

Laboratory Testing in the Era of Direct or Non-Vitamin K Antagonist Oral Anticoagulants: A Practical Guide to Measuring Their Activity and Avoiding Diagnostic Errors

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Semin Thromb Hemost 2015;41:208–227.

Abstract

A new generation of antithrombotic agents has recently emerged. These provide direct inhibition of either thrombin (factor IIa [FIIa]) or FXa, and are increasingly replacing the classical anticoagulants (heparin and coumarins such as warfarin) in clinical practice for a variety of conditions. These agents have been designated several acronyms, including NOACs, DOACs, and TSOACs, respectively, referring to new (novel; non-vitamin K antagonist) oral anticoagulants, direct oral anticoagulants, and target-specific oral anticoagulants, and currently include dabigatran (FIIa inhibitor), and rivaroxaban, apixaban, edoxaban, and betrixaban (FXa inhibitors). The pervading mantra that NOACs do not require laboratory monitoring is countered by ongoing recognition that laboratory testing for drug effects is needed in many situations. Moreover, since these agents “do not require” laboratory monitoring, some clinicians inappropriately take this to mean that they do not affect hemostasis tests. This review aims to briefly review the laboratory studies that have evaluated the NOACs against a wide range of laboratory assays to assess utility for qualitative or quantitative measurements of these drugs, as well as interferences that may cause misdiagnosis of hemostatic defects. Point of care testing, including use of alternate samples such as urine and serum, is also under development but is not covered extensively in this review. The main aims of this article are to provide practical guidance to general laboratory testing for NOACs, as well as to help avoid diagnostic errors associated with hemostasis testing performed on samples from treated patients, as these currently comprise major challenges to hemostasis laboratories in the era of the NOACs.

Keywords

- ▶ NOACs
- ▶ dabigatran
- ▶ rivaroxaban
- ▶ apixaban
- ▶ edoxaban
- ▶ measurement
- ▶ interference

Anticoagulant therapy is typically applied to patients suffering from a variety of conditions, as associated with several prothrombotic risk factors, and primarily to treat or prevent several thromboembolic disorders. These typically include atrial fibrillation, acute coronary syndrome, prevention of venous thromboembolism (VTE) in patients undergoing

major, orthopedic, or cancer surgery, therapy, and prevention of recurrence in patients with previous episodes of VTE.¹ For decades, anticoagulant therapy has been based on the administration of two different classes of drugs, the vitamin K antagonists (VKAs; typically warfarin and acenocoumarol) and heparinoids, including unfractionated heparin (UFH),

published online
February 19, 2015

Issue Theme Anticoagulant Therapy:
Present and Future; Guest Editor: Job
Harenberg, MD.

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Publishers, Inc., 333 Seventh Avenue,
New York, NY 10001, USA.
Tel: +1(212) 584-4662.

DOI <http://dx.doi.org/10.1055/s-0035-1546827>.
ISSN 0094-6176.

low-molecular-weight heparin (LMWH), as well as other heparin-like molecules such as fondaparinux. Although these classes of drugs have been proven effective for both primary and secondary prevention of systemic thromboembolism, they carry several drawbacks and limitations.¹⁻³

Much of this background has been provided by us in a recent review,⁴ and hence will not be covered here again, except as overview. Thus, although VKAs are orally administered, they require careful laboratory monitoring by means of the international normalized ratio (INR) because of high interindividual variability in VKA sensitivity and because of considerable drug and food interactions. In contrast, the heparin drugs are administered parenterally (either intravenously or subcutaneously). Monitoring is typically undertaken for UFH (using an activated partial thromboplastin time [aPTT] or anti-Xa assay) but is not usually required for LMWH.

VKAs act by impairing the liver synthesis of vitamin K–dependent clotting factors (i.e., factors II [FII], VII, IX, and X), thus generating a profound anticoagulant state mirrored by substantial prolongations of both prothrombin time (PT) and aPTT. In contrast, heparins act through selective inhibition of activated coagulation FIIa and FXa, with a more prominent effect on FXa for LMWH and a more prominent effect on FIIa for UFH.

Both classes of drugs are effective clinical anticoagulants.¹ Nevertheless, many drawbacks of both VKAs and LMWH make these drugs a suboptimal means of patient anticoagulation, and this has paved the way to development and commercialization of a new generation of antithrombotic agents. These were originally termed new (or novel) oral anticoagulants (NOACs), and later (as they were no longer new or novel) as direct oral anticoagulants (DOACs), although the terms target-specific oral anticoagulants (TSOACs), direct-specific oral anticoagulants (DSOACs), and non-VKA oral anticoagulants (NOACs) have also been proposed.^{4,5} In keeping with other articles in this issue of the journal, we will adopt the abbreviation NOACs in the current review.

The NOACs have been specifically designed to provide direct inhibition of either FIIa or FXa, and currently include dabigatran (as the only available direct FIIa inhibitor), or rivaroxaban, apixaban, edoxaban, and betrixaban (as direct FXa inhibitors).⁴⁻⁷ These newer agents present several advantages compared with both VKAs and LMWH. First, like VKAs they are oral drugs, and thus avoid the need for injections as required for heparin therapy. Second, they are administered at fixed doses, are reportedly characterized by a more homogenous pharmacokinetic profile to generate a more predictable anticoagulant effect than either VKAs or heparins, thus theoretically requiring no, or perhaps more realistically less stringent, therapeutic measurements.^{4,7-9} The half-life of NOACs is also substantially lower than that of VKAs (i.e., 5–18 vs. 24–48 hours), and this has the theoretical advantage that urgent reversal of the anticoagulant effect may be achieved by simple withdrawal of the drug. On the contrary, there are no currently available antidotes, clinically proven to achieve urgent reversal in the case of overdose or bleeding.

The efficacy and safety profile of the NOACs has also been proven by several studies, indeed concluding that NOACs

display a more favorable risk-benefit profile than either VKAs or LMWH. The similar to superior efficacy in reducing the risk of thrombosis and systemic thromboembolism is coincidentally associated with a much lower likelihood of major bleeding, especially cerebral bleeding, making these agents favored by clinicians and patients alike.^{6,7,10-12}

Nevertheless, the pervading “mantra” that NOACs are also more practical than VKAs or LMWH because they do not require laboratory monitoring is not entirely true, and there are several situations in which laboratory assessment may be useful.^{4,7,9} Although these agents are reported to display predictable pharmacokinetic profiles, their metabolism is complex, and entails enteric adsorption and liver or renal clearance. Thus, there are some drug interactions, as well as differential liver and renal influences,¹³ which may expose patients to a significant risk of over- or undercoagulation. Indeed, a wide range of plasma concentrations can actually be evidenced in population studies, ranging from below 20 to as high as 400 or more ng/mL in different patients at different times postdosage (i.e., trough vs. peak), despite all time points reflecting “therapeutic levels.” Unexpectedly, high or low plasma concentrations may also be evident in subgroups of patients that may alternatively need “personalized” dose adjustments to otherwise prevent bleeds or thrombosis while on standard doses. Finally, unanticipated events such as urgent surgery (or other invasive procedure) and unexpected pregnancy may require an evaluation of the status of a particular patient’s drug level. These considerations define indications for laboratory testing to better define actual levels and/or drug anticoagulant effects. Additional “indications” for laboratory testing include onset of unexpected thrombotic or hemorrhagic events in patients on therapy, patients with extreme of body weight, abrupt impairment of renal and kidney function, suspicion of overdosage, or intoxication, along with assessment of real compliance with therapy.^{4,7,9}

Scope of Current Review

The main aims of this article are (1) to briefly review the laboratory studies that have evaluated the NOACs against a wide range of laboratory assays to assess utility for qualitative (“screening”) or quantitative measurements of these drugs along with test interferences that may cause misdiagnosis of hemostatic defects and (2) to provide practical guidance to laboratory testing for these agents, as well as to help avoid diagnostic errors associated with hemostasis testing performed on samples from treated patients. These concerns currently comprise major challenges to hemostasis laboratories in the era of the NOACs. Assessments related to point of care testing, and alternative sample types such as serum and urine, while mentioned, are largely covered elsewhere in this issue.⁹ Also, although an increasing number of articles are now reporting studies looking at reversal of these agents, we will only briefly overview this for completeness, as a thorough discussion of these studies would require a separate review. This review primarily covers the earlier NOACs, dabigatran, rivaroxaban, and apixaban, since there is substantial experience now with these agents, whereas there is insufficient

information with the newer agents, edoxaban and betrixaban, to provide meaningful guidance. Edoxaban has only been recently cleared by the Food and Drug Administration, and approval by the European Medicines Agency is under current consideration. Betrixaban is still under clinical evaluation.

Laboratory Testing for the NOACs

The “therapeutic-based” testing of drugs including NOACs is classically based on direct assessment of their concentration in serum or plasma using liquid chromatography tandem mass spectrometry (LC-MS/MS) (or analogous) techniques, which are otherwise conventionally regarded as reference methods for establishing the actual concentration of these and other drugs.¹⁴ However, these methods, requiring sophisticated and expensive instrumentation, along with skilled personnel, are unavailable to most routine clinical laboratories, and do not accurately estimate the anticoagulant activity attributable to these drugs (including their metabolites). Even if available, long turnaround times for test results would also make these methods unsuitable for urgent testing. Thus, more practical tests are needed for clinical laboratories required to perform both routine and urgent assessment of NOACs.

As part of our assessment, we performed a Medline search using the following search term: (apixaban OR dabigatran OR rivaroxaban OR edoxaban OR DOAC OR NOAC OR TSOAC) AND (laboratory OR monitoring OR measurement OR test). The rationale for this approach was to perform a “simple” search that any laboratory or clinical practitioner might perform to gain an appreciation of the currently available information. The search was performed without restriction on language, date, or article type, and covered the period up to the end of November 2014. The search retrieved 1,109 records, which

were then manually reviewed by title and abstract for relevance. The following articles were subsequently removed from further consideration: (1) articles that were review or opinion in nature and which did not substantially provide any new or novel data regarding laboratory testing; (2) articles that were restricted to assessments of LC-MS/MS or similar methodologies; (3) articles that could not be retrieved by us, or were in languages or in a form that we could not easily draw meaningful information; and (4) articles describing nonhuman-based studies (e.g., animal studies). Additional articles were added to our review, as either subsequently derived from an evaluation of the above studies (i.e., from the reference list), or else as otherwise known to us. The outcome of this process is summarized in ►Fig. 1. Most articles contained data for dabigatran ($n = 63$) and rivaroxaban ($n = 64$), although a significant number also contained information for apixaban ($n = 26$); very few evaluated edoxaban ($n = 5$). This likely reflected the timeline for development, clinical release, and uptake of these drugs, with the sequence being dabigatran and rivaroxaban, followed by apixaban and then edoxaban. An evaluation of the timeline for these publications would also confirm this conclusion (►Fig. 2), as well as suggesting more information is yet to come (given increasing yearly numbers of publication). Approximately equal number of publications reported on in vitro versus ex vivo data, and a proportion of the studies evaluated reversal of drug effects. The main articles used to draw conclusions in this review are listed in **Appendix A**.

Dabigatran

A total of 63 articles described some form of laboratory-based testing for dabigatran, with many studies including routine coagulation tests (PT and aPTT) and/or direct thrombin

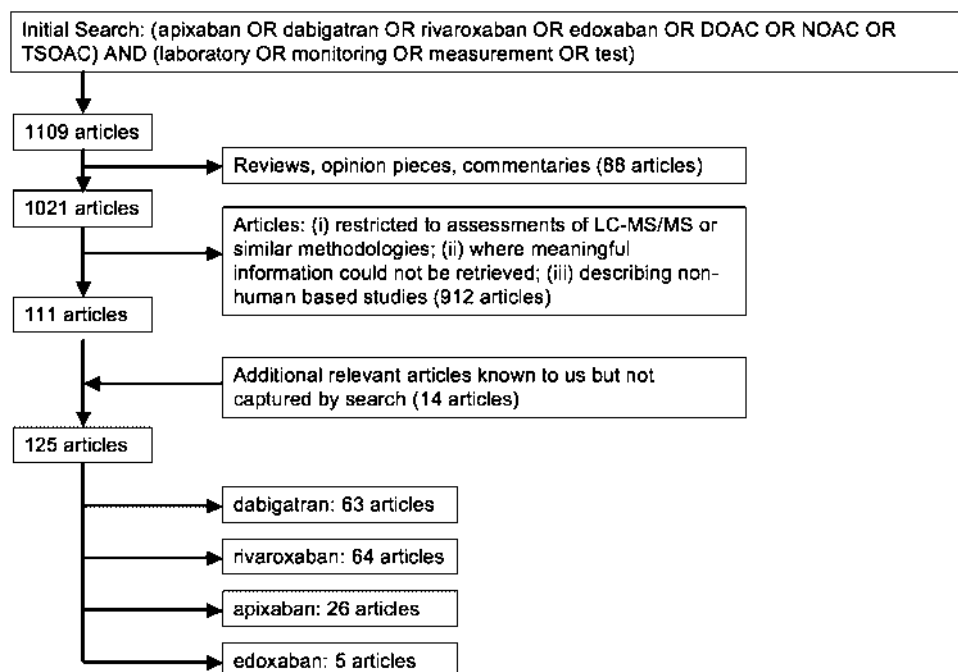


Fig. 1 A summary of the search strategy used to develop the major articles assessed to generate the current report. The final listing of the 125 articles is given in **Appendix A**.

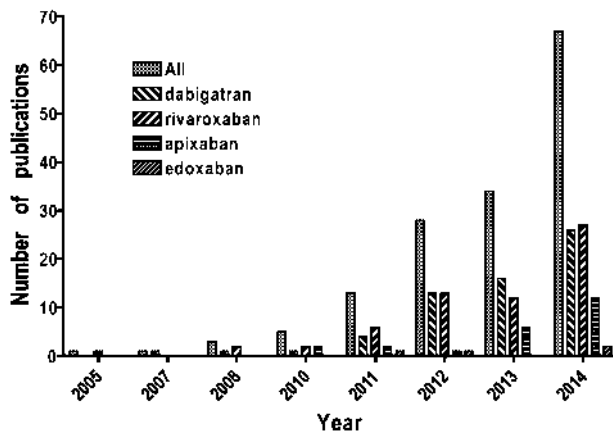


Fig. 2 Year of publication of the major articles is listed in **Appendix A**, as well as by NOAC evaluated.

inhibitor/dilute thrombin time (DTI/dTT) assays. Additional assays assessed included the “standard” TT, “dilute,” or otherwise “modified” PT (dPT/mPT), anti-Xa, ecarin clotting time (ECT) or ecarin chromogenic assays (ECA), factor assays, protein C (PC), protein S (PS), antithrombin (AT), activated PC resistance (APCR), dilute Russell viper venom time (dRVVT), activated clotting time (ACT), prothrombinase-induced clotting time (PICT), platelet function testing, von Willebrand factor (VWF) testing, thromboelastography/rotational thromboelastometry (TEG/ROTEM), and thrombin generation assays (TGA).

Significant prolongations of PT, aPTT, and TT were invariably observed in most instances of measurable dabigatran concentration, although TT was most sensitive, followed by aPTT and PT, which was least sensitive of the three routine assays.^{15–41} There was some reagent-based sensitivity for both PT and aPTT, and there was some variability with respect to results obtained at given dabigatran concentrations for ex vivo data. Thus, although PT and aPTT assays are useful for identifying potential presence of dabigatran drug effect, a normal result for either would not always exclude drug presence. In contrast, excluding occasional technical anomalies, a normal TT would exclude the presence of dabigatran, since this assay is very sensitive to its presence. This is summarized in **Table 1** and **Fig. 3**.

The Hemoclot thrombin inhibitor (HTI) assay (HYPHEN BioMed, Andresy, France), dTT methods, and ECT/ECA methods all showed good sensitivity and were generally reported as useful assays for measuring levels of dabigatran.^{15–17,19–21,24,26,27,29–31,34–37,41–47} The PICT assay was also reported as sensitive, although this assay may require some optimization.^{20,34,35}

Many hemostasis assays are also affected by dabigatran. All clot-based factor assays are affected by dabigatran, although aPTT-based assays (FVIII, FIX, FXI, and FXII) are most affected.^{22,41,48,49} The clot-based PC and PS assays may also be affected, but chromogenic PC and antigen-based (e.g., latex-based free) PS assays are not.^{22,41} AT assays are affected if they are anti-IIa based, but not if they are anti-Xa based.^{22,41} The APCR assays are affected, as are lupus anticoagulant (LA)

assays, and false identification of associated defects are possible.^{4,18,22,40,41,49} On the contrary, due to its sensitivity, the dRVVT may alternatively be used to screen for the presence of dabigatran. The TEG/ROTEM-based assays are also affected, as are TGA and ACT.^{17,19,21,24,26,30,31,36,50–53} The VWF and platelet function tests (platelet function analyzer [PFA], Multiplate, Roche, Basel, Switzerland and light transmission aggregometry [LTA]) are not affected, except for thrombin-mediated effects.^{22,31}

The overall conclusion from these studies in regard to assessing drug effects is that the aPTT can be used for urgent assessment of the presence of dabigatran, although a normal aPTT will not always exclude the presence of the drug. The standard TT is most useful to screen for absence of dabigatran (= normal TT) or potential presence of dabigatran (raised TT), but it is not otherwise useful for assessment of dabigatran level because it is too sensitive. The dRVVT, using a “confirm” LA reagent, also shows good sensitivity to dabigatran and has been proposed a useful screen by some workers, including us.⁴ HTI/dTT and ECT/ECA assays can be used for a more accurate estimation of the anticoagulant effect of the drug and to “quantify” drug level,^{15–17,19–21,24,26,27,29–31,34–37,41–47} although the former tests (HTI/dTT) appear overall better standardized for this purpose than the latter (ECT/ECA).

Rivaroxaban

A similar exploration has been undertaken for rivaroxaban with a similar number of publications ($n = 64$). In contrast to dabigatran, the PT is more generally sensitive to rivaroxaban than the aPTT, although differential reagent sensitivity is observed.^{4,19,24,25,54–69} All TT and ecarin-based assays (i.e., standard TT, dTT, HTI, ECT, and ECA) are insensitive to rivaroxaban.^{24,35,55,61,63} The PICT assay was also reported as sensitive, although this assay may require some major degree of optimization.^{35,56,61,70,71} In general, most publications recommended the use of an anti-Xa assay using a specific rivaroxaban standard, as these assays showed greatest utility.^{55–57,61,62,64,65,68,69,72–80}

Many hemostasis assays are also affected by rivaroxaban. All clot-based factor assays are affected by rivaroxaban, as are the clot-based PC and PS assays.^{55,64,72} Like dabigatran, chromogenic PC and antigen-based (e.g., latex-based free) PS assays are not.^{55,72} Unlike dabigatran, AT assays are affected if they are anti-Xa based, but not if they are anti-IIa based.^{55,60,72} The APCR assays are affected, as are LA assays, and false identification of associated defects is possible.^{4,40,49,60,63,73,74} Again, because the dRVVT is sensitive to rivaroxaban, it may be a useful screen for the presence of drug. TEG/ROTEM-based assays are also affected, as are TGA and ACT.^{19,24,30,31,53,56,57,61,65,68,81–85} VWF and platelet function tests (PFA, Multiplate, and LTA) are not affected.³¹ This is summarized in **Table 1** and **Fig. 3**.

The overall conclusion from these studies in regard to assessing drug effects is that the PT can be used for urgent assessment of the presence of rivaroxaban, although a normal PT will not always exclude its presence. Consensus has also been reached that PT results should be reported as “ratio,” since the use of INR may be misleading in this setting. The

Table 1 Summary of NOAC effects on hemostasis assays

Tests	Dabigatran	Rivaroxaban	Apixaban	Comments
(A) Routine assays (screening)				
PT	-/↑	↑/↑↑	-/↑	Different reagents show different sensitivities; in general, order of sensitivity = rivaroxaban, dabigatran, apixaban; only a few reagents sensitive to apixaban
APTT	↑/↑↑	-/↑	-/↑	Different reagents show different sensitivities; in general, order of sensitivity = dabigatran, rivaroxaban, apixaban
TT	↑↑↑	-	-	Very sensitive to dabigatran; insensitive to anti-Xa agents
Fibrinogen	-/↓	-/↓	-/↓	Most von Clauss methods insensitive; occasional von Clauss methods and PT-based methods will show some false loss
dRVVT	↑↑	↑↑	↑↑	Most dRVVT methods sensitive to all agents
(B) Quantifying assays (measuring)				
DTT/DTI	↑↑	-	-	Very sensitive to dabigatran; not affected by anti-Xa agents including rivaroxaban and apixaban
ECT/ECA	↑↑	-	-	Very sensitive to dabigatran; not affected by anti-Xa agents including rivaroxaban and apixaban
PICT	↑↑	↑↑	↑↑	Requires assay modifications to provide sensitivity to all agents; may require different setups for anti-IIa vs. anti-Xa drugs
Anti-Xa	-	↑↑	↑↑	Sensitive to anti-Xa agents (including rivaroxaban and apixaban); insensitive to dabigatran
(C) Drug interference				
PT factors	↓/↓↓	↓/↓↓	↓/↓↓	All PT factors affected, although most sensitive to dabigatran and rivaroxaban; can also yield impression of factor inhibitor
APTT factors	↓/↓/↓↓	↓/↓↓	↓/↓↓	All APTT factors affected, although most sensitive to dabigatran and then rivaroxaban; can also yield impression of factor inhibitor
Protein C	-/↑	-/↑	-/↑	Chromogenic tests unaffected; clot-based tests may be affected
Protein S	-/↑	-/↑	-/↑	Antigen-based tests (e.g., LIA) unaffected; clot-based tests affected
Antithrombin	-/↑	-/↑	-/↑	Anti-IIa-based methods affected by dabigatran; anti-Xa-based methods affected by other drugs
APCR	-/↑	-/↑	-/↑	APTT-based assays are mostly affected
LA	↑/↑↑	↑/↑↑	-/↑	LA tests are sometimes affected so that prolongation in screen assays exceed prolongation in confirm assays, leading to high screen/confirm assay ratios, and thus false determination of LA. This is especially true of dabigatran and rivaroxaban
VWF	-	-	-	
TEG	↓/↓↓	↓/↓↓	↓/↓↓	Various parameters affected
LTA	- ^a	-	-	
PFA	-	-	-	
TGA	↓/↓↓	↓/↓↓	↓/↓↓	Various parameters affected
(D) Other				
mPT/dPT	↑/↑↑	↑/↑↑	↑/↑↑	Various modifications of the PT may increase sensitivity to all drugs
ACT	↑/↑↑	-/↑	-/↑	

Abbreviations: ACT, activated clotting time; APCR, activated protein C resistance; APTT, activated partial thromboplastin assay; dPT, dilute prothrombin time; dRVVT, dilute Russell viper venom time; DTI, direct thrombin inhibitor (assay); DTT, dilute thrombin time; ECA, ecarin chromogenic assay; ECT, ecarin clotting time; LA, lupus anticoagulant; LTA, light transmission aggregometry; mPT, modified prothrombin time; PFA, platelet function analyzer; PICT, prothrombinase-induced clotting time; PT, prothrombin time; TEG, thromboelastography; TGA, thrombin generation assay; TT, thrombin time; VWF, von Willebrand factor.

^aDabigatran may affect thrombin-induced aggregation.

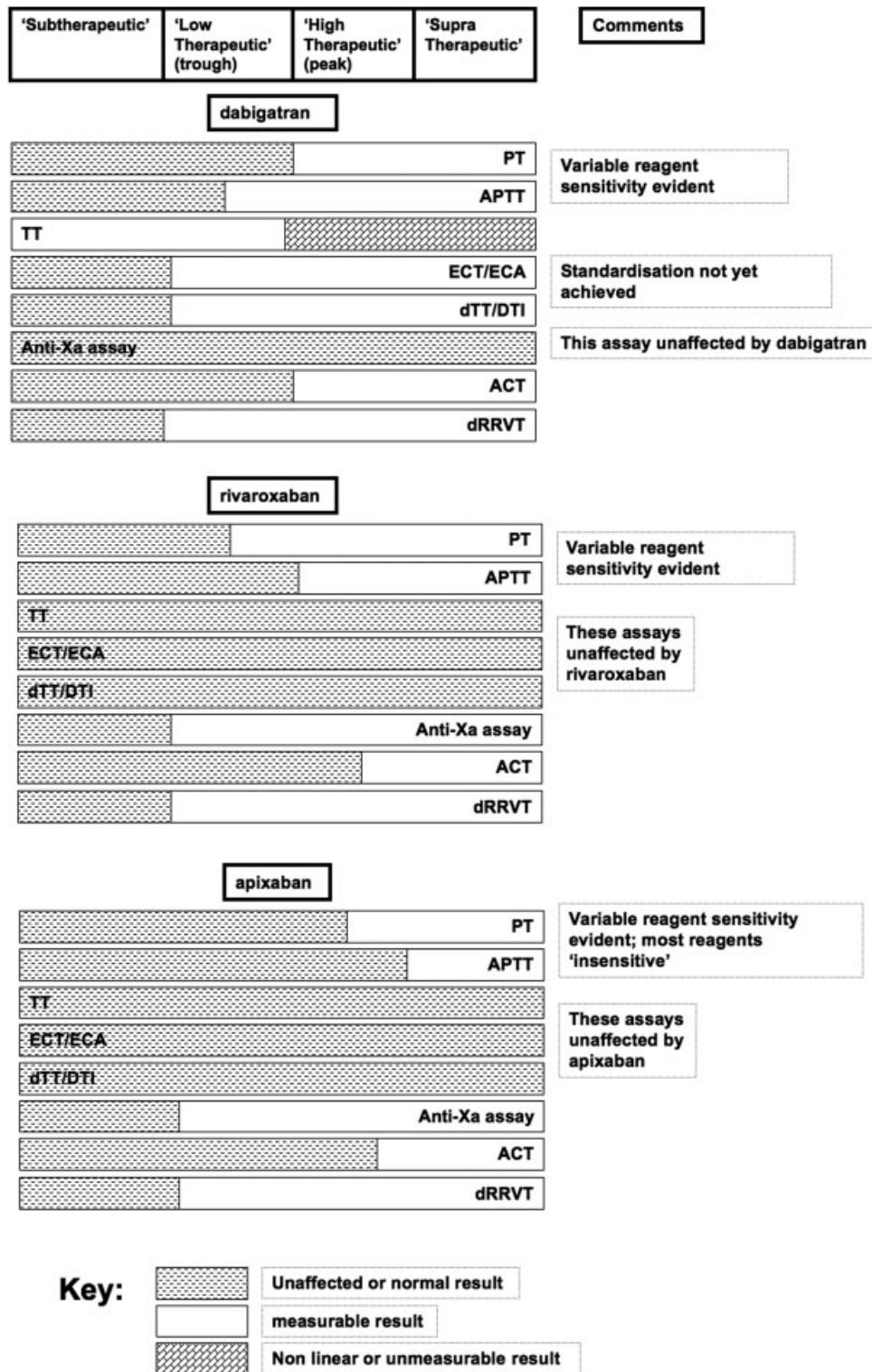


Fig. 3 Potential “clinical utility” of different laboratory tests to assess the main three NOACs (dabigatran, rivaroxaban, and apixaban) shown by relative sensitivity to each drug at “subtherapeutic,” “therapeutic,” and “supratherapeutic” levels. These concepts are a little abstract, as “therapeutic ranges” for these NOAC do not exist; however, based on samples taken from patients at “trough” versus “peak” times, postdosing existing data provide an approximate “therapeutic range” for each NOAC (see Lippi and Favaloro).⁴ Figure represents a modification and expansion of the concept from Hawes et al.²⁶ ACT, activated clotting time; APTT, activated partial thromboplastin time; dRVVT, dilute Russell viper venom time; DTI, direct thrombin inhibitor (assay); dTT, dilute thrombin time; ECT, ecarin clotting time; ECA, ecarin chromogenic assay; PT, prothrombin time; TT, thrombin time.

dRVVT, using a “confirm” LA reagent, has also been proposed as a useful screen by some workers, including us, because it also shows good sensitivity to rivaroxaban.⁴ However, an anti-Xa assay, using a specific rivaroxaban standard, is most useful to measure drug levels.

Apixaban

A similar but less extensive exploration has been undertaken for apixaban with a smaller number of publications ($n = 26$). Apixaban shows least sensitivity of the three main NOACs to routine tests, and most PT and aPTT reagents only show mild sensitivity.^{4,55,86–94} However, some variability is evident and some reagents appear to be sensitive, although most are not. Like rivaroxaban, all TT and ecarin-based assays are insensitive to apixaban, and although the PICT assay was also reported as sensitive, this assay may require some optimization.^{35,55,88,91} In general, most publications recommended the anti-Xa assay using a specific apixaban standard, as these assays showed greatest efficacy.^{55,86–92,95–98}

Many hemostasis assays are also affected by apixaban, although less so than by rivaroxaban. The clot-based factor assays are mildly affected by apixaban,^{55,88,91} so that levels often (but not always) remain in the normal range. Similarly, clot-based PC and PS assays may also be affected, but like the other NOACs, chromogenic PC and antigen-based (e.g., latex-based free) PS assays are not.^{55,88,91} Like rivaroxaban, AT assays are affected by apixaban if they are anti-Xa based, but not if they are anti-IIa based.^{55,88,91} The APCR and LA assays may also be affected by apixaban, and false identification of associated defects are possible, but less likely than for rivaroxaban.^{4,49,55,88,90,92} Again, because the dRVVT is sensitive to apixaban, it may be a useful screen for the presence of drug. The TEG/ROTEM-based assays are also affected by apixaban, as are TGA and ACT.^{32,55,81,88} The VWF and platelet function tests (PFA, Multiplate, and LTA) are not affected.³¹ This is summarized in ►Table 1 and ►Fig. 3.

The overall conclusion from these studies in regard to assessing drug effects is that the routine coagulation tests (both PT and aPTT) cannot be used for urgent assessment of the presence of apixaban, since normal results will not exclude its presence. However, a study from the Subcommittee on Control of Anticoagulation of the International Society of Thrombosis and Haemostasis has recently concluded that standardization of sensitive PT assays produced results that were comparable with most chromogenic anti-FXa assays. This represents a reasonable background for further studies aimed at establishing whether PT may also be used as a first-line test for screening apixaban concentration.⁹¹ The dRVVT, using a “confirm” LA reagent, has also been proposed as a useful screen by some workers, including us, because it also shows good sensitivity to apixaban.⁴ However, an anti-Xa assay, using a specific apixaban standard, is most useful to accurately measure drug levels.

Other NOACs

At variance with the three main NOACs, information on routine or urgent assessment of the other NOACs is scarce, although PT, aPTT, and anti-FXa activity have all been

reported to be prolonged by edoxaban.⁹⁹ However, data to date suggest that the anti-FXa assay may be most suited for its assessment, albeit potentially requiring modification to optimize the assay.

A Practical Approach to Laboratory Assessment of NOACs

Given the background data, and our own experience with these agents, our recommended approach is similar to that we recently published, and in line with most other major expert recommendations.⁴

As regard to dabigatran and other potential FIIa direct oral inhibitors, the aPTT is generally a reliable screening test in patients taking this drug, especially when rapid and reliable information about the potential risk of overcoagulation is needed. The standard TT is also useful for exclusion of dabigatran (normal TT). The dRVVT is also a useful screening test. However, for an accurate estimation of the anticoagulant effect, the HTI or dTT (or ECT/ECA where this assay is available and has been optimized) is recommended.

As regard to anti-FXa inhibitors, the PT would represent a reliable screening test in patients taking rivaroxaban, especially when rapid and reliable information about the potential risk of overcoagulation is needed, but with some notable exceptions related to reagent sensitivity. The PT is generally not recommended for screening of patients taking apixaban due to poorer sensitivity. However, sensitive PT reagents are available and can be used for this purpose.⁹¹ The dRVVT is a useful screening test for both rivaroxaban and apixaban. However, for a more accurate estimation of the anticoagulant effect of anti-FXa inhibitors, specific anti-FXa assays (using the specific drug as calibrator) are the preferred means.

Taken together, the available published evidence allows the development of tentative algorithms for urgent and routine measurement of NOACs (►Fig. 4). Before embarking on routine or specialized testing of the NOACs, all laboratories must assess, and if required optimize, all assays for such use (►Fig. 4A). All test results must also be considered in light of respective assay sensitivities and with respect to timing of last dose of NOAC (►Fig. 4A).

When urgent screening of NOACs are required and when the drug being assessed is known, a simplified testing approach is reasonable and may entail the use of aPTT for dabigatran (and other anti-FIIa inhibitors) and PT for rivaroxaban (►Fig. 4B), for screening purposes. Where available, the dRVVT may also be useful to screen for these NOACs. No conventional clotting test seems generally suitable for apixaban and edoxaban, although the dRVVT appears to be an acceptable option (at least for apixaban), and ongoing studies will confirm the reliability of using sensitive PTs for the screening of apixaban anticoagulant activity. As regard to the accurate estimation of the anticoagulant effect, this may be accomplished for dabigatran with DTI/HTI/dTT (or a suitable ECT/ECA) and for all anti-FXa drugs with anti-FXa assays, using specific calibrators, with some residual doubts about the effectiveness of these tests for measuring the anticoagulant effect of edoxaban.

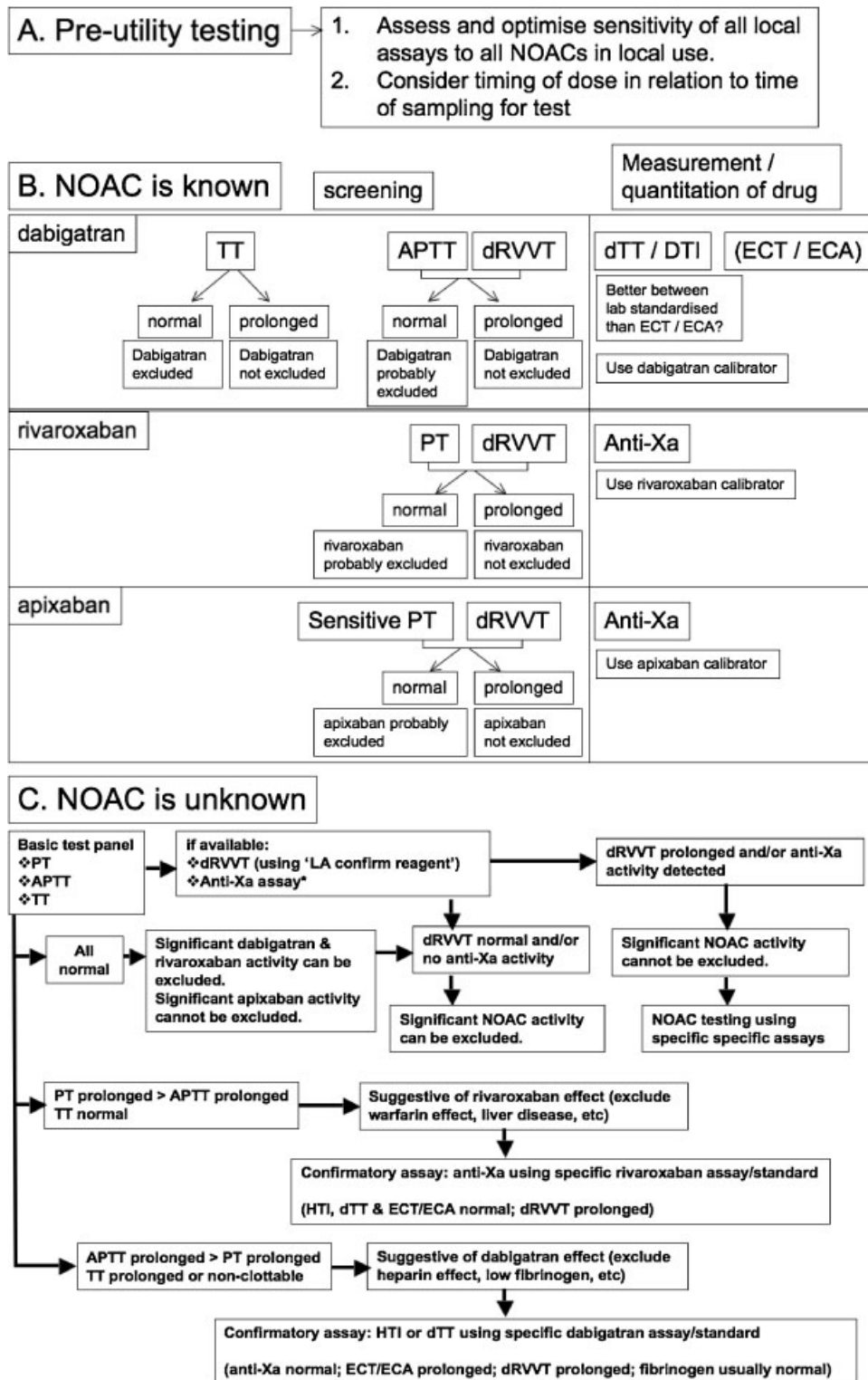


Fig. 4 Proposed approach/algorithms to hemostasis investigation of patients on NOACs. (A) Requirements before initiating clinical testing. (B) Suggested test approach for the urgent screening of dabigatran, rivaroxaban, and apixaban, when the drug being assessed is known. (C) Suggested test approach for the urgent screening of dabigatran, rivaroxaban, and apixaban, when the drug being assessed is not known. Refer to text for further explanation. Modified from Lippi and Favaloro.⁴ aPTT, activated partial thromboplastin time; dRVVT, dilute Russell viper venom time; DTI, direct thrombin inhibitor (assay); dTT, dilute thrombin time; ECT, ecarin clotting time; ECA, ecarin chromogenic assay; PT, prothrombin time; TT, thrombin time.

When urgent screening of NOACs is required and when the drug being assessed is not known, a more complex or comprehensive testing approach is required (►Fig. 4C). To some extent, the approach undertaken depends on tests available at local sites. All coagulation laboratories tend to perform PT and aPTT assays, with these typically available 24 hours/day. Many laboratories can also perform TT assays. Accordingly, these assays are recommended as a preliminary screen, and normal results will provide sufficient evidence for excluding significant effects from dabigatran and rivaroxaban (assuming the laboratory has validated relative sensitivities to these drugs). Normal PT and aPTT assays may not discount an apixaban (or edoxaban) effect, due to low sensitivity. The dRVVT assays are also often available in many coagulation laboratories, being used for LA testing. In our experience, this assay, using the confirmation reagent to reduce possible LA interference, can provide additional information, and a normal dRVVT can be used to exclude a significant dabigatran, rivaroxaban, and apixaban effect. The pattern of results from these four clot-based assays, readily available in many coagulation laboratories, can also inform on the possible drug present (►Tables 1 and 2), given that each drug provides a different pattern.

An anti-Xa assay can also be performed. In a preliminary screen approach, any anti-Xa assay will do, providing the laboratory recognizes the sensitivity of this assay for the different NOACs. For example, even a “standard” anti-Xa assay using a LMWH heparin standard will provide some information, with no activity discounting all anti-Xa NOACs (as well as UFH and LMWH), and low levels suggesting some anti-Xa NOAC (or UFH/LMWH) activity, and high levels suggesting a greater potential anti-Xa NOAC/UFH/LMWH influence. Naturally, if the specific assays are available, and should drug levels require quantification, then specific quantitative assays should instead, or additionally, be performed (►Fig. 4C). Indeed, the use of the panel of assays shown here will also help determine the likely drug, given the different patterns observed (►Table 1). Indeed, the test patterns for the NOACs are also different to those of classical anticoagulants (VKAs/UFH/LMWH) (►Table 2), so a particular test pattern for an unknown drug can likely inform on the identity of that drug.

In regard to recent local (Australasian) experience, the DTI/dTT assays have been found to be much more sensitive to dabigatran, and more reproducible in this setting than existing ECT/ECA assays in use.⁴¹ In addition, data from the commercial DTI (HTI) and in-house dTT assays were almost interchangeable.⁴¹ Based on these data, we would currently recommend the DTI/dTT method(s) ahead of the ECT/ECA methods for assessment of dabigatran. However, better harmonization of the ECT/ECA assays in other localities may provide additional support for use of these assays in place of DTI/dTT assays in those geographies. Moreover, in some countries, the ECT/ECA assays may be cleared by regulators of in vitro diagnostic products, but DTI/dTT assays may not be. We have previously reported on differential geographical hemostasis testing based on regulatory issues.¹⁰⁰

In regard to PICT assays, although these have been shown sensitive to most NOACs, they may require some further degree of optimization, and perhaps separate methodological assays for anti-IIa and anti-Xa NOAC agents.^{9,20,34,35,56,61,70,71,91}

Assay Interferences

In regard to assay interferences, all NOACs affect most clot-based assays, and anti-IIa agents and anti-Xa agents will also, respectively, affect assays based on anti-IIa and anti-Xa activity (e.g., AT).^{18,22,41,49,55,60,63,72–74,88,90,92,101} In general, LA testing should be avoided while patients are on NOAC therapy. In particular, testing for LA while patients are treated with dabigatran or rivaroxaban will provide false-positive LA results on most samples, with prolongation in LA screening assays often yielding higher values than prolonged confirm assays, with resultant high test screen/confirm ratios.^{4,22,41,49,55,63,73,74,88,90,92} Although apixaban is less likely to yield a false-positive LA, prolonging screen and confirm LA assays more concordantly, it may be safer to avoid LA testing also in these patients. Factor assay testing should also be avoided while patients are on NOAC therapy, with dabigatran and rivaroxaban in particular affecting all clotting factors and potentially yielding false-low values. Again, although apixaban is less likely to yield a false-low factor

Table 2 Summary of test patterns for NOACs and conventional anticoagulants

Coagulation test	Effect of					
	Anti-IIa NOAC dabigatran	Anti-Xa NOAC rivaroxaban	Anti-Xa NOAC apixaban	VKAs	UH	LMWH
PT/INR	+	++	(+)	++	–/+	–
aPTT	++	+	(+)	+	++	–/+
TT	++	–	–	–	++	+/-
Anti-Xa	–	++	++	–	++	++
dRVVT	++	++	++	++	–/++	–

Abbreviations: APTT, activated partial thromboplastin time; dRVVT, dilute Russell viper venom time; INR, International Normalized Ratio; LMWH, low-molecular-weight heparin; NOAC, non-vitamin K antagonist oral anticoagulant; PT, prothrombin time; TT, thrombin time; UH, unfractionated heparin; VKAs, vitamin K antagonists.

Note: UH at therapeutic levels will not affect PT or dRVVT when these reagents contain a heparin neutralizer; however, excess UH will affect these assays when neutralizer limits are exceeded.

Source: Modified from Lippi and Favaloro.⁴

level, it may also be safer to avoid factor testing in these patients. The clot-based PC and PS assays should also be avoided on patients on any NOAC; however, chromogenic PC and antigenic PS assays seem to be unaffected. AT assays are affected by the NOACs, with anti-IIa–based assays affected by dabigatran and anti-Xa assays affected by the anti-Xa agents. If AT testing is required, the alternative assays will need to be employed to obtain an accurate test result. The APCR assays should also be avoided on patients on NOAC treatment. In general, platelet function and VWF tests (excluding FVIII) are unaffected by the NOACs, as these are not clot based. Finally, TEG/ROTEM and TGA are affected and should also be avoided, except, of course, if drug effects are specifically being assessed.

Reversal of NOACs

As mentioned early in this review, an increasing number of studies have reported on reversal on NOACs using many laboratory assays. As also noted earlier, a full discussion on these studies would require another separate review. However, a brief overview is important for completeness. In general, there are no specific antidotes currently available, although a few are in clinical trials.¹⁰² Accordingly, most studies to date have reported on several currently available prothrombin complex concentrates or “bypassing” agents (including factor eight inhibitor bypass activity) and activated FVIIa).^{30,36,50,51,82,83,103–112} Of interest, all agents have been shown to correct some but not all hemostasis tests. In general, these agents do not “correct” the specific activity. Therefore, reversal agents, in general, do not correct TT/dTT/DTI for dabigatran, nor do they correct anti-Xa assays for anti-Xa agents. Most studies have instead employed global assays such as PT, aPTT, TEG/ROTEM, and TGA, and many parameters within TEG/ROTEM and TGA are “corrected,” at least partially, by most agents. Whether this will translate to effective treatments for bleeding, or whether the specific antidotes in development will abrogate their need in some cases, will need to await future evaluation.

Conclusion

The development and marketing of NOACs has revolutionized the historical approach to anticoagulant therapy.^{113,114} Although these novel classes of agents present several advantages over traditional VKAs and heparins, the claimed benefit that laboratory monitoring is not needed cannot be taken to mean that measurement of their effect will never be required. There are several situations in which routine and urgent measurement of NOACs is required, with these likely to further increase as NOACs continue to replace conventional anticoagulants for increasing numbers of indications in increasing numbers of patients. In a world with limited resources, the use of simple and economical means for therapeutic drug assessments represents the most logical approach (–Fig. 4), provided that the sensitivity of all tests to all NOACs are known, and if required optimized, and providing time of last dose is taken into account.

The dRVVT remains an underappreciated test for its potential contribution to laboratory assessment of NOACs. In many cases, the sensitivity of the dRVVT is higher than that for the routine assays (PT and aPTT). As the dRVVT is used widely in LA detection, it should not be difficult for laboratories to assess the sensitivity of the reagent in use in their laboratory against the DOACs.^{4,8,41,49,55,63} Again, given potential variable sensitivities evident against the different NOACs with different dRVVT reagents, laboratories will need to properly evaluate the dRVVT assay in use in their laboratory against all the NOACs in use in their locality.

Finally, of additional interest, it can be noted that the NOACs can be assessed in nonplasma samples and by POC test methods. Thus, Harenberg et al have extensively shown that the measurement of dabigatran, rivaroxaban, and apixaban is feasible in serum samples of patients, and reliable at least for the latter two agents, and is also possible in urine.^{9,115–119} These may provide additional opportunities when plasma samples are unavailable, and where additional plasma collection is impractical or not possible.

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