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Published in:
International Journal of Laboratory Hematology

DOI:
10.1111/ijlh.12992

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

Received: 16 December 2018 | Revised: 18 January 2019 | Accepted: 25 January 2019

DOI: 10.1111/ijlh.12992

REVIEW ARTICLE

WILEY ISLH International Journal of Laboratory Hematology

An update on laboratory assessment for direct oral anticoagulants (DOACs)

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Abstract

The first direct oral anticoagulant (DOAC) to be approved for clinical use was dabigatran, a direct thrombin inhibitor, in 2010. Since that time, four additional DOACs, all direct anti-Xa inhibitors, have been approved, including rivaroxaban, apixaban, edoxaban and betrixaban. Our knowledge about the effect of DOACs on laboratory testing, as well as the use of the laboratory for measuring DOACs has been an evolving process. These drugs are not routinely monitored in the same fashion as coumadin, but there is an increasing demand on the laboratory to have the capacity to adequately assess DOAC anticoagulant effect (pharmacodynamics) or levels (pharmacokinetics) in either emergent or the routine situations. This manuscript provides an update on laboratory guidance and progress of methods for measuring DOACs.

KEYWORDS
direct oral anticoagulants, laboratory guidance, laboratory practice, laboratory testing

1 | INTRODUCTION

The direct oral anticoagulants (DOACs) are a relatively new class of drugs prescribed in patients with or at risk for venous thromboembolism (VTE) or atrial fibrillation. Historically, anticoagulation of VTE patients was based on heparin, heparin derivatives, or oral vitamin K antagonists. These historic anticoagulant choices are associated with limitations or inconveniences such as the need for infusion (unfractionated heparin, UFH) or subcutaneous injection (low molecular weight heparin, LMWH), dietary influences (warfarin), and frequent or episodic monitoring for potential dose adjustment. These nuisances are somewhat mitigated with DOACs, as these orally administered drugs require no routine or episodic monitoring.[1-8] Recent clinical publications, however, would suggest that laboratory drug measurements may be warranted in certain populations, such as in the elderly, those at extremes of body weight, those requiring drugs that impact certain metabolic pathways, and those with renal impairment or poor responders.[1-9] Moreover, DOAC-treated patients that require acute intervention or in emergent situations, such as bleeding, acute stroke, trauma, surgery, may require assessment of their coagulation status to assure appropriate management.[10-13] Moreover, shortly after their approval status in the United States and Europe (dabigatran etexilate in 2010), it became readily apparent that the effect of these drugs on laboratory testing, specifically coagulation assays (eg, prothrombin time and other assays), was widely variable depending on drug type, reagent used, and test applied.

As the prescribing of DOACs has increased, so has our knowledge of this class of anticoagulants on coagulation assays. The initial published data, usually made available by pharmaceutical scientists, offered somewhat limited information to clinical laboratories as either the published testing methods used were not widely available (eg, ecarin clotting time) or were modified assays (eg, raw or drug-calibrated anti-Xa) or a single reagent was used for analysis (eg, Neoplastine prothrombin reagent from Diagnostica Stago). In addition, early knowledge about DOACs and their impact on coagulation assays was often misconstrued and thus guidance documents for both clinicians and laboratory professionals generated have since been either refuted or refined. The purpose of this document is to align our current knowledge on the assessment and impact of DOAC anticoagulation in the laboratory. Whether DOAC patients should be monitored or episodically measured is beyond the scope of this document and will not be addressed.

TABLE 1 DOAC characteristics[1-8,14,16,17]

	Dabigatran	Apixaban	Betrixaban	Edoxaban	Rivaroxaban
Manufacturer	Boehringer Ingelheim	Bristol-Myers-Squibb/Pfizer	Portola Pharmaceuticals	Daiichi Sankyo	Janssen Pharmaceuticals/ Bayer Healthcare AG
Trade name	Pradaxa	Eliquis	Bevyxxa	Savaysa Lixiana	Xarelto
Target	Bound and free thrombin	Bound and free factor Xa	Bound and free factor Xa	Bound and free factor Xa	Bound and free factor Xa
Bioavailability	3%-7%	~50%	~35%	~60%	~80%-100%
Clearance	~80% renal	~55% fecal	~85% fecal	~50% renal	~70% renal
Protein binding	~35%	~90%	~60%	~55%	~95%
Tmax	1.5-3 h	3-4 h	~3-4 h	1-2 h	2-3 h
Half-life[a]	~13 h	~12 h	~19-27 h	~12 h	~ 5-13 h
$C_{through}$[b,c]	60 (35-95) ng/mL (mean; 25-75th percentile)	63 (22-177) ng/mL (median; 5-95th percentile)	12 ng/mL (mean)	19 (10-39) ng/mL (median; IQR)	32 (6-239) ng/mL (mean; 10th-90th percentile)

[a]Half-life is dependent on renal function, with increased half-live associated with renal impairment
[b]Values listed for doses associated with prevention and recurrence of deep vein thrombosis and pulmonary embolism.
[c]For betrixaban, values are based on 80 mg once-daily dose for thromboprophylaxis in adult hospitalized patients.

1.1 | Direct oral anticoagulants—the currently approved drugs

Direct oral anticoagulants are approved (albeit may be regionally limited) for stroke prevention in nonvalvular atrial fibrillation (NVAF), treatment of VTE, secondary prevention of VTE, and/or thromboprophylaxis after knee/hip surgery.[1-14] (Table 1) Dose may differ for each DOAC and indications, and readers are encouraged to seek other sources for peak and tough levels for all DOAC indications.

2 | DOAC LABORATORY INTERFERENCE

Direct oral anticoagulants function as anticoagulants by inhibiting activated serine proteases, specifically thrombin and activated factor X (FXa), and for this reason, these drugs affect commonly used global coagulation assays as well as select special coagulation assays.[15-18] Different aPTT and PT reagents show varying responsiveness to each of these agents.[19] The sensitivity toward anti-IIa or anti-Xa agents is activator, phospholipids, and buffer dependent in such a manner that the conditions in which the enzymatic assays are performed interfere with the potency of inhibition of the DOAC.[20] In general, direct thrombin inhibitors (dTI) tend to prolong the APTT more than the PT while direct FXa inhibitors (DXa) drugs prolong the PT to a greater extent than the aPTT. Likewise, one-stage factor activity and inhibitor (ie, Bethesda) assays that are dependent on either the aPTT or PT are affected by DOAC presence and the degree of interference depends on reagent responsiveness, specific DOAC present, and DOAC concentration.[15-17,21-23] DOAC-affected one-stage factor activity results spuriously underestimate factor activity which may or may not demonstrate nonparallelism. Furthermore, false-positive inhibitor titers based on one-stage aPTT or PT factor assays may be reported. Chromogenic factor activity assays based on FXa generation (ie, factor VIII, IX, and X assays) may be underestimated in the presence of direct Xa inhibitors, but direct thrombin inhibitors have no effect on these assays. Chromogenic FXIII activity will be underestimated in the presence of dabigatran.

Thrombin time assays are exquisitely sensitive to the presence of dabigatran such that prolongation may be evident even at trough levels while DXa agents have no effect on the thrombin time.[21] The effect of dabigatran on functional fibrinogen measurements is very method dependent although most fibrinogen assays are not affected by any of the DOACS.[24]

Thrombophilia testing may be significantly affected by even small amounts of DOACS, the effect of which are drug, concentration, test methodologic, and reagent dependent. (Table 2) Tests used to screen and confirm the presence of a lupus anticoagulant that are based on the aPTT or Russell viper venom (RVV) based may be variably affected by the presence of any DOAC, leading to falsely elevated or positive results. aPTT-based methods are generally affected more by dabigatran than dXa agents while both classes of DOACS impact RVV-based assays.

Chromogenic antithrombin activity assays that are based on FXa generation are overestimated in the presence of FXa inhibitor drugs and assays based on FIIa generation overestimated by dabigatran. Clot-based protein C and S assays are overestimated by all DOACS. In DOAC-treated patients, chromogenic protein C and free protein S antigen assays are recommended due to their lack of DOAC interference. Assays for activated protein C resistance are typically overestimated in the presence of any DOAC. With certain methodologies, such as a prothrombinase-based activated protein C resistance (APCr) assay, therapeutic levels of dabigatran can elevate results to the extent that an abnormal APCr result is falsely extended into the normal range.[25,26] The main advantage of noscarin-based or APTT-based APC resistance assay is that these methods are not influenced

TABLE 2 Effect of DOAC on thrombophilia testing[11,15-21,23-25]

	Test Bias	Dabigatran (ng/mL)	Apixaban (ng/mL)	Betrixaban (ng/mL)	Edoxaban (ng/mL)	Rivaroxaban (ng/mL)
Antithrombin-IIa method[a]	False ↑	~25	No effect	No effect	No effect	No effect
Antithrombin-Xa method[a]	False ↑	No effect	~40-110	No effect	~120-270	~100-130
Protein C-clot based[a]	False ↑	~25	>750	Unknown	~280	~22
Protein C—chromogenic	–	No effect	No effect	No effect	No effect	No effect
Protein S-activity[a]	False ↑	~25	~470	~30	~270	~220
Protein S—antigen	–	No effect	No effect	No effect	No effect	No effect
LA screen[a]	↑	~25	~80-110	~10	~20-35	~10-30
LA confirm[a]	↑	~25	~80	~10	~20	~20-30
LA ratio[a,b]	False ↑	~25[a,b]	~200->750[a,b]	~50[a,b]	~30->500[a,b]	~10->720[a,b]
APCr[a,b,c]	Biased Ratios[c]	~25-200[a,b]	>740[a,b]	Unknown	>300[a,b]	>300[a,b]

This table reflects minimum DOAC concentration that affected thrombophilia testing, from cited studies. These DOAC concentrations may not be reflective of all reagents or methods.

[a]Reagent and method dependent APCR.

[b]Method dependent, including no DOAC effect on test result.

[c]APCr ratio bias can be either falsely low or falsely high with some methods, resulting in mis-identifying of V Leiden mutation or missing V Leiden mutation respectively.

APCR: activated protein C resistance ratio.

by dXa agents. RVV-based APCr assays are influenced by both dTI and dXa agents.

With the exception of apixaban, even DOAC trough levels may have significant impact on thrombophilia testing. Therefore, to obtain accurate results, whenever possible, hemostasis testing should be performed 4-5 days after DOAC treatment is discontinued, but this is not a recommended clinical point of view. Switching from a DOAC to low molecular weight heparin for a transient period of time may be an alternative when testing must be completed. If optimum patient care requires testing is performed while patients are on DOAC therapy, results must be interpreted with caution. However, possibilities to avoid this interference are now possible with the coming of adsorbent agents able to remove DOAC from the plasma sample (see game changers section).

3 | LABORATORY GUIDANCE—MYTHS AND FACTS

Tripodi, in a 2016 publication, described various pros and cons as to whether or not to measure DOACs.[12] This was followed by another article Tripodi published in April 2018 which essentially disputed previously published notions as to why laboratories do not perform DOAC testing such as: test availability, rapid result turn-around-time (TAT), difficulty in test performance and interpretation, result variability, lack of cutoff values, poor definition of tests, and finally the fact that conventional tests (eg, PT and APTT) have been recommended to be an acceptable means for assessing DOACs.[12] Not related to the Tripodi paper are additional confounders (in the United States) which include a lack of FDA approved methods for quantifying DOACs, and a general reluctance to provide tests that may be infrequently used.

Three articles published in 2018 provided guidance directed to either laboratories or clinicians regarding laboratory measurement of DOACs.[11,16,27] On behalf of the International Council for Standardization in Haematology (ICSH), a document was published in 2018 to provide laboratory guidance for assessing DOACs.[16] Prior to manuscript publication, this document was shared with pharmaceutical and in vitro diagnostic companies for their comments and input. This open-access document provides recommendations addressing the preanalytical (and general patient and laboratory recommendations), analytical and postanalytical phases of coagulation testing in patients on DOACs. (Table 3) The anti-FXa DOAC betrixaban was approved just prior to manuscript submission. Subsequent publication about the effect of betrixaban on coagulation testing was published in 2018.[23] As expected, and consistent with other DOACs, there is a wide degree of variability on betrixaban concentration and global assay responsiveness.

The ICSH recommendations for the preanalytical phase include nonemergent trough blood collection if routine DOAC assessment is desired. The optimal sample for DOAC measurement is collected in 3.2% sodium citrate (alternative material acceptable for tandem mass spectrometry), with the resultant whole blood or plasma

stability dependent on the DOAC, test, and storage container and temperature. The analytical recommendations include the limited use of screening assays such as the prothrombin time (PT) and activated partial thromboplastin time (APTT), while recognizing the use for other assays such as the thrombin time and heparin-related anti-Xa assays that may provide some assessment to the presence of DOACs with the DOAC result to be reported in ng/mL. Other analytical recommendations include assigning tandem mass spectrometry as the gold standard for DOAC quantitation, reporting of active DOAC metabolites, and the use of drug-calibrated tests (eg, ecarin clotting time, ecarin chromogenic assay, dilute thrombin time, DOAC calibrated anti-FXa) are suitable as alternative, rapid methods to tandem mass spectrometry measurements. The guidance document recommends the steps required for method validation or verification of performance depending on whether the assay is an LDT or approved commercial product, respectively. Lastly, the ICSH committee recommended that external quality assurance (EQA) have two dispatches per year, with at least two samples per dispatch.

While the ICSH document provides guidance for the laboratory, there are some limitations, as the changes in the DOAC arena have frequently and rapidly, including laboratory methods for DOAC measurements, including point-of-care methods, novel methods, or modified global assays. The ICSH DOAC committee plans these issues in a short communication in 2019.

Douxfils and colleagues published their recommendations on DOAC measurements directed for clinicians.[11] However, there are salient points that clearly are targeted for the clinical laboratory. Included are the capacity for DOAC testing (coagulation analyzers ability to perform a particular test or method), and the requirement for interpreting the results correctly. Previously, we noted that as the expertise lies within the laboratory for laboratory testing, so should the interpretation of laboratory results in patients receiving DOAC therapy.[15,18]

Tripodi and colleagues published recommendations from various Italian clinical and laboratory societies.[27] The key features of these recommendations are (a) "Undetectable anti-FXa activity probably excludes clinically relevant drug concentrations," (b) "dedicated tests for DOAC should be urgently set up in all clinical laboratories and made available to clinicians," and (c) "…it is useful to measure DOAC not only before, but also after administration of the antidotes."[27]

Reversal strategies that are specific (Idarucizumab, Praxbind, Boehringer Ingelheim for dabigatran and andexanet alpha, AndexXa Portola for rivaroxaban and apixaban) or nonspecific (three or four factor, activated or nonactivated prothrombin complex concentrates,

TABLE 3 ICSH Laboratory Measurements of DOAC recommendations[16]

	Recommendations
General patient	Trough drug level assessment for nonemergent situations, with results reported in ng/mL.
	Each reported DOAC has accompanying comment with expected trough levels based on published studies.
General laboratory	Internal quality control (IQC) performed at least once daily during testing performance.
Sample	3.2% sodium citrate preferred sample for coagulation studies, to be processed within 4 h of collection. Plasma or serum can be used for liquid chromatography-mass spectrometry/mass spectrometry.
	Plasma samples for dabigatran not tested within 24 h of collection, and samples for anti-Xa DOACs not tested within 8 h of collection should be frozen. Frozen plasma can undergo three freeze–thaw cycles.
	For thrombin time testing (dabigatran), plasma samples are stable for 4 h at room temperature.
Screening test	The PT and/or APTT not reliable measurements of DOAC, especially apixaban, and should not be used to quantify DOAC concentrations.
	In a patient with known DOAC exposure, a prolonged PT or APTT should be considered secondary to drug effect until proven otherwise.
	A normal TT excludes the presence of significant dabigatran concentration.
	TEG, ROTEM, and other POC not sufficient for assessing DOACs.
	Nonspecific POC methods may not have sufficient responsiveness to detect DOAC presence.
	Urine screening tests may provide a rapid qualitative and semiquantitative of recent DOAC exposure.
Tandem mass spectrometry	LC-MS/MS to be the gold standard test for measuring DOAC concentration, with active metabolites being reported.
Other quantitative method	Drug-calibrated DTT, ECA, ECT, and anti-FIIa chromogenic methods are suitable methods to provide quantitation of dabigatran.
	Drug-calibrated anti-FXa is suitable methods to provide quantitation of anti-Xa DOACs.
	Antithrombin supplement anti-FXa methods should not be used for DOAC assessment.
Method performance	Prior to test implementation and result reporting, DOAC method validation or verification of performance is required requiring a written protocol, to include precision, accuracy, linearity, and other parameters as necessary.
External quality assessment	Each laboratory must enroll in a DOAC EQA program, with at least two sample per dispatch, performed at least twice annually.

APCCs or PCCs, respectively) were not fully addressed, as there is limited to no published information on those reversal strategies on coagulation testing in the setting of specific or nonspecific DOAC reversal. Andexanet alpha, an anti-Xa reversal agent for DOAC (rivaroxaban and apixaban only in the US) does not require pre- or post-treatment assessment of anti-Xa activity as the FDA summary report[28] indicated "…mean % change in baseline levels did not correlate with hemostatic outcomes." and thus the FDA is stating that no anti-Xa monitoring is required. It is unclear whether the European Medicines Agency will also adopt such language. There is currently an open, phase 3B, clinical trial (NCT02329327) for andexanet alpha use in bleeding patients, with anticipated recruitment of ~300 patients.[29] The primary endpoint is no longer a laboratory measurement as noted in ANNEXA-A, ANNEXA-R, and ANNEXA-4 studies, but rather a primary outcome demonstrating excellent to good hemostasis at 12 hours postadministration (previous outcome measure was at 24 hours but was modified in 2018). Perhaps surrogate laboratory measurements will be additionally provided, but not part of the clinical trial outcome. Of note, possible interaction of andexanet alpha with TFPI has been reported which may tilt the balance to a prothrombotic state.

4 | LABORATORY GUIDANCE—THE NEEDS

Laboratories may also require additional guidance on procedures that should be followed in situations of bridging or overlapping therapy. For example, if a dabigatran-treated patient requires heparin anticoagulation (eg, with hospital admission), what strategies can be used to monitor UFH anticoagulation? Since both dabigatran and UFH influence the APTT, patient safety during this bridging period requires UFH monitoring other than the traditional APTT. The need for alternative testing is also true for the anti-FXa DOACs when anti-FXa methods are the primary UFH monitoring tool. For those laboratories that may consider implementing a DOAC measuring method, the ICSH guidance document provides little detailed information about the specifics of testing. As such, an alternative source that details cookbook-type instructions for rapid measurements (eg, ecarin clotting time, and anti-FXa chromogenic measurements) has been published, although not in an open-access format.[30]

5 | LABORATORY DOAC TESTING: THE GAME CHANGERS?

There are several developments in the area DOAC testing that provide better options for assessing DOACs in acute and nonacute settings. This includes modifications of existing platforms, creation of new testing platforms, and alternative collection methods that will be discussed below.

DOAC Dipstick (DOASENSE GmbH Heidelberg, Germany): A urine dipstick method that can screen and segregate dabigatran from anti-Xa DOACs.[31] The dipstick also features a creatinine pad to assess renal function. Limitations include unreadable pads due to urine color, the lack of correlation with plasma DOAC concentrations, and delay between drug ingestion and urine detection (1 hour longer to detect in urine than in blood). However, the proposed use of these urine dipsticks is in acute settings, such as a bleeding patient in the emergency department or patient requiring emergent intervention (eg, surgery or thrombolysis) with either known or unknown DOAC medication history. As urine is readily available, and the read time for this point-of-care method mere seconds, this may provide rapid assessment of a DOAC-treated patient. A postmarket clinical trial[32] is currently underway to compare DOAC Dipstick qualitative measurements with tandem mass spectrometry DOAC measurements in urine.

Dried blood spot collection for DOAC testing: This method allows for at-home blood collection (by fingerstick) that can be scheduled at the patient's convenience while still maintaining timed collection integrity (recommended trough timed collections).[33] The dried blood spot sample then can be easily mailed to designated laboratory for tandem mass spectrometry measurement. The use of dried blood spot collections has been commonly used for decades as a means of screening for metabolism disorders in neonates.[34]

TEG 6S (Haemonetics Corporation, Braintree, MA): The newer version of the thromboelastogram (TEG), the TEG 6S uses resonance-frequency microfluidic technology in lieu of the viscoelastic properties of clotting blood using cups and pins.[35] The single use, four-channel cartridges currently contain kaolin (channel 1), heparinase (channel 2), tissue factor (channel 3), and abciximab (channel 4). The NOAC assay is a cartridge in development replaces heparinase in channel 2 with ecarin and replaces tissue factor in channel 3 with factor Xa. The use of ecarin and factor Xa will allow specificity when assessing dabigatran and antifactor Xa drugs, respectively. In the study evaluating patients on dabigatran, rivaroxaban, and apixaban, the ROC analysis yielded a sensitivity of 92% and 94% for anti-Xa DOAC and dabigatran, respectively.[35] The TEG 6S provided germane results (clotting time, R) within 5 minutes of test initiation.

DOAC-Stop: (Haematex Research, Hornsby NSW, Australia)[36-39] The impact of direct oral anticoagulants (DOACs) on laboratory assays used for thrombophilia testing (eg, antithrombin, protein S, protein C, lupus anticoagulant, and activated protein C resistance) is a well-known issue and may cause false-positive and false-negative results. Therefore, the correct interpretation of tests that are performed in patients taking DOACs is mandatory to prevent misclassification and the subsequent clinical consequences. DOAC-Stop is the first device able to remove DOACs from a plasma sample. There is now another device on the market that looks similar (eg, DOAC-remove which is also an absorbent tablet to put into the plasma)[39] or other that are not yet marketed but look more convenient for daily use (eg, the Hemofilter, which is a filter able to provide a plasma free of platelet and DOACs).[40] The adsorbent procedure appeared to be an effective and simple way to overcome the interference of DOAC on coagulation tests and should facilitate the interpretation of thrombophilia screening tests in patients taking DOACs. Filters appear more convenient for the daily use than the tablets.

ST Genesia (Diagnostica Stago, Asnières sur Seine Cedex, France) is an automated analyzer for thrombin generation testing and may be a "game changer" for the assessment of DOAC in the future.[41] Preliminary observations showed that thrombin generation testing is affected by all anticoagulant drugs and therefore it could be the candidate assay. The test has been found to be sensitive to all kind of anticoagulants and may best represent interindividual response more so than exploring merely plasma drug concentrations. In addition to providing the interindividual response to an antithrombotic drug, thrombin generation testing is also able to explore in more detail the impact of anticoagulants on the coagulation process.

6 | CONCLUSION

Following the introduction of DOACs, there has been an evolution of published information regarding the need and methods to measure their concentration in the laboratory as well as their impact on coagulation assays including methods to block this interference. This document serves to align our current knowledge on the assessment and impact of DOAC anticoagulation in the laboratory.

CONFLICT OF INTEREST

RCG involved in expert testimony on dabigatran and rivaroxaban testing, received honoraria from Siemens Healthcare Diagnostics, is on advisory board for Roche Diagnostics, Boehringer Ingelheim and NovoNordisk, consultant for Grifols Diagnostic Solutions, and board member of International Council for Standardization in Haematology (ICSH). DMA consultant for rivaroxaban litigation and received honoraria from Bayer, Baxalta, and Siemens Healthcare Diagnostics. JD received honoraria or grants from Daiichi Sankyo, Diagnostica Stago, Roche Diagnostics, Bayer Pharmaceutical CEO of Qualiblood sa, and Bristol-Meyer-Squibb.

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How to cite this article: Gosselin RC, Adcock DM, Douxfils J. An update on laboratory assessment for direct oral anticoagulants (DOACs). *Int J Lab Hematol*. 2019;41(Suppl. 1):33-39. https://doi.org/10.1111/ijlh.12992