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REVIEW

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Comprehensive review of the impact of direct oral anticoagulants on thrombophilia diagnostic tests: Practical recommendations for the laboratory

Romain Siriez[1] | Jean-Michel Dogné[1] | Robert Gosselin[2] | Julie Laloy[3] | François Mullier[4] | Jonathan Douxfils[1,5]

[1]Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for LIfe Sciences (NARILIS), University of Namur, Namur, Belgium

[2]Thrombosis and Hemostasis Center, Davis Health System, University of California, Sacramento, California

[3]Department of Pharmacy, Namur Nanosafety Center (NNC),, Namur Research Institute for Life Sciences (NARILIS), University of Namur, Namur, Belgium

[4]Laboratory Hematology, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for LIfe Sciences (NARILIS), CHU UCL Namur, Université catholique de Louvain, Namur, Belgium

[5]Qualiblood s.a, Namur, Belgium

Correspondence
Jonathan Douxfils, Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for LIfe Sciences (NARILIS), University of Namur, Namur, Belgium.
Email: jonathan.douxfils@unamur.be

Abstract

There is a laboratory and clinical need to know the impact of direct oral anticoagulants (DOACs) on diagnostic tests to avoid misinterpretation of results. Although the regulatory labelling documents provide some information about the influences of each DOAC on diagnostic tests, these are usually limited to some of the most common tests and no head to head comparison is available. In this paper, we report the impact of DOACs on several thrombophilia tests, including assessment of antithrombin, protein S and protein C activity assays, detection of activated protein C resistance and assays used for lupus anticoagulant. Results are compared and discussed with data obtained from literature. The final goal of this comprehensive review is to provide practical recommendations for laboratories to avoid misdiagnosis due to oral direct factor Xa (FXa) or IIa (FIIa) inhibitors. Overall, oral direct FXa (apixaban, betrixaban, edoxaban and rivaroxaban) and FIIa (dabigatran) antagonists may affect clot-based thrombophilia diagnostic tests resulting in false-positive or false-negative results. An effect on FIIa-based thrombophilia diagnostic tests is observed with dabigatran but not with anti-FXa DOACs and conversely for FXa-based thrombophilia diagnostic tests. No impact was observed with antigenic/chromogenic methods for the assessment of protein S and C activity. In conclusion, interpretation of thrombophilia diagnostic tests results should be done with caution in patients on DOACs. The use of a device/chemical compound able to remove or antagonize the effect of DOACs or the development of new diagnostic tests insensitive to DOACs should be considered to minimize the risk of false results.

KEYWORDS

apixaban, betrixaban, dabigatran, edoxaban, rivaroxaban, thrombophilia

1 | INTRODUCTION

Since approximately 10 years, direct oral anticoagulants (DOACs) were approved for several thromboembolic disorders.[1] The key advantages of these anticoagulants are the absence of routine monitoring, an oral administration with fixed doses and more predictable pharmacokinetic profiles.[1-3] Nevertheless, although the clinical usefulness of blood measurements to assess the level of DOACs anticoagulation is subject to debate in the literature, clinicians and scientists agree that there is a laboratory and clinical need to know the impact of these anticoagulants on diagnostic tests to avoid misinterpretation of results.[1,3-6] While the regulatory labelling documents

of these medicines provide some information about the influences of each DOAC on diagnostic tests, these are usually limited to some tests, with few information about the reagents used and lack appreciation of this impact in relation with the "on-therapy" ranges of these drugs.

The objective of this comprehensive review is to provide an in-depth overview of the literature on the impact of oral direct factor Xa (FXa) and factor IIa (FIIa) inhibitors on screening coagulation tests and specialized thrombophilia tests, including antiphospholipid syndrome (APS) assays, antithrombin (AT) assays, protein S (PS) and protein C (PC) assessment and the detection of activated protein C resistance (APC-R). Finally, this document aims at providing practical recommendations and solutions for laboratorians to deal with interference encountered in daily practice to avoid diagnostic errors.

2 | IMPACT OF DOACs ON SCREENING COAGULATION TESTS

The impact of DOACs has been evaluated on screening coagulation tests in several original articles. These tests include (a) prothrombin time and activated partial thromboplastin time; (b). thrombin time; and iii. fibrinogen assays (Figure 1).[1,3,4,7-13]

2.1 | Prothrombin and activated partial thromboplastin time

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are used to evaluate the bleeding risks of an individual in some clinical situations like emergency, bleedings and other thromboembolic complications. The impact of DOACs on these tests is of utmost importance since it may directly mislead the direction for the subsequent exploration of an isolated PT or aPTT prolongation. Hence, a prolonged PT and/or aPTT in patient with known DOAC exposure may be expected. Nevertheless, physicians have to keep in mind that these chronometric assays can be impacted by other physiologic conditions potentially impacting the diagnostic approach (Figure 2). Additionally, the laboratory must also be aware of the numerous preanalytical variables associated with the patient, specimen collection, transportation, processing or storage that may also affect the PT and APTT. The most complicated feature of the DOAC interference on PT and aPTT is the variability between molecules and the inter-reagent variability.[7-11,13,14]

Therefore, there are no general rules with regard to DOAC exposure to the PT and/or aPTT impact as this depends on the reagents used at the laboratory facilities, the prescribed DOAC taken by the patient and the plasma concentration at time of blood collection. Thus, for the use of PT and aPTT testing for the DOAC anticoagulant assessment, the laboratory medicine specialist and the clinician should collaborate to establish an institutional protocol on which appropriate laboratory test should be used based on clinician needs in order to provide a multidisciplinary approach for a more accurate test interpretation (Figure 2).

Finally, considering the impact of DOACs on PT and aPTT, clotting factors activity measurements may be falsely underestimate. Indeed, Douxfils et al and Siriez et al reported a mean decrease of ±35% at 100 ng/mL of edoxaban, ±12% at 100 ng/mL of apixaban, ±42% at 150 ng/mL of betrixaban for the aPTT-based clotting method. They also reported for the PT-based clotting method, a mean decrease of ±18% of 100 ng/mL of edoxaban, ±11% of 100 ng/mL of apixaban, ±24% at 150 ng/mL of betrixaban.[8-10] Furthermore, the variability between molecules and the inter-reagents variabilities are also observed and could be explained by differences in the ionic force, the pH of the buffer solution, the source of activator (human or rabbit tissue factor; kaolin, silica or ellagic acid as surface activator) or even the PL composition.[1,4,7-11,14,15] This is especially important for high plasma levels of FVIII and FIX (aPTT-based assays) which are associated with an increased risk of venous thromboembolism.[16,17] Therefore, multi-dilution analysis using insensitive (to DOACs interferences) reagents should be considered to reduce the impact of the DOACs' presence in the sample.

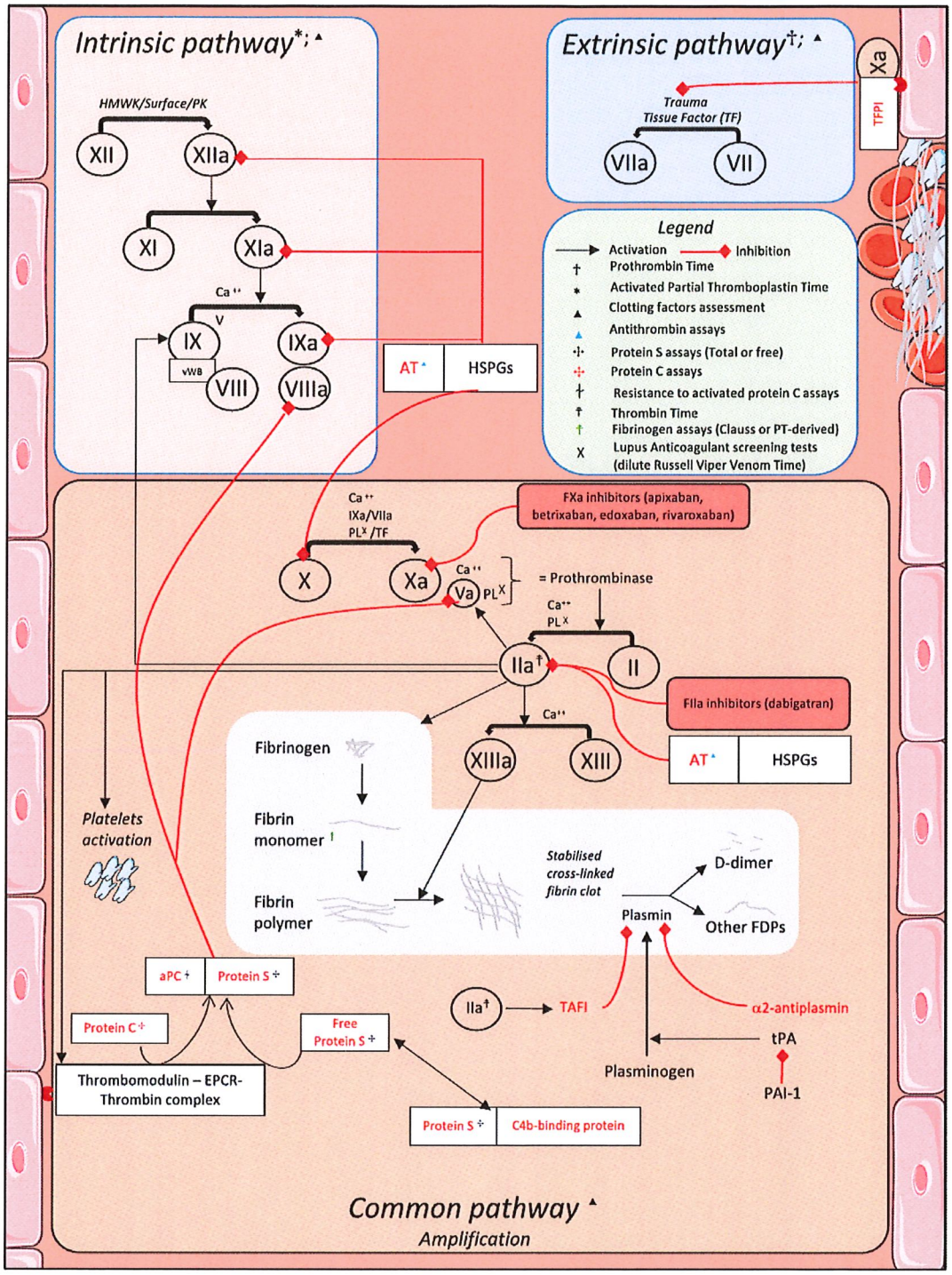
2.2 | Thrombin time

Thrombin time (TT) reflects the conversion of fibrinogen to fibrin and will allow clinician to detect fibrinogen-related abnormalities such as congenital or acquired deficiencies of fibrinogen (hypofibrinogenemia), abnormal fibrinogen molecules (dysfibrinogenemia), or poorly polymerized fibrin (as seen in disseminated intravascular coagulation [DIC]).[18] Nevertheless, this test is sensitive to the presence of thrombin inhibitors that may be present in the plasma. Therefore, the presence of direct FIIa inhibitors such as dabigatran will lead to an abnormal prolongation of TT [1,11,19] either at clinically relevant or trivial levels, depending on the instrument and the used reagents.[4] Therefore, practically all patients on dabigatran may have a TT result outside the normal range. Conversely, direct FXa inhibitors have no impact on the TT.

2.3 | Fibrinogen assays

In addition to TT, testing for fibrinogen abnormalities can be performed with other chronometric measurements such as PT

FIGURE 1 Summary of the coagulation cascade. aPC, activated protein C; AT, antithrombin; EPCR, endothelial protein C receptor; FDPs, fibrin degradation products; HMWK, High-molecular-weight kininogen; HSPGs, heparan sulphate proteoglycans; PAI-1, plasminogen activator inhibitor-1; PK, Prekallikrein; PL, phospholipids; TAFI, thrombin-activatable fibrinolysis inhibitor; TF, tissue factor; tPA, tissue-type plasminogen activator



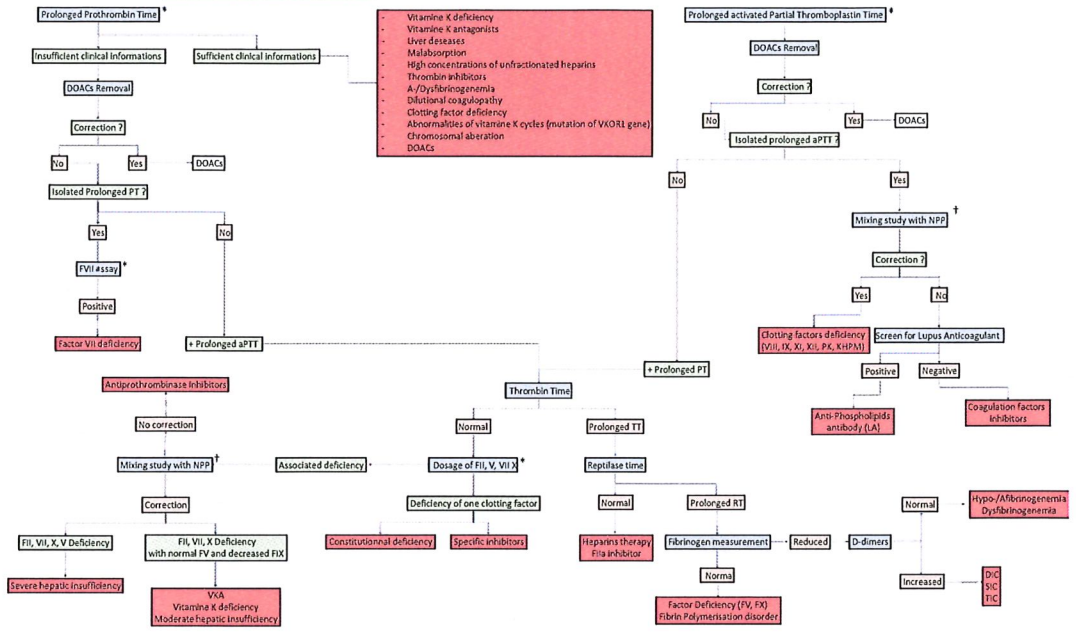


FIGURE 2 Diagnostic scheme of plasma samples with insufficient clinical information. According to the used PT or aPTT reagent, the sensitivity of prothrombin time and activated partial thromboplastin time assay can fluctuate and move out of the ratio range of 1.2. The presence of factor Xa inhibitors or dabigatran can concentration-dependent falsely prolong chronometric assays leading to useless investigation and false-positive or false-negative diagnostics. *Tests potentially impacted by the presence of DOACs (apixaban, rivaroxaban, edoxaban, betrixaban or dabigatran), depending on reagents and analysis. †The impact of the DOACs is depending of the dilution used. At most the dilution is important, at least the impact of DOACs is important. As we can observe, the impact of DOACs can lead to unnecessary investigations. Furthermore, the management of emergency bleeding in patient under DOACs remains problematic due to the lack of clinical information and the impact on routine and diagnostic tests. Only chromatographic methods with spectrometry could give information about the DOAC but the turnaround time and the availability limit its use in routine. DOACs, direct oral anticoagulants; PT, Prothrombin time ; aPTT, activated partial thromboplastin time

or aPTT. Although PT and aPTT can detect severe fibrinogen deficiencies, these assays recognize poor sensitivities to mild fibrinogen deficiency or dysfunction. Currently, functional assay (eg Clauss method) is commonly used for testing fibrinogen abnormalities. That assay, based on the thrombin clotting time, requires a high concentration of thrombin (ranging from 35 to 200 U/mL) to initiate clot formation.[18] The fibrinogen concentration is then obtained from a reference curve.[18] Although direct FXa inhibitors do not impact fibrinogen assays,[4,7-10,14] the intake of oral direct FIIa inhibitor dabigatran just as other parenteral direct thrombin inhibitors could induce a false underestimation depending on drug concentration and assay reagent (Fibri-Prest® and STA®-Fibrinogen-5 are reported as not influenced according to the results reported by Douxfils et al and Lindhal et al contrary to Fibrinogen C® and Multifibren U®).[11,13,14,20] This could be explained by different dilutions of samples, pipetting schemes or even by the amount of thrombin introduced in the test, which is quite lower in the reagents Fibrinogen C® and Multifibren U® compared to the other reagents. The consequence is that the amount of dabigatran encountered during the intake interval may be sufficient to interfere with the measurement of fibrinogen if these reagents are used. Finally, unaffected quantitative immunoassays such as ELISA could be considered but have to be correlated with other fibrinogen assays.[18]

3 | IMPACT OF DOACs ON SPECIALIZED HEMOSTASIS TESTS

Thrombophilia, a hypercoagulable state leading to a thrombotic tendency, could be related to a loss-of-function (eg antithrombin-; PS or PC deficiency) or a gain-of-function mechanisms (eg APC-R) as well as possibly related to acquired mechanisms (eg lupus anticoagulants [LAC]).[7,21,22] Among thrombophilia testing for such hemostatic abnormalities (Figure 1), DOACs are well known to have a potential impact on the assessment of selected assays for (a) antithrombin assays; (b) protein S activity (clot-based); (c) protein C activity (clot-based); (d) activated protein C resistance assays and (e) antiphospholipid syndrome (clot-based) testing [6,7,11-13,15,23-25] making immunological or less sensitive (to DOAC interference) assays more

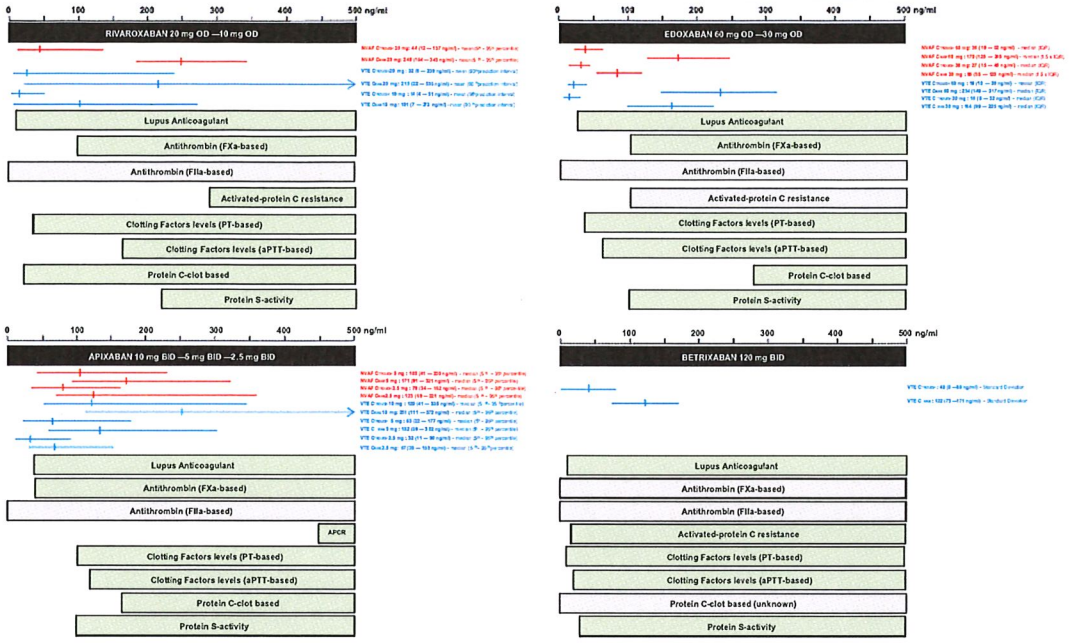


FIGURE 3 Impact of factor Xa inhibitors related to indications on diagnostic assays. Laboratory testing of factor Xa inhibitors and their impacts on diagnostic assays. Red and blue lines represent plasma concentrations at peak and through in NVAF and VTE, respectively. Green boxes represent ranges of concentrations that can impact diagnostic assays and lead to misdiagnosis while grey boxes represent unaffected assays (see original article for reagents used). For clotting factors measurement, boxes represent the dynamic range of quantitation (the dynamic range of quantitation is defined as range covering from the lowest observed limit of quantification to the maximal concentration tested or supposed) of PT and aPTT for sensitive reagents. $C_{through}$, minimum plasma concentration during the dosing interval; C_{max}, maximum plasma concentration during the dosing interval; VTE, venous thromboembolism; NVAF, non-valvular atrial fibrillation; OD, once daily; bid, twice daily; APC-R, activated protein C resistance. *Notes:* Data on plasma concentrations were extracted from Douxfils et al and Gosselin et al[2,4,7,9,10] for apixaban, edoxaban, rivaroxaban and from Siriez et al[8] for betrixaban

suitable for diagnostic purposes.[9,10] An overview of impacted tests is provided in Figures 3-4, in Table 1 and in Tables S1-S6. The subsequent sections will focus exclusively on the hypercoagulable state of the Virchow's triad. Hemodynamic changes (stasis, turbulence) as well as endothelial injury or dysfunction will not be discussed in this review.

3.1 | Assessment of antithrombin activity

3.1.1 | Principle

Antithrombin is a natural anticoagulant inhibiting serine protease, including FXa, FIIa, FIXa, FXIa, FXIIa, kallikrein and plasmin (Figure 1).[26-28] Although prevalence of AT deficiency in general population is extremely low (0.02-0.17%), this deficiency can lead to a 5- to 50-fold increased risk for venous thrombosis. Furthermore, severe AT deficiency is generally associated with neonatal mortality, greatly underestimating the number of observed cases.[11,26,28] Measurement of AT levels can be performed by immunological (quantitative assays) or more commonly, by functional assays (qualitative assays). As qualitative AT deficiency report normal ranges of antithrombin, activity assays are recommended by International Society of Thrombosis and Haemostasis (ISTH) guidelines.[28] Functional AT assays are chromogenic assays based on the principle of FIIa (human or bovine) or FXa (human) inhibition in the presence of heparin. The heparin binds AT leading to a complex antithrombin-heparin with residual concentration of FIIa or FXa is then available to cleave a specific chromogenic substrate which releases p-nitroaniline, producing a yellow colour. The measured absorbance at 405 nm is inversely proportional to the AT activity concentration in the plasma sample.[26,28]

3.1.2 | Impact of DOACs on antithrombin activity measurement

Due to the principle of the assay, AT measurements based on thrombin chromogenic assays are not influenced by direct FXa

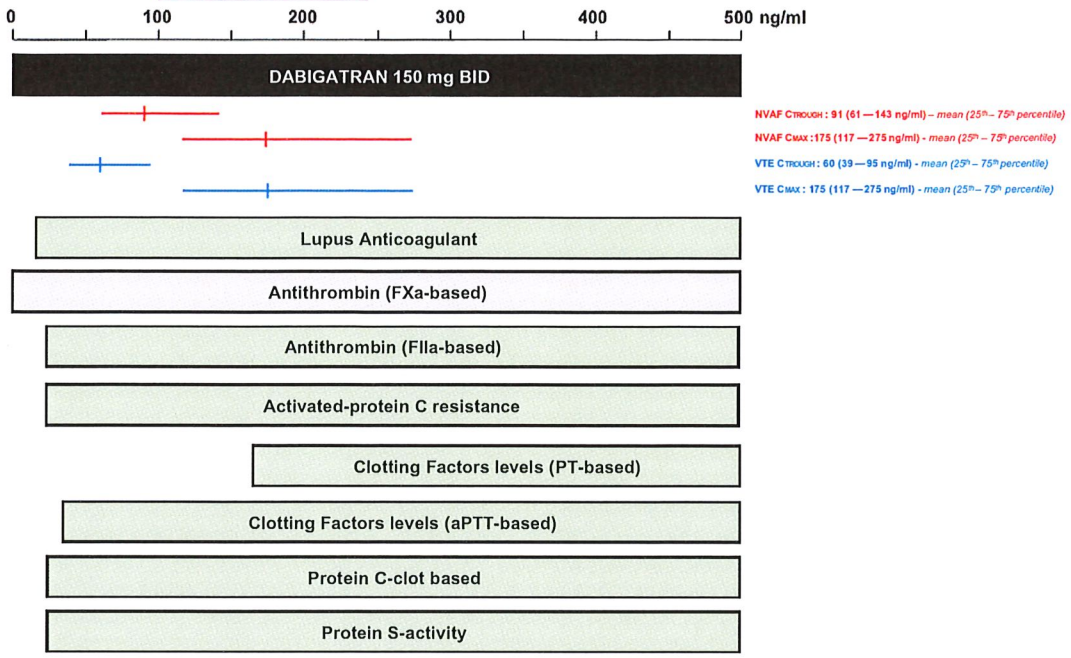


FIGURE 4 Impact of factor IIa inhibitors related to indications on diagnostic assays. Laboratory testing of factor IIa inhibitors and its impacts on diagnostic assays. Red and blue lines represent plasma concentrations at peak and through in NVAF and VTE, respectively. Green boxes represent ranges of concentrations that can impact diagnostic assays and lead to misdiagnosis while grey boxes represent unaffected assays (see original articles for reagents used). For clotting factors measurement, boxes represent the dynamic range of quantitation (the dynamic range of quantitation is defined as range covering from the lowest observed limit of quantification to the maximal concentration tested or supposed) of PT and aPTT for sensitive reagents. $C_{through}$, minimum plasma concentration during the dosing interval; C_{max}, maximum plasma concentration during the dosing interval; VTE, venous thromboembolism; NVAF, non-valvular atrial fibrillation; OD, once daily; bid, twice daily. These DOAC concentrations may not be reflective of all reagents or methods. *Notes:* Data on plasma concentrations were extracted from Douxfils et al,[11] Favaloro et al,[1] Gessoni et al,[35] Bonar et al[12] and Gosselin et al[2]

inhibitors (apixaban, edoxaban, rivaroxaban and betrixaban) and inversely for the AT measurements using FXa-based chromogenic assays are not influenced by dabigatran.[1,14,24] Therefore, AT activity in the presence of FXa or FIIa DOACs using FXa- or FIIa-based enzyme assays, respectively, lead to an overestimation of reported AT activity by inhibiting the key enzyme of the assays.[2] For example, Douxfils et al report an increase of 0.09 IU/mL per 100 ng/mL of rivaroxaban with the Coamatic-LR® (FXa-based) which was then confirmed by Hillarp et al Rivaroxaban has, on the opposite, no impact on Berichrom®ATIII and Stachrom®ATIII, two thrombin-based AT assays.[7,15] An increase of 22% was observed at high dabigatran concentrations (>900 ng/mL) with Stachrom®ATIII and Berichrom®ATIII.[11,20] Van Blerk et al, in a multicenter study performed in 192 laboratories in Belgium, reported a mean factitious elevation of AT activity of ±15%-20% at 250 ng/mL of dabigatran with the Berichrom®ATIII and the Stachrom®ATIII, respectively.[13] An overestimation of 25%–45% of AT levels was observed in presence of 290 ng/mL of rivaroxaban with HemosIL®Liquid-Antithrombin, Coamatic®Antithrombin or Innovance®Antithrombin and several studies reported similar results for apixaban and edoxaban with an estimated increase of 10% per 100 ng/mL.[1,6,8-10,12-15,20,29-31]

3.2 | Assessment of protein S activity

3.2.1 | Principle

PS is a multifunctional glycoprotein acting as antithrombotic factor thanks to activated protein C (aPC)-dependent and independent mechanism. Measurement of PS activity is required when PS deficiency is suspected. With an estimated prevalence ranging from 0.03% to 0.1%, this deficiency can lead to a 10-fold higher risk of venous thrombosis.[21,27,32] PS exists in two forms: an active free form (40%) and a complex inactive form bound to complement protein C4b-binding protein (C4bBP) (60%). Amount of free PS may be investigated by antigenic methods (eg enzyme-linked immunosorbent assay [ELISA] or latex immunoassay [LIA]) while

TABLE 1 Summary of recommended assays for the measurement of direct oral anticoagulants in plasma

Tests	Apixaban	Betrixaban	Dabigatran	Edoxaban	Rivaroxaban	Comments
Prothrombin time (s)	+	+	+	+	+	All clotting assays are impacted by DOAC with a variability between molecules and an inter-reagents variability. Interpretation of such tests should be done with caution in presence of DOACs.
Activated partial thromboplastin time (s)	+	+	+	+	+	
Fibrinogen						
Clauss method	=	-	=	=	=	Clauss method seems less impacted by DOAC unlike PT-derived measurement.
PT-derived	=	-	-	-	-	
Coagulation factor activity assays	-	-	-	-	-	PT and aPTT reference curves being impacted by DOAC, coagulation factors' levels are underestimated. The use of insensitive reagent with adequate dilution of plasma sample is recommended.
Antithrombin activity						
Antigenic assays	=	=	=	=	=	The presence of anti-IIa influence thrombin-based antithrombin assays while anti-Xa influence FXa-based antithrombin assays leading to an overestimation of antithrombin activity. Thrombin-based assays should be preferred for antithrombin activity measurement in presence of FXa inhibitors and conversely for FIIa inhibitor. Immunological assays for antithrombin are not impacted by DOACs and could be used for a quantitative assessment.
Anti-Xa based	+	+	=	+	+	
Anti-IIa based	=	=	+	=	=	
Protein C activity						
Antigenic assays	=	=	=	=	=	Clot-based assays for evaluation of protein C activity should be avoided in presence of DOAC. Chromogenic assays are recommended. Immunological assays for protein C are not impacted by DOACs and could be used for a quantitative assessment.
Clot-based test	+	+	+	+	+	
Chromogenic test	=	=	=	=	=	
Protein S activity						
Clot-based test	+	+	+	+	+	Clot-based assays for evaluation of protein S activity should be avoided in presence of DOAC. Immunological assays for protein S are not impacted by DOACs and could be used for a quantitative assessment.
Protein S free Ag	=	=	=	=	=	
Lupus anticoagulant testing: LA-sensitive aPTT and DRVVT (screening, mixing, confirmatory)	+	+	+	+	+	Antiphospholipid syndrome cannot be evaluated in presence of DOAC. DRVV screen, DRVV confirm and LA-sensitive aPTT should be avoided in presence of DOAC. The use of Taipan snake venom time and ECT time could be an alternative. Furthermore, the use of DOAC removals could avoid the influence of FXa and FIIa inhibitors.
Activated protein C resistance	+	+	+	+	+	DOAC interferes with APTT-based assay for APC-R assessment. Pefakit APC-R could be an alternative except for edoxaban and dabigatran.

Note: +: Impacted tests, should be avoided or used with caution in presence of DOAC; −: Underestimated tests, should be avoided or used with caution in presence of DOAC; =: Unimpacted tests, could be used in presence of DOAC.

PS activity may be investigated by clot-based (PT, aPTT, or dRVVT based) methods.[4,25,27,32]

All clot-based assays are chronometric assays using PS-deficient plasma with aPC or activator of protein C to measure the aPC co-factor activity of PS (free physiologically active form of PS).[4,27,32]

3.2.2 | Impact of DOACs protein S activity measurement

The presence of anti-Xa or anti-IIa inhibitors falsely suggests an elevated activity of PS by prolonging clotting time of PT-based, aPTT-based or dRVVT-based unlike antigenic assays which are

not impacted.[2,6,12,22,25,31] For rivaroxaban, an overestimation of approximately 15% per 100 ng/mL can be observed with STA®-Staclot®Protein S.[7,32] For apixaban, an overestimation of 13% per 100 ng/mL was observed with STA®-Staclot®Protein S and an overestimation of 30% was also observed with edoxaban for higher concentration (from 250 ng/mL).[9,10] Protein S activity reagent is not influenced until 10 ng/mL of betrixaban but higher concentration (from 30 ng/mL) leads to overestimation of 10%.[8]

3.3 | Assessment of protein C activity

3.3.1 | Principle

Protein C, a natural anticoagulant, is cleaved and activated by the complex formed by thrombin, thrombomodulin and endothelial PC receptor and converted into aPC which, in presence of P S, inhibit FVa and FVIIIa toning down the amplification phase of the coagulation (Figure 1).[21,27] The measurement of PC activity is required to detect PC deficiency, an risk factor of venous thrombosis with a prevalence of 0.2% that could increase risk of thromboembolism by 5 to 10-fold.[33,34] Except immunoassays (eg ELISA or LIA) that measure antigen of PC, clot-based (PT or aPTT-based) or chromogenic assays are commonly used to assess the PC activity. Clot-based assays are based on the in situ activation of PC into aPC by specific snake's venom (eg Protac™, a single chain glycopeptide obtained from *Akistrodon contortrix contortrix*) which degrades, after initiation of coagulation by addition of calcium, FVa and FVIIIa prolonging clotting time initiated by the contact pathway. The prolongation of the clotting time is proportional to PC present. Chromogenic assays rely on the cleavage of synthetic substrate by aPC with the liberation of chromogenic compound without requiring PL or contact activator. Although functional assay could be performed with both of these tests, current recommendation is to prefer chromogenic assays which are less impacted than clot-based assays by FVIII elevation, LAC or FV_{Leiden}.[1,4,6,7,9,12,19,33,35]

3.3.2 | Impact of DOACs on protein C activity measurement

The presence of anti-Xa or anti-IIa DOACs will falsely suggest an elevated activity of PC by prolonging clotting time of PT or aPTT.[1,6,7,9,10,29,31] For example, according to a study of Gosselin et al, more than 15% of overestimation could be observed with 276 ng/mL of edoxaban, 22 ng/mL of rivaroxaban or 25 ng/mL of dabigatran with clot-based assays.[2,24,36]

Conversely, chromogenic assays, such as HemosIL® Protein C, Berichrom®, STA-Stachrom® PC are not impacted by DOACs and could be used for measurement of PC activity.[10,24,33]

3.4 | Assessment of activated protein C resistance (APC-R)

3.4.1 | Principle

Resistance to aPC is usually due to a mutation of FV_{Leiden} inducing the replacement of arginine into glutamine. That replacement prevents cleavage of FV_{Leiden} leading to a poor response to aPC and an increased risk of VTE.[21] Indeed, heterozygous FV_{Leiden} mutation lead to a fourfold risk of developing a first VTE while homozygous FV_{Leiden} mutation lead to a 50-fold risk of developing a VTE.[21] Polymerase Chain Reaction (PCR) and chronometric assays can be used to diagnostic an FV_{Leiden}.[30,35,37]

The first chronometric assay used the ratio between baseline aPTT and aPTT added with purified exogenous aPC. The addition of aPC inactivates FVa and FVIIIa results in an increased clotting time of the aPTT in sample without FV_{Leiden} mutation. By contrast, in a sample with mutation, the prolongation in the clotting time is less marked. Nevertheless, any factors that could impact aPTT assays (abnormal factor levels, LA, anticoagulants treatments, ...) could interfere with the correct interpretation of this test..[30,35,37]

Second modified assays dilute plasma sample with FV-deficient human plasma (1:4) containing polybrene, a heparin inhibitor, to improved sensitivity and specificity and to normalize factor levels. Nevertheless, that assay was still influenced by LAC and oral anticoagulants.

Third-generation assays used Russell's viper venom from *Daboia russelii* to overcome the influence of contact pathway and venom from *Agkistrodon contortrix* to activate endogenous PC but without dilution in FV-deficient human plasma. However, with these 3rd-generation assays, low FII, FV and fibrinogen levels, and anticoagulants may interfere with correct test result interpretation.[30,35,37]

A FXa-based clotting time with purified fibrinogen, FIIa and PS in presence of purified aPC was also proposed as another APC-R assay to avoid interference with aforementioned factors deficiencies. Despite that advantage, the presence of LAC and anticoagulants still impacted test results interpretations.

Finally, researchers developed plasma-based functional clotting assay with the replacement of FXa by noscarin, a FVa-dependent and PL-independent prothrombin activator isolated by *Notechis scutatus*. A dilution with FV-deficient plasma (1:4) is performed to overcome to factors deficiencies/elevations and the PL independence avoid impacts of LAC.[30,35,37,38]

3.4.2 | Impact of DOACs on activated protein C resistance assays

On one hand, the assessment of APC-R based on aPTT or dRVVT seems to be impacted by DOACs[15] despite exceptions occurred with apixaban and rivaroxaban which seem not impacting aPTT assay.[39-41]

On the other hand, DOACs seem not interfering with prothrombinase-based assay as the Pefakit APC-R FV_{Leiden}. Nevertheless, Lindahl et al highlighted a major impact of dabigatran from 50 ng/mL, and to a lesser extent, edoxaban, due to their impact on thrombin (edoxaban has a small inhibitory activity on thrombin). These results are supported by Douxfils et al and Favresse et al where concentration of edoxaban around 100 ng/mL and 20 ng/mL of dabigatran may provide false-negative results.[6,7,9,25,35] In contrast, Hillarp et al reported that edoxaban does not impact Pefakit APC-R FV_{Leiden}. These contrary results could be explained by the high lot-to-lot variability of PL concentrations but further investigations are required.[1,7,9,10,29,30]

3.5 | Determination of antiphospholipid syndrome

3.5.1 | Principle

Antiphospholipid syndrome is induced by the presence of immunoglobulins binding PL and proteins providing a disruption of coagulation process and may promote venous/arterial thrombosis and pregnancy morbidity.[42,43] Although little-known, recent studies estimate the incidence at five cases per 100 000 persons per year and the prevalence at 40-50 cases per 100 000 persons. The current criteria for the classification of APS are based on clinical presentation and laboratory testing.[44] The laboratory components include anticardiolipin antibody testing and anti-beta 2 glycoprotein I (anti-ß2GPI) antibody testing commonly detected by ELISA and assessing for LAC.[45] There are multiple methods for LAC testing, but the current recommendations consider two types of assays of differing methodologies.[45] Currently, and according to external quality assurance, the most frequent assays for detection of APS consist of derivative aPTTs containing lower level of PL and dRVVT. Literature also reports the use on a lower scale (<5% of laboratories) of Kaolin Clotting Time (KCT), Silica Clotting Time (SCT) and Platelet Neutralization Procedure (PNP). Finally, the use of other snake venom derivatives (eg ecarin or Taipan snake venom) could be considered but international standardization, implementation and clinical validation of these tests are still required.[4,9,23,46,47]

Derivative aPTTs with low concentration of PL paired with a classical aPTT are widely used tests for screening LAC. Two kinds of tests may be used: (a) for the screening of LAC using aPTT reagents that contain low PL concentrations making them very sensitive to the inhibition of these PL by LAC antibodies and (b) aPTT-based test in which specific PL are added to correct the clotting time by saturating the antibodies (ie Staclot®-LA). The difference between the two conditions should not exceed a certain amount of time as specific by reagent instructions for use, to classify the patient as LAC-negative.

The dRVVT uses the venom from a snake, *Daboia russelii*, as an activator of the endogenous FX present in the sample conferring the advantage of being insensitive to deficits in FVII, FVIII, FIX and contact factors. The reagent is also composed of PL and calcium ions to trigger the clotting reaction. The principle is quite simple and relies on the capacity of the antibodies against PL present in test plasma to bind the PL from the reagent and consequently prolong clotting time. To ensure that the prolongation of the clotting time is due to the presence of these antibodies, the test is performed with two different concentrations of exogenous PL. Thus, a "screen test" is first performed with a low concentration of PL to suspect the presence of LAC antibodies. If the screen ratio between the patient's clotting time and a reference pool plasma is at least equal to the cut-off value then a "confirm test", with high concentration of PL, is performed vs the reference pool plasma. A normalized ratio is calculated, that is ratio screen on ratio confirm, and have to be lower than the cut-off value to exclude LAC. According to guidelines for LAC detection,[45] the cut-off values have to be determined locally considering the relatively large inter-laboratory cut-off variations.

3.5.2 | Impact of DOACs on lupus anticoagulants assays

Due to an increased rate of recurrent thrombotic events compared with vitamin K antagonist therapy, European Medicines Agency (EMA) do not recommend the use of DOACs for patients with a history of thrombosis and who are diagnosed with APS, especially for patients that are triple positive (for LAC, anticardiolipin antibodies and anti-ß2GPI antibodies). That recommendation is supported by a randomized phase 3 open-label noninferiority study involving the comparison of rivaroxaban and warfarin. Data are not yet fully available for others DOACs but currently suggest similarly increased risk of recurrent thrombotic events.[48] Nevertheless, DOACs are still used to treat patients with thromboembolism, typically irrespective or prior to antiphospholipid antibody assessment. Therefore, the accurate diagnostic of APS is mandatory to avoid inappropriate use of DOACs in this population.[42]

Direct oral anticoagulants often prolong the screening tests above the cut-off limit, leading to unnecessary laboratory investigations due to this first false-positive interpretation. This increases the costs related to additional reagent use and technician time.[19,22,43,49,50] As the sensitivity of the screen and confirm tests differs (ie the screen test is more sensitive to the presence of DOACs than the confirm test), the normalized ratio may still be above the cut-off, generating false-positive results, or on the contrary it can be reduced, generating false-negative results. This is explained by the disproportionality between the sensitivities of the screen and confirm reagents which may sometimes exceed 20%. This difference in term of sensitivity is concentration- and drug-dependent meaning that this error cannot be anticipated or corrected universally to address all LAC testing platforms.

Several studies reported these observations, showing that the presence of DOACs generates false-positive or false-negative results.[7,9-11,22,51] Dabigatran, edoxaban and rivaroxaban may impact both dRVVT screen and confirm assays. However, typically the

impact is more pronounced on the screening assay more so than on the confirmatory test leading to an increased normalized ratio and false-positive result interpretation.[9,12,30,43,47,49,50,52,53] Apixaban, on the contrary, showed a different impact on dRVVT screen and confirm from others DOACs. Indeed, at therapeutic concentrations, and until 500ng/mL, apixaban will have a lower impact on dRVVT screen than on the dRVVT confirm tests, leading to reduced normalized ratio and, therefore to a potential false-negative interpretation.[49] Ratzinger et al reported a significant impact of all DOACs on the diagnosis of LAC, even at C_{trough}, leading to false-positive results. For that reason, several authors recommend that, under DOACs, requests for dRVVT testing should be averted, even at DOAC C_{trough} to avoid false-positive or false-negative result interpretations.[2,4,7-11,24,36,45,49,53]

Next to the impacts on dRVVT, DOACs also showed a more modest impact on aPTT-based assays by acting on FXa of FIIa.[2,43,50] Some studies reported that Staclot®-LA, containing hexagonal phase phosphatidylethanolamine, could be used since the result is not affected at therapeutic concentration of FXa inhibitors but not for dabigatran which influence the interpretation at concentration as low as 50 ng/mL. This difference remains obscure but could be linked to its composition and concentrations of PL.[1,4,7-11,19,30,36,45,49,51,52] Finally, Haematex Research has developed reagents for which the sensitivity for DOACs do not differ between the screen and the confirm reagent allowing the use of the DRVT-LS/DRVT-LR to diagnose LAC. Further investigations are required to see how these reagents could be implemented in the testing algorithm of LAC testing.[51]

4 | STRATEGIES AND RECOMMENDATIONS TO AVOID THE INTERFERENCES OF DOACS ON DIAGNOSTIC TESTS

Considering that DOACs may impact almost all routine and diagnostic tests to varying degrees, laboratories have to resort to strategies to avoid interferences. Laboratories have to keep in mind that the influences of these drugs can lead to misdiagnosis and either false-positive and false-negative results. To reduce the impact of these drugs, some solutions are available (Figure 5).

4.1 | To antagonize the effects of DOACs

Reversal agents such as idarucizumab, andexanet alfa and ciraparantag were developed as reversal agents for patients with bleeding events associated with treatment with DOAC anticoagulants. However, there have been several studies suggesting their use as an in vitro neutralizing agent to mitigate DOACs' interferences on coagulation testing.

Idarucizumab (Boehringer Ingelheim), a humanized antibody fragment approved in the United States and in Europe in 2015, has demonstrated a prevention of any interference of dabigatran in coagulation assays preventing false-positive or false-negative diagnostic readouts without interfering with coagulation assays and without requiring interruption of patient anticoagulation therapy.[1,29,31,49,54] Jacquemin et al demonstrated that the addition of 125 µg/mL

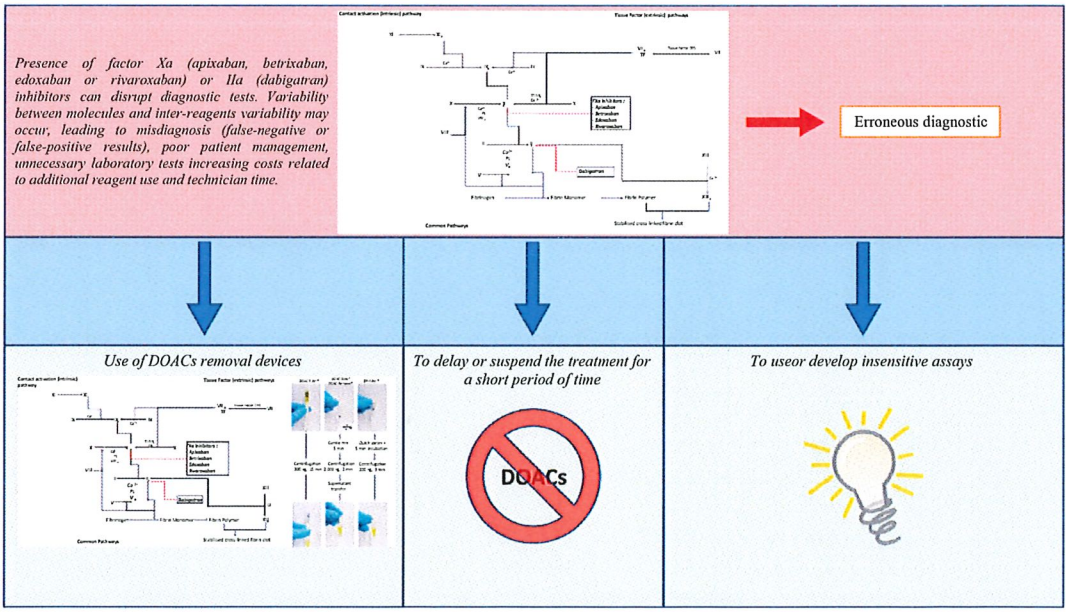


FIGURE 5 How to deal with anticoagulated plasma?

idarucizumab fully neutralizes the activity up to 1500 ng/mL dabigatran and that in case of overdosage, the addition of 625 µg/mL idarucizumab could inhibit up to 8000 ng/mL without interfering with routine coagulation tests.[1,31,54]

Andexanet alfa, a recombinant antidote for both direct FXa inhibitors and low molecular weight heparins approved in the United States in May 2018, was also proposed to potentially correct the impact of these drugs although this molecule is currently expensive, not yet marketed worldwide, and not yet widely available for laboratory tests.[1,54] Nevertheless, recent studies evaluated the use of andexanet compared with DOAC-Stop® on LAC testing, factor VIII and factor XI and APC-R. These studies highlighted that andexanet alfa induced prolongation of clotting times due to its anti-Xa effect and produced an "over-correction" of the screen/confirm ratio than the baseline that could lead to potential false-negative results. For clotting factors measurement, andexanet alfa leaves some residual interference leading to false low values compared to the use of DOACs-Stop® (see section above). Finally, andexanet alfa was unable to neutralize the interference of rivaroxaban on APC-R, creating even more false-positive results..[41,53,55]

Ciraparantag, a cationic synthetic molecule initially developed as potential reversal for UFH, LMWH and fondaparinux, is presented as a potential universal reversal for FXa inhibitors and dabigatran and is still under FDA review. Nevertheless, binding calcium chelators (eg sodium citrate), ciraparantag could distort the results of clot-based coagulation assays.

Unfortunately, there are no commercial reagents including these reversals agents. Therefore, all investigations required "home-made" reagents making their use quite expensive (3300$ for 100 mg of andexanet alfa, 3500$ for 2.5 g of idarucizumab and 841$ for 100 mg of ciraparantag).[49]

4.2 | To remove the presence of DOACs

To avoid the impact of DOACs on diagnostic tests, the use of absorbent products, such as activated charcoal, is proposed to remove the drugs from the samples with a minimal impact on the clotting capability of the sample.[56] Some studies showed that this technique could be as efficient as the addition of idarucizumab for removing dabigatran or andexanet alfa for removing rivaroxaban.[4,31,41,49,53,54,57] DOAC-Stop® (Haematex Research) and DOAC-Remove® (5-Diagnostics AG) were developed in this perspective. According to the manufacturer, one tablet of DOAC-Stop® or DOAC-Remove® is added per 1 mL plasma (minimal required volume ranging from 1.0 to 1.5 mL), mixed for 5 minutes (DOAC-Stop®) or 10 minutes (DOAC-Remove®) and centrifuged 2 minutes at 2000 g (DOAC-Stop®) or 2-5 minutes at 2500 g (DOAC-Remove®) to obtain plasmas devoid of DOAC until 500 ng/mL (lower than limit of quantification).[29,57] Recent study by Exner et al, or Favresse et al, suggest its use to eliminate the impact of DOACs on diagnostic tests.[29,54,57,58] These results are consistent with the results of Platton et al where PT, DOAC-specific anti-Xa assay, FVIII and dRVVT of samples from patients on rivaroxaban or apixaban where normalized after treatment with DOAC-Stop®.[41,53,54,56,58] More recent studies of Favaloro et al on the use of DOACs-Stop® for LAC testing, FVIII and FIX measurement and APC-R in case of rivaroxaban interferences support these findings.[41,53,55]

However, the use of DOAC-Stop® seems to interfere with TGT parameters such as time to peak, endogenous thrombin potential, peak height or mean velocity rate index.[57] Furthermore, DOAC-Stop® seems to possess procoagulant side effect that could also lead to interferences.[57] The mechanism by which procoagulant changes in plasma devoid of anticoagulant remains unclear. It may be explained, in part, by a decreased level of tissue factor pathway inhibitor (TFPI), an anticoagulant physiological protein,[57] the activation of contact pathway by the charcoal, by the caption of small molecule such as citrate or even by the presence of residual platelets. These impacts are all the more important as the used volume of plasma is small (<1.0 mL).[57] In summary, the adsorption of DOACs seems to be a promising, easily accessible and cheap solution (<6 euros/minitab) despite the minor procoagulant effect but the use of DOAC-Stop® should warrant caution.[29,57] Further studies need to corroborate these results.

More recently, filters coated with absorbent product (DP-Filter® (UNamur) or (DOAC-Filter®, [Diagnostica Stago]) have been developed and are currently under investigation. These devices are easy easier to use than raw material (ie charcoal) and the filter avoid the passing of potential residues, which might potentially interfere with the measurement (the concentration of DOAC in these residues can be very high), as observed with DOAC-Stop® or DOAC-Remove®. For these devices, there is a slight loss of volume after centrifugation (±100 µL). These methods appeared to be an effective, ergonomic and simple way to overcome the interference of DOACs on coagulation tests and to avoid erroneous results.[29,58,59]

4.3 | Discontinuation of the therapy

Currently, recommendations are to stop, at least 2 days, the treatment of DOACs to avoid interference for low-risk patients. Nevertheless, the discontinuation of the treatment is not clinically recommended and exposes patient to a risk of thromboembolic recurrence. Furthermore, stopping treatment of DOACs do not always allow the concentration to fall below 30 ng/mL (eg patient with moderate renal impairment or antiarrhythmic patients requiring a longer interruption), even after 24 or 48 hours, and depending on the test, this may still impact the diagnostic tests.[2,29,49,60] Our consideration is to avoid thrombophilia testing as much as possible with DOACs treatments if results induce management modifications. In which case, laboratories should consider other strategies such as the use of neutralizing agents such as activated charcoal.

4.4 | Optimal thrombophilia testing in DOAC-treated patients

According to the impact of DOACs on thrombophilia testing, general recommendations should be implemented in the laboratories. We would advise avoiding the use of thrombophilia testing in DOAC-treated patient. If results may induce clinical management changes, laboratories have to keep in mind that, without using strategies (especially activated charcoal and filtering mechanisms given their ease of use and cost) to avoid interferences, DOACs can falsely disturb AT, PS, PC, APC-R assays and LAC determination if no pre-treatment of the sample is realized to reduce their impact. Additional recommendations could be considered for each thrombophilia testing:

- To evaluate AT deficiency, adequate reagents should be used meaning that tests based on thrombin should be used in patients on direct FXa inhibitors and that tests based on FXa should be used in patients with dabigatran.[1,6,8,9,12-15,20,29-31] This recommendation is supported by the latest guidelines of the ISTH. It is also recommended to use antigen assay which is not impacted by the presence of DOACs and repeat testing at least 4 weeks later to confirm the deficiency.[28] Although complementary assays as PT, aPTT and PS and PC activity measurements are recommended to help for the interpretation of results, these tests can also be impacted by DOACs.[28]
- As previously reported, and according to recommendations for PS measurements, clot-based PS activity assays during treatment with DOACs should be avoided.[6,12] Currently, results in some literature suggest to test at C_{trough} although test interference is still possible.[1,3,4,9,10,19] Recommendations for primary screening assay for PS suggest the use of immunological assays which are not impacted by DOACs.[1,3,4,9,10,19,27]
- As recommended by current ISTH guidelines, clotting-based PC assay should be avoided in patients taking DOAC in favour of unimpacted chromogenic assays. Although PT, aPTT and PS and AT measurement are recommended to help for the interpretation of results, these tests could be biased by the presence of DOACs.[33,34] Results and literature about assessment of PC activity suggest that the use of immunological assays or chromogenic assays not involving FX or FII are not impacted by presence of DOACs.[1,4,6,7,9,12,19]
- Current recommendations and guidelines from ISTH regarding to APC-R consist to avoid APC-R assays in patients taking FXa or IIa inhibitors [38] unless the assays are known to be insensitive (eg prothrombinase-based assay Pefakit APC-R FV_{Leiden} with apixaban and rivaroxaban).[6,7,9,25,35] Current guidelines also recommend genetic analyses, which are easily automated, for FV_{Leiden} which are DOAC-free interference.[38] Although mutations other than FV_{Leiden} would not be detected, these are quite rare (FVR2 haplotype, $FV_{Liverpool}$ $FV_{Cambridge}$ and $FV_{Hong\text{-}Kong}$) and associated with a lower thrombotic risk than FV_{Leiden}. Nevertheless, this method is cost-effective and remains time-consuming in some labs.[21,25,38]
- LAC detection for determination of APS during DOACs treatment remains a challenge. Currently, as confirmed by guidelines from the Clinical and Laboratory Standards Institute (CLSI) of 2014, LAC testing is not recommended in patients on DOACs since ratio between screen and confirm may be increased, and in most cases, leading to false-positive result interpretations.[1,4,9,10,12,19,30,47,49,50,52] The newly available guidelines from ISTH on LAC detection support our previous consideration about the use of antidotes (idarucizumab and andexanet alfa) or neutralizers (activated charcoal) to quench in vitro the activity of anticoagulants.[60] They also report the promising use of a combination of ecarin clotting time (ECT) and Taipan snake venom time (TSVT). Both venoms directly activate prothrombin and converts it to thrombin. Taipan is PL and calcium-dependent while Ecarin is not. These properties could allow the assessment of LAC with a screen/confirm concept and may overcome the presence of anti-Xa (rivaroxaban, apixaban or edoxaban).[1,23,47] Then, TSVT and ECT could be considered, as already recommended by Van Os et al, Arachchillage et al and Douxfils et al.[4,7,9,23,47] However, these assays are not available in clinical practice and still required international standardization, implementation and clinical validation.[4,8-10,23,43,46,47,51,56]

Finally, we would recommend to laboratories to provide a comprehensive thrombophilia tests and interpretations panel along with known or suspected drugs interferences, not isolated to DOACs. This panel of tests and used reagents could be accompanied by practical recommendations for either (a) alternative methods and/or strategies to consider if the test result induces a modification in patient management or (b) repeat testing once patient is off therapy or iii. other considerations. Moreover, each laboratory should have its own written policy in place for addressing samples obtained from patients with known DOAC exposure and thrombophilia testing. That allows for both clinician education and the ability of laboratories to cancel inappropriate testing in the absence of alternative strategies or neutralizing methods.

5 | CONCLUSIONS

In conclusion, coagulation tests are in most cases deeply impaired by DOACs making results unreliable. Overall, chronometric and chromogenic assays requiring FX and/or FIIa are impacted by DOACs. This impact is concentration and reagents dependent requiring each manufacturer to report the sensitivity and impact of their reagents to each DOACs. The use of devices or chemical compounds able to remove or antagonize the effect of DOACs or the development of new diagnostic tests insensitive to DOACs should be considered. These represent the easiest and cheapest options to avoid interferences and further minimize the risk of false results without discontinuation of anticoagulation.

CONFLICT OF INTEREST

Among the authors, J. Douxfils is CEO and founder of QUALIblood s.a., a contract research organization manufacturing the DP-Filter, is co-inventor of the DP-Filter (patent application number: PCT/ET2019/052903) and reports personal fees from Stago, Roche,

Roche Diagnostics and Daiichi-Sankyo, outside the submitted work. J-M. Dogné is co-inventor of the DP-Filter (patent application number: PCT/ET2019/052903). RC. Gosselin is part of advisory board for Biomarin. He is expert testimony for dabigatran and rivaroxaban testing and reports personal fees from Machaon Diagnostics Laboratory. He is also speaker honoraria for Diagnostica Stago and Siemens Healthcare Diagnostics and consultant for Grifols Diagnostic and uniQure. F. Mullier reports institutional fees from Stago, Werfen, Nodia, Sysmex and Bayer. He also reports speaker fees from Boehringer Ingelheim, Bayer Healthcare, Bristol-Myers Squibb-Pfizer, Werfen, Aspen, Sysmex and Stago, all outside the submitted work. The other authors have no conflicts of interest to disclose.

AUTHOR'S CONTRIBUTIONS

Romain Siriez wrote the first draft of the manuscript with the support of Jonathan Douxfils and Jean-Michel Dogné. Jonathan Douxfils supervised the project. Authors provided critical feedback and helped shape the research, analysis and manuscript. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

ORCID

Romain Siriez https://orcid.org/0000-0002-5003-6939
Robert Gosselin https://orcid.org/0000-0002-5669-8722
Jonathan Douxfils https://orcid.org/0000-0002-7644-5298

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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