers. Blood Transfus 2017; 15(6): 562-7.

ACTIVATED PROTEIN C RESISTANCE

TABLE I. Features of Commercially Available APC Resistance Assays

Assay name(s)	Clotting mixture	APC source	Interferences			
			Factor levels	LA	Xa inh.	DTI
Chromogenix Coatest APC Resistance (the "original" assay)	Colloidal silica Phospholipids Calcium	Purified APC	+ [16]	+ [17]	+	(+)
Chromogenix Coatest APC Resistance V (the "modified" or "second-generation" assay)	Colloidal silica Phospholipids Calcium Factor V-deficient human plasma Polybrene (heparin inhibitor)	Purified APC	– [18]	+	+ [19]	+ [20]
Siemens ProC Ac R (formerly also available as LifeTherapeutics GradiLeiden)	RVV-X Phospholipids Calcium Heparin inhibitor	*A. c. contortrix* protein C activator (activates protein C in patient plasma)	+ [21]	(–) [21]	(+)	(+)
Aniara Hemoclot Quanti V-L	Purified factor Xa Phospholipids Calcium Purified fibrinogen, factor II, and protein S Heparin inhibitor	Purified APC	–	+	+	(+)
Pentapharm Pefakit APC-R Factor V Leiden (also available as Sekisui Acticlot Protein C Resistance)	RVV-V Factor V-deficient human plasma Noscarin, a prothrombin activator (Va dependent, calcium, and phospholipid independent) Polybrene (heparin inhibitor)	Purified APC	– [22]	– [22]	(–) [19]	+ [23]

Kadauke S, Khor B, Van Cott EM. Activated protein C resistance testing for factor V Leiden. American journal of hematology 2014; 89(12): 1147-50.

ACTIVATED PROTEIN C RESISTANCE

Dabigatran interferes in all APCr assays due to its inhibitory action on thrombin

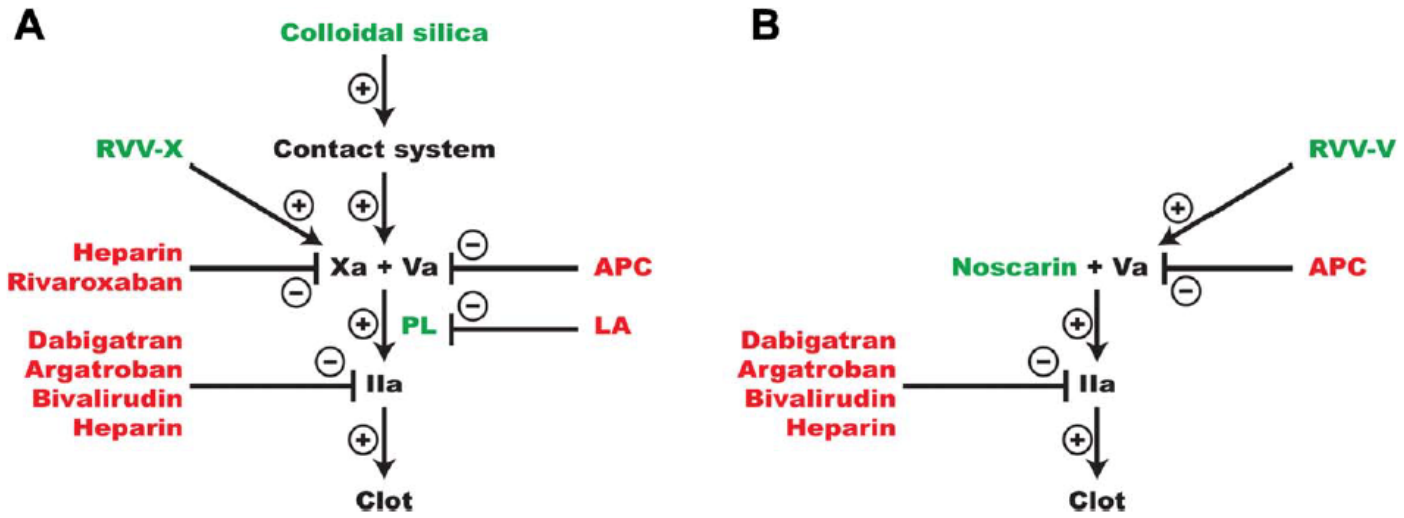


Figure 1. Simplified overview of major components of APC resistance assays discussed in this article. Components which either activate (+) or inhibit (−) the indicated clotting factor [or phospholipid (PL), in the case of lupus anticoagulant (LA)] are shown. (A) Interactions of components of the first four assays listed in Table I are shown. (B) Interactions of components of the fifth assay listed in Table I are shown. RVV-X, RVV factor X activator; RVV-V, RVV factor V activator. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Kadauke S, Khor B, Van Cott EM. Activated protein C resistance testing for factor V Leiden. American journal of hematology 2014; 89(12): 1147-50.

ACTIVATED PROTEIN C RESISTANCE

And this effect in already obtained at very low concentrations of dabigatran

Table I - Results from the *ex vivo* study.

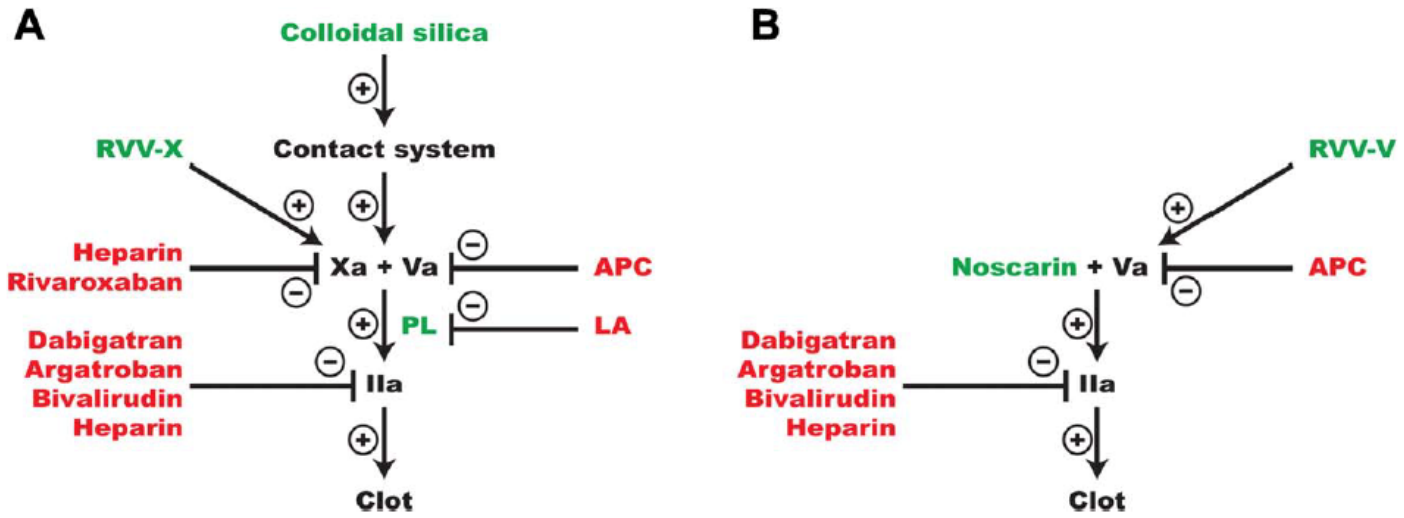
	DAB concentration	APCr ratio	p	p	p
O	0 ng/mL	3.69±0.41			
A	<100 ng/mL	5.38±0.66	O *vs* A p<0.01		
B	100-200 ng/mL	6.88±0.88	O *vs* B p<0.005	A *vs* B p<0.05	
C	>200 ng/mL	7.77±1.02	O *vs* C p<0.001	A *vs* C p<0.01	B *vs* C P<0.05
	DAB concentration	**APCr ratio**			
O1	0 ng/mL	1.34±0.08			
A1	<100 ng/mL	1.79±0.11	O1 *vs* A1 p<0.05		
B1	100-200 ng/mL	2.23±0.14	O1 *vs* B1 p<0.01	A1 *vs* B1 p<0.05	-
C1	>200 ng/mL	2.74±0.83	O1 *vs* C1 p<0.001	A1 *vs* C1 p<0.01	B1 *vs* C1 p<0.05

APCr: activated protein C resistance; DAB: dabigatran etexilate; FV wild-type homozygous subjects: groups O, A, B and C. FV Leiden heterozygous patients: groups O1, A1, B1 and C1.

Gessoni G, Valverde S, Valle L, et al. Effect of dabigatran on a prothrombinase-based assay for detecting activated protein C resistance: an ex vivo and in vitro study in normal subjects and factor V Leiden carriers. Blood Transfus 2017; 15(6): 562-7.

ACTIVATED PROTEIN C RESISTANCE

On the other hand, rivaroxaban do not interfere with APCr assays that shortcut X(a) such as noscarin-based assays



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ACTIVATED PROTEIN C RESISTANCE

On the other hand, rivaroxaban, (apixaban), edoxaban* and betrixaban do not seem to interfere with APCr assays that shortcut X(a) such as noscarin-based assays

***For edoxaban conflicting results have been reported – the FDA Pharmacology review mentions an anti-thrombin activity of edoxaban…**

Table 1. APCr ratios in FV wild-type subjects, FVL heterozygous, and FVL homozygous carriers

Rivaroxaban (ng/mL)	APCr ratio		
	FV wild-type	FVL heterozygous	FVL homozygous
Ex vivo study			
0	3.89 ± 0.43	1.34 ± 0.11	–
<100	3.92 ± 0.45	1.31 ± 0.13	–
100–200	3.83 ± 0.55	1.36 ± 0.16	–
200–300	3.92 ± 0.46	–	–
>300	3.93 ± 0.46	–	–
In vitro study			
0	4.48 ± 1.04	1.48 ± 0.14	1.03 ± 0.04
50	4.55 ± 0.97	1.46 ± 0.17	1.10 ± 0.07
100	4.63 ± 0.95	1.47 ± 0.15	1.01 ± 0.05
200	4.69 ± 0.87	1.43 ± 0.15	1.02 ± 0.07
300	4.65 ± 1.06	1.45 ± 0.16	1.01 ± 0.06
400	4.66 ± 1.15	1.47 ± 0.15	1.01 ± 0.05

Data are expressed as mean ± 2standard deviation.
APCr, activated C protein resistance; FV, Factor V; FVL, Factor V Leiden.

Gessoni G, Valverde S, Valle L, et al. Effect of dabigatran on a prothrombinase-based assay for detecting activated protein C resistance: an ex vivo and in vitro study in normal subjects and factor V Leiden carriers. Blood Transfus 2017; 15(6): 562-7.
Hillarp A, Strandberg K, Baghaei F, et al. Effects of the oral, direct factor Xa inhibitor edoxaban on routine coagulation assays, lupus anticoagulant and anti-Xa assays. Scandinavian journal of clinical and laboratory investigation 2018: 1-9.
Douxfils J, Chatelain B, Chatelain C, et al. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost 2016; 115(2): 368-81.

ACTIVATED PROTEIN C RESISTANCE

However, rivaroxaban and betrixaban (and probably edoxaban) interferes with the aPTT-based APCr assay…

…While aPTT-based methods seem to be less sensitive to the presence of apixaban

drug levels above 400 ng/mL

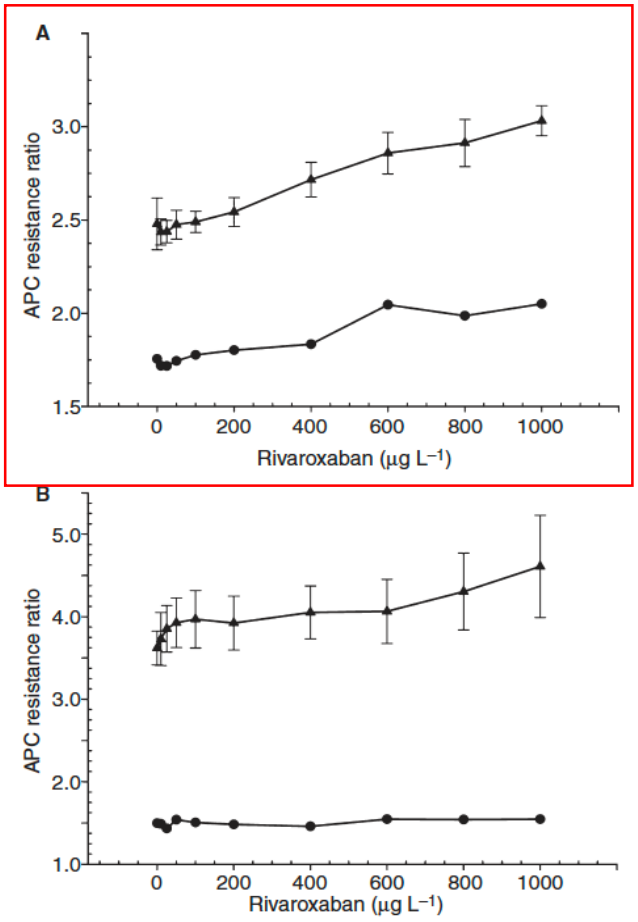


Fig. 4. Effect of rivaroxaban on coagulation-based APC resistance assays. (A) APTT-based assay (Coatest APC Resistance V). (B) Assay based on activation at the prothrombinase level (Pefakit APC Resistance Factor V Leiden). The results from nine individuals with normal phenotype (▲) and one individual with an APC-resistant phenotype (●) are expressed as mean ratio ± SD.

Hillarp A, Baghaei F, Fagerberg Blixter I, et al. Effects of the oral, direct factor Xa inhibitor rivaroxaban on commonly used coagulation assays. Journal of thrombosis and haemostasis : JTH 2011; 9(1): 133-9.

Douxfils J, Chatelain C, Chatelain B, et al. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost 2013; 110(2): 283-94.

Siriez R, Evrard J, Dogne JM, et al. Betrixaban: Impact on Routine and Specific Coagulation Assays-A Practical Laboratory Guide. Thromb Haemost 2018; 118(7): 1203-14.

ANTITHROMBIN ,FIBRINOGEN, PROTEIN C AND S

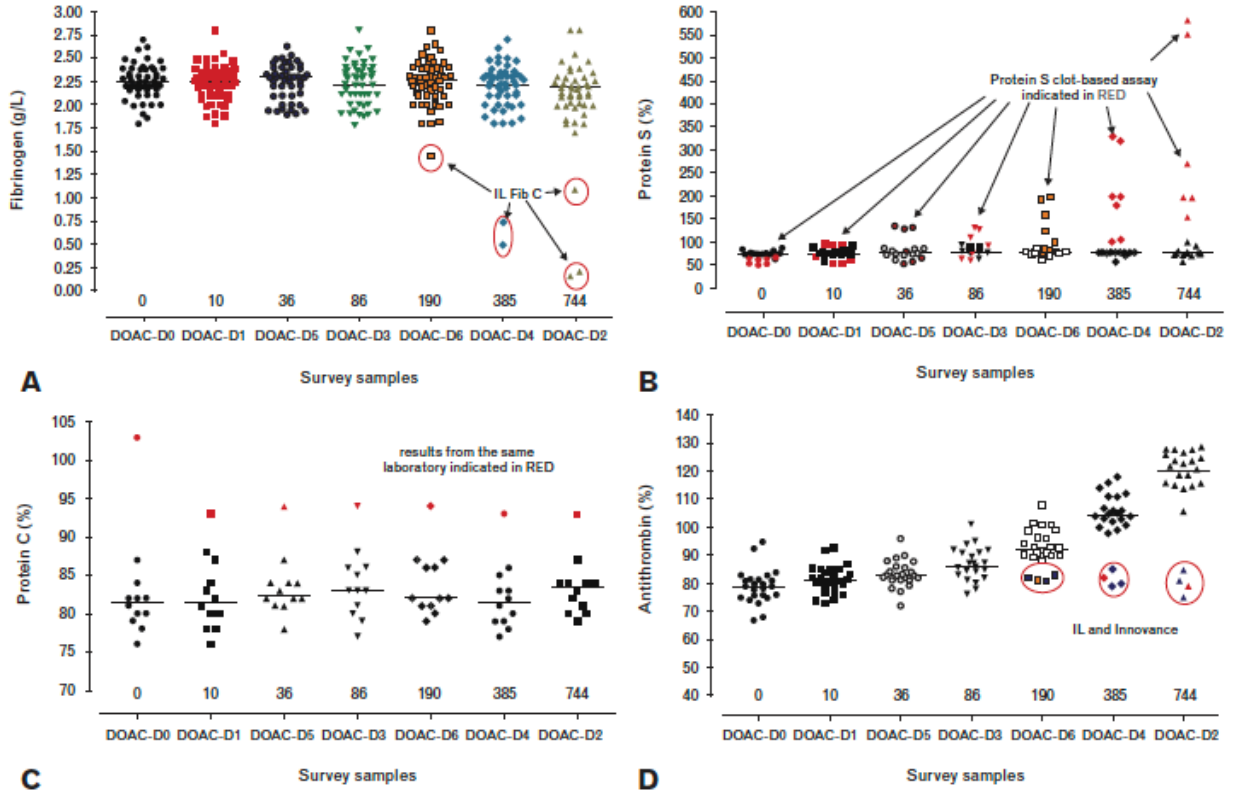


Fig. 4 Dabigatran and fibrinogen, PS, PC and AT test results. Participant-reported values (y-axis) for fibrinogen (A; g/L), PS (B; %), PC (C; %) and AT (D; %) test results for each survey sample (DOAC-DO to DOAC-6) shown separately in order of increasing concentration of dabigatran and as identified on the x-axis (median survey results shown above x-axis).

- **Clot based assays for protein S and thrombin based antithrombin assays are influenced by the presence of dabigatran at therapeutic levels**
- **Chromogenic protein C assays are not influenced while clot-based are influenced**

Bonar R, Favaloro EJ, Mohammed S, et al. The effect of dabigatran on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology 2015; 47(4): 355-64.

ANTITHROMBIN ,FIBRINOGEN, PROTEIN C AND S

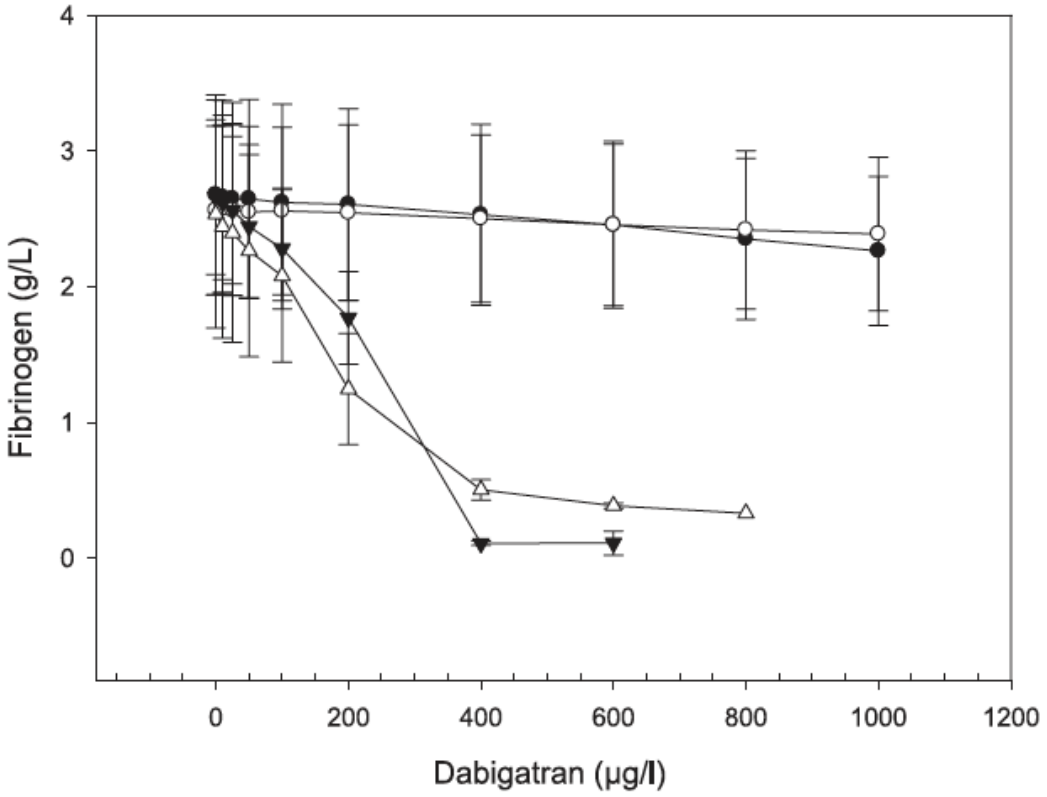


Figure 4: Effects of dabigatran on fibrinogen concentration results. Four different assays were evaluated; Fibri-Prest (●), Fibrinogen C (▼), Dade Thrombin (○) and Multifibren U (Δ). Results are shown as g/l of 10 different healthy donors ± SD.

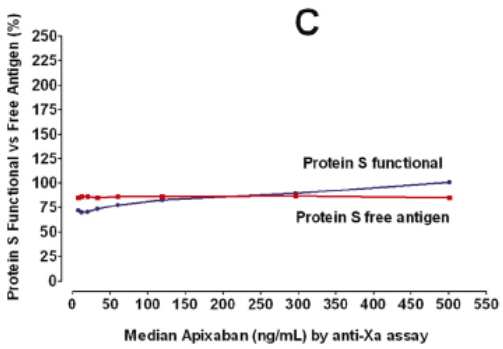
- **Fibrinogen measurement using reagents that contain low levels of thrombin to trigger the clot process are also influenced at higher dabigatran concentrations**

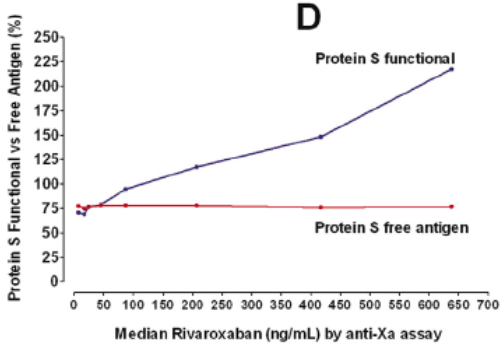
Lindahl TL, Baghaei F, Blixter IF, et al. Effects of the oral, direct thrombin inhibitor dabigatran on five common coagulation assays. Thromb Haemost 2011; 105(2): 371-8.

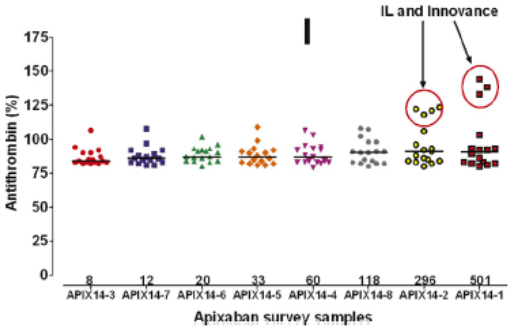
ANTITHROMBIN ,FIBRINOGEN, PROTEIN C AND S

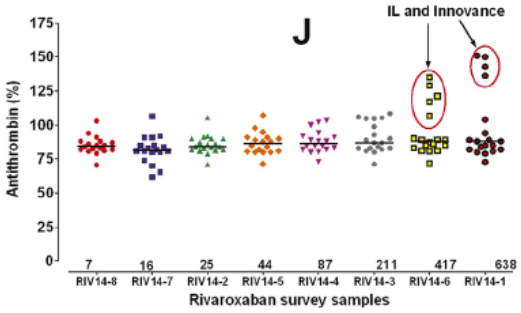
- **Clot based assays for protein S and FXa-based antithrombin assays are influenced by the presence of rivaroxaban and edoxaban at (supra)therapeutic levels**

- **Functional protein S is not influenced by apixaban (except at higher levels)**





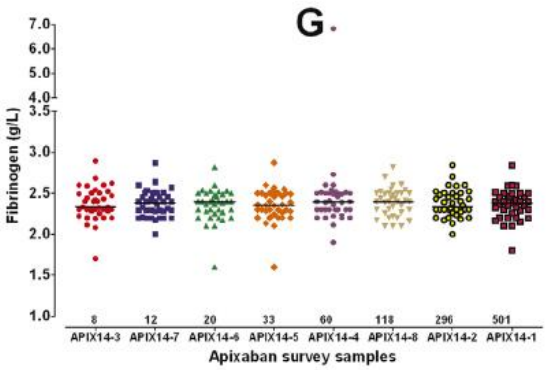


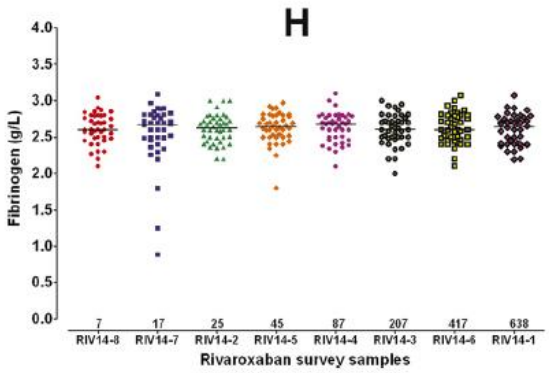


Bonar R, Favaloro EJ, Mohammed S, et al. The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology 2016; 48(1): 60-71.
Douxfils J, Chatelain C, Chatelain B, et al. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost 2013; 110(2): 283-94.
Douxfils J, Chatelain B, Chatelain C, et al. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost 2016; 115(2): 368-81.

ANTITHROMBIN ,FIBRINOGEN, PROTEIN C AND S

- **Fibrinogen measurement using the Clauss method is not influenced by the presence of apixaban, edoxaban or rivaroxaban**
- **The PT-derived measurement of fibrinogen may be influenced and should be avoided**

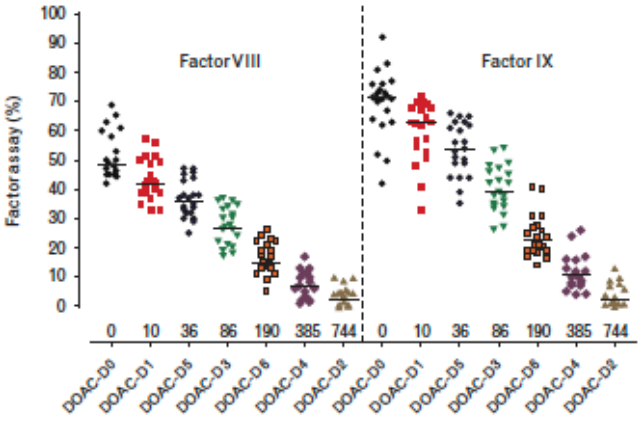


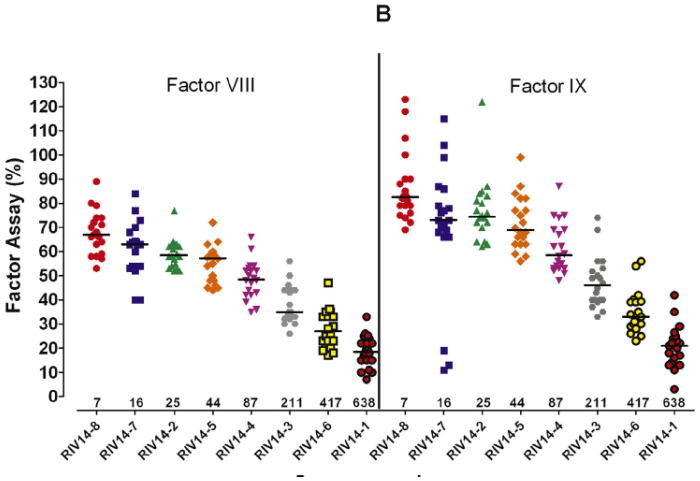


Bonar R, Favaloro EJ, Mohammed S, et al. The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology 2016; 48(1): 60-71.
Douxfils J, Chatelain C, Chatelain B, et al. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost 2013; 110(2): 283-94.
Douxfils J, Chatelain B, Chatelain C, et al. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost 2016; 115(2): 368-81.

CLOTTING FACTORS

- **Measurement of clotting factors in influenced according to the sensitivity of the triggering reagents (PT or aPTT) and the sensitivity of this reagents to the drugs**
- **For FVIII and FIX, dabigatran shows more interference than DFXaI**





Bonar R, Favaloro EJ, Mohammed S, et al. The effect of dabigatran on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology 2015; 47(4): 355-64.

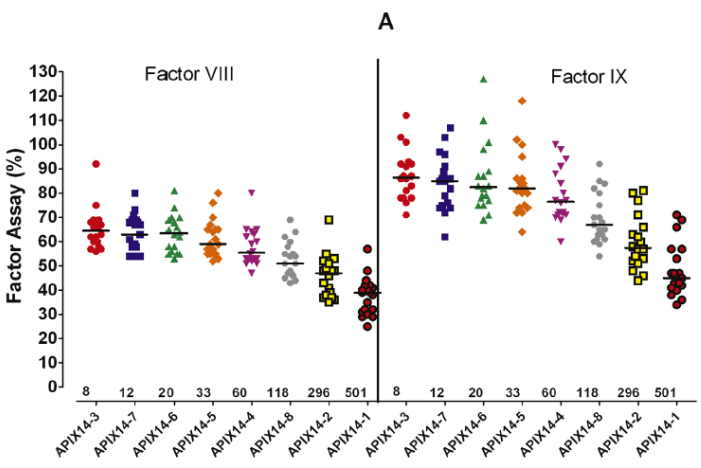
Bonar R, Favaloro EJ, Mohammed S, et al. The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology 2016; 48(1): 60-71.

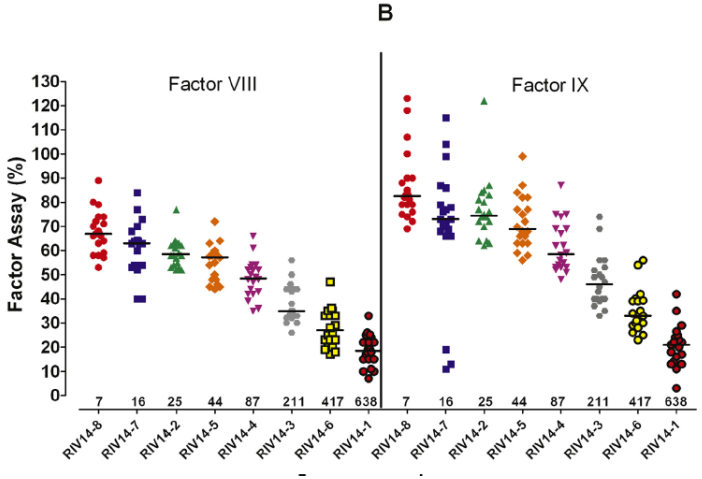
Douxfils J, Chatelain C, Chatelain B, et al. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost 2013; 110(2): 283-94.

Douxfils J, Chatelain B, Chatelain C, et al. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost 2016; 115(2): 368-81.

CLOTTING FACTORS

- **Among DFXaI, apixaban showed less interference than rivaroxaban**
- **Edoxaban and betrixaban should be somewhere in between**





Bonar R, Favaloro EJ, Mohammed S, et al. The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology 2016; 48(1): 60-71.

Douxfils J, Chatelain C, Chatelain B, et al. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost 2013; 110(2): 283-94.

Douxfils J, Chatelain B, Chatelain C, et al. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost 2016; 115(2): 368-81.

How to avoid the interference?

- **Some groups reported to test at trough…**
  - **But interference are still possible since levels at trough may still interfere with the tests**
- **Some other recommend to skip/miss a dose of the anticoagulant**
  - **It is safe? What a… is not protected?**
  - **Even with one … in some patients (i.e. those with low … metabolism…)**

- **The use of the reversal ag… has even been proposed**
  - **Maybe in your lab but not in mine…**
- **Other options have to be find!**

Ratzinger F, Lang M, Belik S, et al. Lupus-anticoagulant testing at NOAC trough levels. Thromb Haemost 2016; 116(2): 235-40.
Jacquemin M, Toelen J, Schoeters J, et al. The addition of idarucizumab to plasma samples containing dabigatran allows the use of routine coagulation assays for the diagnosis of hemostasis disorders. Journal of thrombosis and haemostasis : JTH 2015; 13(11): 2087-92.
Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. Journal of thrombosis and haemostasis : JTH 2018; 16(2): 209-19.

How to avoid the interference?

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journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Simple method for removing DOACs from plasma samples

arce J.[b], Xavier R.[c], Ahuja M.[a]

edical Centre, Perth, Australia

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ORIGINAL ARTICLE

WILEY ISLH International Journal of Laboratory Hematology

The adsorption of dabigatran is as efficient as addition of idarucizumab to neutralize the drug in routine coagulation assays

M. Jacquemin[1,2] | J. Toelen[2] | L. Fe[1] | J. Schoeters[2] | J. Van Horenbeeck[2] |
I. Vanlinthout[2] | M. Debasse[2] | T. Va

Evaluation of the DOAC-Stop® Procedure to Overcome the Effect of DOACs on Several Thrombophilia Screening Tests

Julien Favresse[1] Benjamin Lardinois[1] Lina Sabor[1] Bérangère Devalet[2] Julie Vandepapeliere[2]
Maximilien Braibant[3] Sarah Lessire[4] Bernard Chatelain[1] Hugues Jacqmin[1] Jonathan Douxfils[5,6]
François Mullier[1]

Exner T, Michalopoulos N, Pearce J, et al. Simple method for removing DOACs from plasma samples. Thrombosis research 2018; 163: 117-22.
Jacquemin M, Toelen J, Feyen L, et al. The adsorption of dabigatran is as efficient as addition of idarucizumab to neutralize the drug in routine coagulation assays. International journal of laboratory hematology 2018; 40(4): 442-7.
Favresse J, Lardinois B, Sabor L, et al. Evaluation of the DOAC-Stop® Procedure to Overcome the Effect of DOACs on Several Thrombophilia Screening Tests. TH Open 2018; 02(02): e202-e9.

How to avoid the interference?

- **How does it work?**

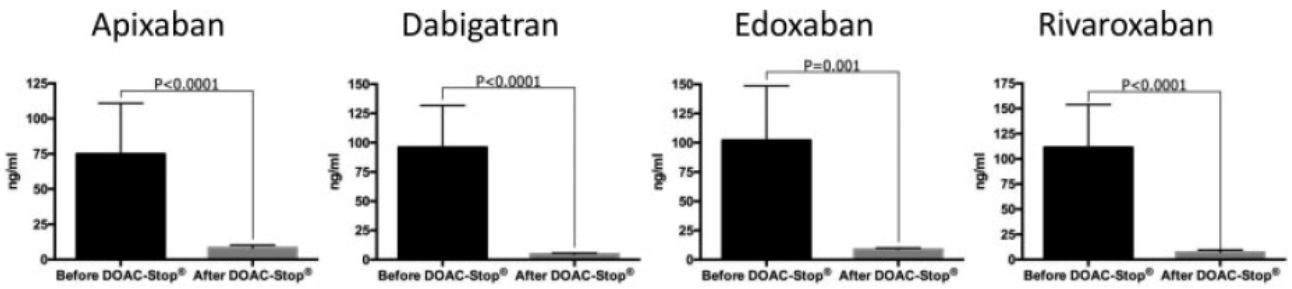

Fig. 1 Impact of the DOAC-Stop® adsorbent treatment on apixaban, dabigatran, edoxaban, and rivaroxaban concentrations. The mean (and 95% confidence interval) of each direct oral anticoagulant is presented before and after the DOAC-Stop® treatment.

Favresse J, Lardinois B, Sabor L, et al. Evaluation of the DOAC-Stop® Procedure to Overcome the Effect of DOACs on Several Thrombophilia Screening Tests. TH Open 2018; 02(02): e202-e9.

How to avoid the interference?

- **Is it effective?**

Pefakit APC-R factor V Leiden
(noscarin and FV-deficient plasma)

Table 1 Impact of the DOAC-Stop® adsorbent treatment on common thrombophilia screening tests

	Apixaban		Dabigatran		Edoxaban		Rivaroxaban		Controls	
	80 ng/mL (10–316)		73.5 ng/mL (2–406)		136.5 ng/mL (21–354)		76.5 ng/mL (7–456)			
	Before DOAC-Stop®	After DOAC-Stop®	Before DOAC-Stop®	After DOAC-Stop®	Before DOAC-Stop®	After DOAC-Stop®	Before DOAC-Stop®	After DOAC-Stop®	Before DOAC-Stop®	After DOAC-Stop®
Antithrombin (%)	95.7	98.6	99.5	97.2	95.6	101.3	98.7	100.3	89.8	91.4
	$p = 0.003$ ($n = 26$)[a]		$p = 0.09$ ($n = 30$)		$p = 0.01$ ($n = 10$)[a]		$p = 0.05$ ($n = 27$)		$p = 0.14$ ($n = 19$)	
Free protein S (%)	104.5	101.7	99.58	97.26	99.3	99.8	101	100.2	89.2	88.3
	$p = 0.08$ ($n = 24$)		$p = 0.005$ ($n = 31$)[a]		$p = 0.71$ ($n = 10$)		$p = 0.48$ ($n = 25$)		$p = 0.48$ ($n = 19$)	
Protein C (%)	105	104.9	104.8	104.7	122.7	123.2	121.0	122.5	98.5	100.8
	$p = 0.87$ ($n = 24$)		$p = 0.96$ ($n = 32$)		$p = 0.86$ ($n = 10$)		$p = 0.11$ ($n = 25$)		$p = 0.28$ ($n = 19$)	
APCR	4.0	4.3	6.6	4.6	4.5	4.2	4.1	4.1	3.6	3.5
	$p = 0.02$ ($n = 20$)[a]		$p < 0.0001$ ($n = 30$)[a]		$p = 0.001$ ($n = 10$)[a]		$p = 0.98$ ($n = 26$)		$p = 0.22$ ($n = 19$)	
PTT-LA (s)	42.8	38.1	55.3	37.5	38.3	34.4	42.1	35.1	36.2	36.1
	$p = 0.0007$ ($n = 26$)[a]		$p < 0.0001$ ($n = 27$)[a]		$p = 0.02$ ($n = 8$)[a]		$p < 0.0001$ ($n = 31$)[a]		$p = 0.84$ ($n = 19$)	
dRVVT screen (s)	55.5	40.6	80.2	42.1	59.3	37.7	77.0	40.9	37.7	38.1
	$p < 0.0001$ ($n = 27$)[a]		$p < 0.0001$ ($n = 31$)[a]		$p = 0.04$ ($n = 8$)[a]		$p < 0.0001$ ($n = 31$)[a]		$p = 0.43$ ($n = 18$)	
dRVVT confirm (s)	49.90	36.8	61.4	38.0	51.5	36.2	52.3	36.8	37.4	36.6
	$p < 0.0001$ ($n = 27$)[a]		$p < 0.0001$ ($n = 29$)[a]		$p = 0.04$ ($n = 8$)[a]		$p < 0.0001$ ($n = 31$)[a]		$p = 0.09$ ($n = 20$)	
dRVVT ratio	1.1	1.1	1.2	1.1	1.1	1.1	1.4	1.1	1.0	1.1
	$p = 0.05$ ($n = 27$)		$p = 0.0005$ ($n = 27$)[a]		$p = 0.08$ ($n = 8$)		$p < 0.0001$ ($n = 31$)[a]		$p = 0.07$ ($n = 18$)	

Abbreviations: APCR, activated protein-C resistance; dRVVT, dilute Russell's viper venom time.
Notes: The minimal, median, and maximal DOAC concentration is indicated in the first line of the table. The mean (and 95% confidence interval) of each parameter is presented before and after the DOAC-Stop® treatment.
[a]p-Value <0.05.

Favresse J, Lardinois B, Sabor L, et al. Evaluation of the DOAC-Stop® Procedure to Overcome the Effect of DOACs on Several Thrombophilia Screening Tests. TH Open 2018; 02(02): e202-e9.

HOW TO AVOID THE INTERFERENCE?

- **Is it free of interference?**

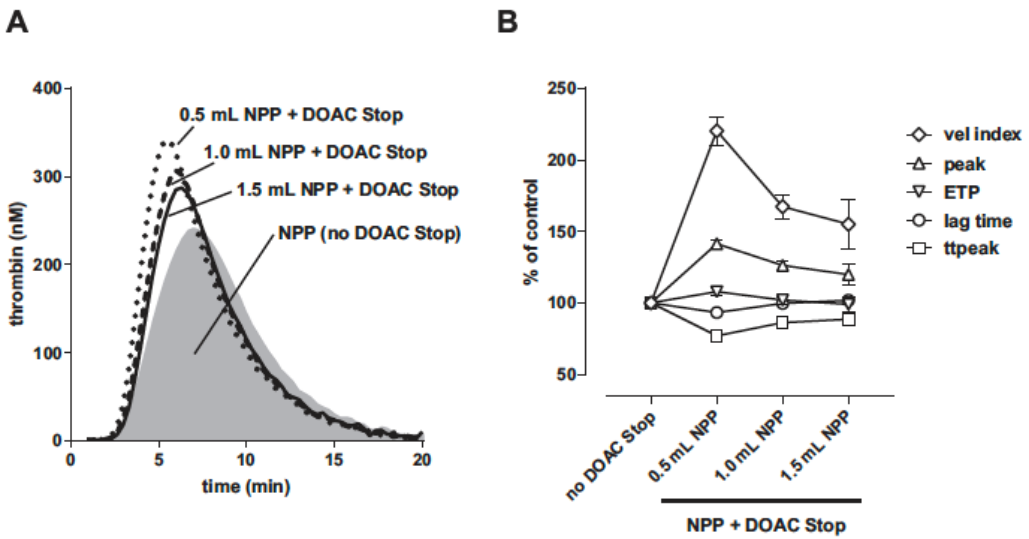

Fig. 2. Procoagulant side effect of DOAC Stop. Different volumes of normal pooled plasma (NPP) were treated with one minitab DOAC Stop. Samples were subjected to CAT and measured against calibrator wells containing untreated NPP. Representative curves are shown in panel A. In panel B, CAT parameters (in % of untreated NPP) of 10 separate treatments of the same NPP batch with DOAC Stop (mean ± SD).

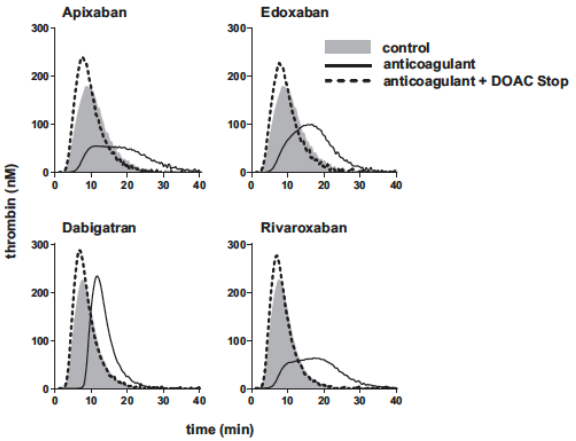

Fig. 1. Neutralization of the anticoagulant effect of DOACs by DOAC Stop. NPP was spiked with 80 ng/mL DOAC. Buffer-spiked NPP, DOAC-spiked NPP and DOAC-spiked NPP treated with DOAC Stop (1 mL plasma per tablet) were subjected to CAT and measured against calibrator wells containing untreated NPP.

Kopatz WF, Brinkman HJM, Meijers JCM. Use of DOAC Stop for elimination of anticoagulants in the thrombin generation assay. Thrombosis research 2018; 170: 97-101.

CONCLUSIONS

- **DOACs interfere with a lot of coagulation assay due to their mode of action**
- **The interference depends on the therapeutic agent, the plasma levels and the reagents/methodologies**
- **Solutions are now available with activated charcoal but seems to activated the coagulation (activation of contact pathway? Residual platelets? Adsorption of citrate?)**

Due to the high inter-reagent/methodology variation regarding the sensitivity, all the manufacturers should update their package leaflet to inform about the interference of all anticoagulant agents on their methodology

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