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Direct Oral Anticoagulants (DOACs) in the Laboratory: 2015 Review

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ABSTRACT

Direct oral anticoagulant therapies, including direct anti-Xa and thrombin inhibitors have recently been introduced and may have advantages over vitamin K antagonists such as warfarin. This review describes briefly the clinical utility and mechanism of action of these agents. Detailed information is provided on effect of these agents on routine assays including the APTT and PT as well as their impact on specialty laboratory assays. Also included are the use of drug specific assays and a discussion of alternative methods to determine relative drug concentration, such as evaluating drug calibrators in APTT and PT assays and using heparin calibrated anti-Xa assays to measure direct Xa inhibitors.

Contents

Introduction

Oral anticoagulant agents recently approved in both the United States (US) and Europe include dabigatran etexilate (Pradaxa®, Boehringer-IngelheimPharma GmbH & Co., Ingelheim, Germany), rivaroxaban (Xarelto® - Janssen and Bayer HealthCare), apixaban (Eliquis, Bristol-Myers Squibb/Pfizer), and edoxaban (Savaysa, Daiichi Sankyo, Inc). Dabigatran is a direct inhibitor of thrombin while rivaroxaban, apixaban, and edoxaban are direct inhibitors of activated factor X (FXa). This new class of anticoagulants has been referred to as non-vitamin K or novel oral anticoagulants (NOACs), target-specific oral anticoagulant agents (TSOACs), or direct oral anticoagulant agents (DOACs). The International Society for Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) for the control of anticoagulation recommends the term DOACs [1].

The most common clinical indications for these rapid-acting anticoagulant drugs includes stroke prevention in non-valvular atrial fibrillation, thromboprophylaxis in hip or knee replacement surgery, and for the treatment as well as secondary prevention of venous thromboembolic disease (VTE), including deep venous thrombosis and pulmonary embolus. It is estimated that 3 million individuals in the US suffer atrial fibrillation, as many as 900,000 could be affected with

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VTE annually, and approximately 300,000 undergo hip and 700,000 knee replacement yearly [2–4]. In clinical trials, DOACs been shown to be at least as effective as warfarin, but with a reduced incidence of intracranial hemorrhage [5–10]. As each of these agents has predictable pharmacodynamics, pharmacokinetics and wide therapeutic windows, routine therapeutic monitoring is not required [9,11]. Although therapeutic ranges have not been validated by the pharmaceutical companies that manufacturer DOACs, information about drug concentration is available in select FDA summary reports and some published studies [12–16].

DOACs are rapidly-acting, target-specific anticoagulants that inhibit both free and bound activated serine proteases, unlike heparin that can inhibit only free proteases [17,18]. This is of clinical importance because bound thrombin and FXa retain activity. For example, activated factor X (FXa) within the prothrombinase complex (bound FXa) is 300,000 fold more efficient in converting prothrombin to thrombin, than is circulating (free) FXa. The ability to inactivate bound serine proteases makes the anticoagulant action of DOACs more robust than warfarin or heparin. DOACs have relatively short half- lives and multiple clearance mechanisms including both hepatic and renal clearance, although dabigatran is cleared exclusively through the kidneys. Given the many advantages of DOACS, their use may be favored over both warfarin and heparin and it is likely that over time DOACs will be prescribed to millions of patients annually [10].

Laboratory Assays and DOACS - An Overview

DOACs present unique challenges with both routine screening as well as specialty assays of coagulation, and, although routine monitoring is not required, drug specific assays to measure plasma concentration are available. Effect on routine and specialty assays as well as assays to monitor DOACs will be discussed in subsequent sections.

Routine coagulation screening assays, including the prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin clotting time (TCT) are widely available on a routine and emergent basis in most clinical laboratories. These assays are not a reliable measure of DOAC anticoagulant effect. This is because the sensitivity of the PT and APTT varies considerably based on reagents used, as well as the specific DOAC being measured [19–24]. [Fig. 1] Given this, PT or APTT results (in seconds), in the presence of a given concentration of DOAC, cannot be standardized across laboratories [21–23]. Furthermore, because DOACs inhibit both free and bound serine proteases, a given prolongation of the clotting time, such as PT in seconds, when a patient is on warfarin, does not equate to the same level of anticoagulation when a patient has the same prolongation of PT but is on a direct Xa DOAC [25]. Patients can be fully anticoagulated on apixaban for example, with only a slight elevation of the PT [26]. The traditional TCT is exquisitely sensitive to the presence of dabigatran, with even trough levels resulting in "no clot detected" with some reagent systems [27]. Direct Xa DOACs will not prolong the TCT.

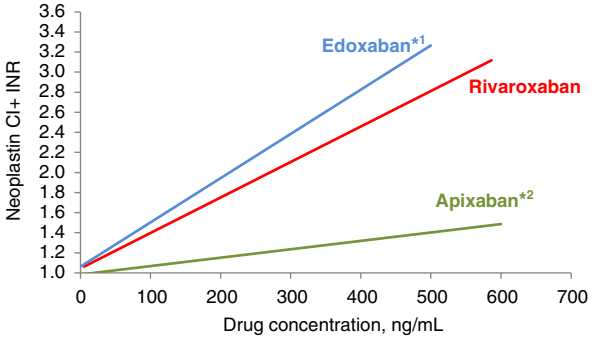

Fig. 1. Difference sensitivity of a single PT reagent (Neoplastin CI +, Diagnotisca Stago, Parisppany, NJ) to anti-Xa DOAC enriched pooled normal plasma. (Edoxaban data extrapolated from reference [15]; apixaban data unpublished observation DMA and RG, January 2015).

Although laboratory monitoring is not required when patients are administered DOACs, there are several clinical situations where determination of the level of anticoagulation may be of value, such as a patient experiencing hemorrhage or thrombosis, or requiring an emergent surgical procedure while on therapy [19,20]. DOAC concentrations can be accurately measured using a variety of laboratory methods [19, 20,23,28,29]. Mass spectrometry, when calibrated with each drug to be measured, is considered the gold-standard method and demonstrates good accuracy and precision over a broad concentration range although this test is not widely available [28]. More rapid methods including the dilute thrombin time, ecarin methods and chromogenic anti-Xa assays are potentially suitable means to measure DOACs, but must employ calibrators and controls specific for (or referenced against) the DOAC being measured [19,20,22,28,30]. Despite their availability, problems associated with existing assays used to quantitate DOACs include lack of: 1) FDA- approved DOAC calibrators or kits, 2) validated expected therapeutic plasma concentrations, and 3) knowledge of plasma concentrations associated with increased thrombotic or hemorrhagic risk. Furthermore, clot-based and chromogenic assays demonstrate variation between instrument/reagent systems, and also lack specificity [30,31].

If drug-specific assays are not available, it has been recommended that the relative sensitivity of a laboratory's PT and APTT to various types and concentrations of DOACs be determined locally. To accomplish this, commercially available calibrator material specific for the drug to be measured is assayed in the local laboratory against routine APTT and PT assays. This practice has been proposed by both the British Committee for Standards in Haematology (BCSH) as well as the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, although published studies verifying this practice were not available when these recommendations were made [32,33]. We used drug specific calibrators to assess PT and APTT DOAC sensitivity and compared this to sensitivity determined using well-characterized patients samples with drug concentrations measured by LC/MS-MS. With the exception of manufactured dabigatran calibrator evaluated using 2 APTT reagents (SynthASil and PTT-A), the use of drug-specific calibrators over-estimated reagent sensitivity compared to sensitivity determined using patients samples [34]. A patient's level of anticoagulation may be greatly underestimated when response to APTT and/or PT is based on a manufacturer's calibrator rather than samples from patients actually on drug. [Fig. 2A-C] This discrepancy likely reflects variation in the citrate concentration of the manufactured calibrators compared to that used for APTT and PT assays or may be due to the lyophilization process applied to the calibrator material.

Other laboratory methods, such as thromboelastographic measurements or endogenous thrombin potential assays have also been explored in patients taking DOAC, but their clinical use is not widely appreciated [20,35].

Dabigatran – Routine Screening Assays

The APTT is more responsive to dabigatran than is the PT while the TCT is exquisitely responsive. The APTT, however, cannot reliably distinguish therapeutic from subtherapeutic levels of dabigatran. In a study evaluating 7 APTT reagents (including 2 of the most common reagents used in the US, Actin FS and SynthasIL), we demonstrated that 18% of patients had a normal APTT despite measureable on-therapy dabigatran levels using LC-MS/MS [27]. This finding does not support the recommendation in the "Practice Guide" from the American Society of Hematology adapted in part from the American College of Chest Physicians Evidence-Based Clinical Practice Guideline on Antithrombotic and Thrombolytic Therapy (9th Edition), which states that in a patient on dabigatran who is bleeding, "a normal APTT is an indicator that

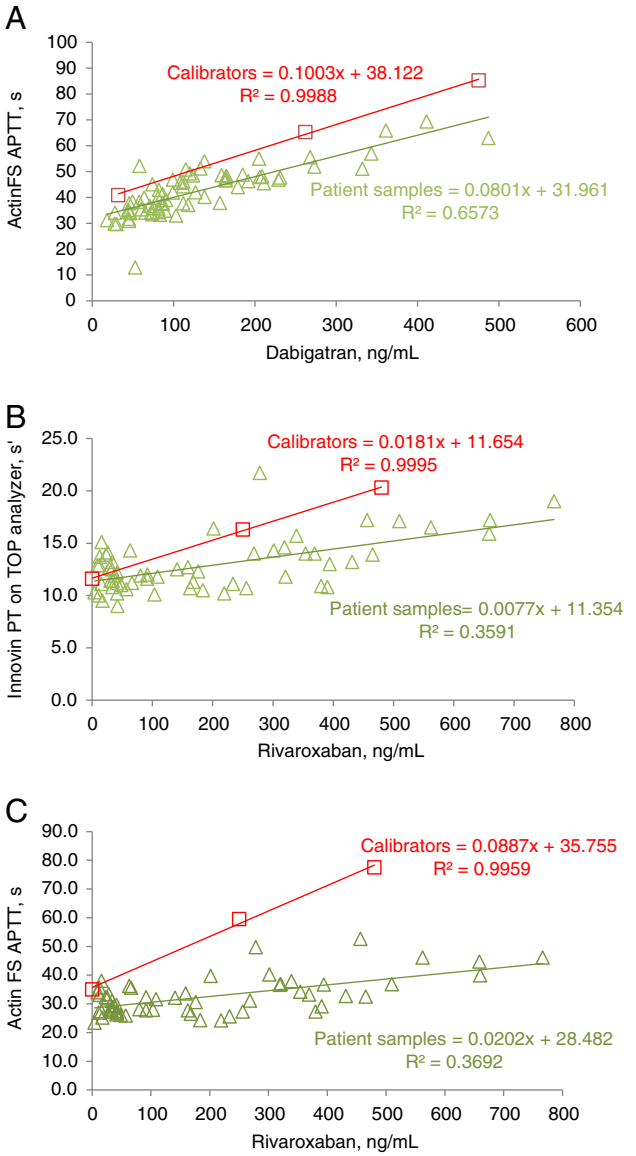

Fig. 2. A-C: Comparison of using commercial DOAC drug specific calibrators (open squares) versus samples (open triangles) from patients taking these dabigatran (2A) or rivaroxaban (2B-C) for determining reagent sensitivity for PT and APTT. The use of calibrators tended to over estimate the sensitivity of both dabigatran (2A) and rivaroxaban (2C) for Actin FSL (Siemens Healthcare Diagnostics, Newark, DE), and overestimated the sensitivity of Innovin (Siemens) for PT testing (2B) when used on the ACL TOP700 (Instrumentation Laboratory, Bedford, MA).

dabigatran would be unlikely to contribute to bleeding" [36]. Furthermore we demonstrated that, depending on reagent used, 15 to 35% of patients with plasma dabigatran levels >100 ng/mL had a normal APTT, which is at variance with the British Committee for Standards in Haematology guidance which states that "above 100 ng/mL, the APTT is invariably prolonged" [32,33]. The thrombin time is exquisitely responsive to dabigatran and we demonstrated that a normal thrombin time indicates no or minimal plasma dabigatran levels are present [27,37].

Dabigatran – Drug Specific Assays

Dabigatran plasma concentration can be assessed using a variety of assays including; a dilute thrombin time (HEMOCLOT THROMBIN INHIBITORS [Hyphen BioMed, France], an ecarin clotting time (Ecarin Reagent, Diagnostica Stago, Asnieres France) or ecarin chromogenic assay (ECA-T Kit; Diagnostica Stago, Asnieres, France), as long as each assay employs a specific dabigatran standard curve [19,20,22,23,32,38,39]. In a multicenter study, each assay demonstrated a linear relationship with drug concentration determined by LC/MS-MS, although accuracy was questionable as the measured dabigatran levels varied in a statistically significant manner, even when the same method was used by different laboratories [40].

Dabigatran - Interference in Specialty Coagulation Assays

Dabigatran will interfere with most APTT-based and some PT-based assays, depending on the drug concentration. Only very high dabigatran levels cause PT-based assay interference. Dabigatran interference in laboratory testing produces factitious results that are not representative of its physiologic effects. (Table 1) In a published study using dabigatran spiked into normal plasma, we demonstrated that relatively low levels of dabigatran (i.e. at 75 ng/mL which is below the typical on therapy range) yield mixing study results consistent with an APTT inhibitor and relatively high levels of dabigatran (i.e. at 200 ng/mL which is the higher end of the therapeutic range) will cause inhibitor effect in the PT as well as the APTT assay [41]. Surprisingly, further prolongation of the mix was evident with incubation suggesting the presence of a time and temperature-dependent inhibitor. APTT-based factor assays, specifically factors VIII, IX, and XI can show significant factitious decreases in activity due to dabigatran effect, depending on the concentration present. (Table 1) In our study, at dabigatran concentrations of 200 to 300 ng/mL factor VIII activity fell below 10 IU/dL and drug effect could not be diluted out to achieve normal factor levels, and a Bethesda titer

Table 1
The effect of DOACs on routine and specialty coagulation assays.

Assay	Direct Thrombin Inhibitor	Direct Xa Inhibitor [§]
APTT	Prolonged ↑↑	Prolonged ↑[¥]
PT/INR	Prolonged ↑	Prolonged ↑↑[#]
TCT	Prolonged ↑↑↑	No effect
Clauss Fibrinogen	No effect or factitiously low[¶]	No effect
AT Activity		
a. FXa based	a. No effect	a. Factitiously overestimated
b. FIIa based	b. Factitiously overestimated	b. No effect
PC Activity		
a. Clot based	a. Factitiously overestimated	a. Factitiously overestimated
b. Chromogenic	b. No effect	b. No effect
PS Activity		
a. Clot based	a. Factitiously overestimated	a. Factitiously overestimated
b. Free PS Ag	b. No effect	b. No effect
APTT-based APCR with added FV deficient plasma	Factitiously elevated ratio	Factitiously elevated ratio
APTT- based factor assays, one stage	Factitiously low FVIII, IX, XI	Factitiously low FVIII, IX, XI[†#]
PT- based factor assays, one stage	Factitiously low FII, V, VII, X[†]	Factitiously low FVII, X, V, II[#]
Chromogenic FVIII activity	No effect	Factitiously low
APTT Mixing Study	Incomplete correction	Incomplete correction
PT Mixing Study	Incomplete correction	Incomplete correction
LA Tests	Possible to misclassify as LA	Possible to misclassify as LA

APTT-activated partial thromboplastin time, PT/INR-prothrombin time/international normalized ratio, AT-antithrombin, PC-protein C, PS-protein S, APCR-activated protein C resistance, F-factor, LA-lupus anticoagulant.
[†] Slight increase in clotting time; ↑↑ moderate increase in clotting time; ↑↑↑ marked increase in clotting time.
[¶] Effect is method-dependent, most fibrinogen assays show no effect.
[†] When drug levels are supratherapeutic.
[§] Assay effect due to edoxaban is hypothesized based on drug action.
[#] The potential for demonstrating effect is reagent dependent.
[¥] Apixaban has little to no effect on the APTT.

was measurable. This effect may occur with other APTT-based factor assays, including factor IX and factor XI activity assays [41,42]. Therefore, APTT-based screening tests, mixing studies, factor activity analysis, and even Bethesda assays may yield spurious results in the presence of dabigatran, leading to a false impression of either a factor deficiency, or the presence of a specific factor inhibitor [29,41]. Clot-based methods for protein C, protein S, and APC resistance are significantly affected by even small amounts of dabigatran, resulting in factitious overestimation of protein C and protein S activities and a falsely elevated APC resistance ratio. The reptilase time, also a clot-based assay similar in methodology to the thrombin time, is not affected by dabigatran. FXa–based antithrombin assays are unaffected by the presence of dabigatran, but a factor IIa–based assay factitiously overestimates antithrombin concentration. Dabigatran may cause false positive lupus anticoagulant (LA) results but will not affect ELISA-based antiphospholipid assays [41,43]. Chromogenic or latex immunoassays including plasminogen activity, quantitative D-dimer, free protein S antigen, chromogenic protein C activity, von Willebrand factor antigen, and von Willebrand factor activity (ristocetin cofactor), are not affected by the presence of dabigatran [41–43]. Laboratories with limited access to patient information (eg, reference laboratories) may require a mechanism (e.g. by performing TCT) to rule out presence of dabigatran in order to avoid misinterpretation of laboratory results.

Anti-Xa DOACs- Routine Screening Assays

While overall, PT reagents are more responsive to anti-Xa DOACs in a concentration-dependent manner than APTT reagents, the response varies by specific anti-Xa DOAC and PT reagent used [19,20,22,24,26,40]. [Fig. 1] For example, therapeutic levels of apixaban demonstrate essentially no effect on APTT and little effect on PT assays, rivaroxaban or edoxaban that often elevate both the APTT and PT [41–50]. Given a similar mechanism of action between these agents, the basis for this difference is unknown. APTT and uncalibrated PT of unknown responsiveness cannot reliably determine DOAC concentration. In a study of patients on rivaroxaban 32% of the patient samples demonstrated a normal PT with “on therapy” plasma levels of drug [47]. As such, a normal PT should not be used to signify that most or all of rivaroxaban has been cleared. Some reagents are sufficiently responsive to rivaroxaban (and possibly edoxaban) such that they can be used to measure drug concentration if used with drug-specific calibrators [44].

Direct Anti-Xa DOAC – Drug Specific Assays

Direct anti-Xa DOACs can be accurately measured using modified chromogenic anti-Xa assays that employ drug specific calibrators although there are no FDA-approved kits available [22,23,31,40,45,51]. This same methodology is used to measure indirect FXa inhibitor drugs including heparin, LMWH and fondaparinux. Contrary to popular belief, the use of a drug-specific calibrator does not limit or improve the assay’s specificity to that particular drug. All direct and indirect FXa inhibitor drugs will be detected in a chromogenic anti-Xa assay regardless of the calibrator used. Quantifying direct Xa inhibitors agents with an assay calibrated with UFH, LMWH or fondaparinux is not recommended, although these methods have the potential to exclude the presence of DOACs at significant concentration [23,31]. [Table 1; Fig. 3] While these heparin-calibrated anti-Xa measurements have a linear relationship to anti-Xa DOAC concentration, significant limitations exist using these methods to quantify anti-Xa DOACs, specifically; 1) assays to measure indirect Xa inhibitors are measured in IU/mL and direct Xa DOACs are measured in ng/mL and there is no direct relationship between these units of measure, 2) there is significant variability in measured drug concentration, as demonstrated with rivaroxaban, between different manufactured anti-Xa kits, 3) the therapeutic range, at least for apixaban and rivaroxaban, far exceeds the typical calibration range for UFH or LMWH and is often in the 5 – 9 IU/mL range and 4) the assay

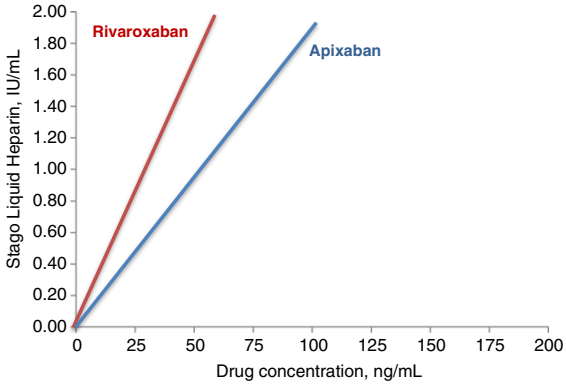

Fig. 3. Sensitivity of low molecular weight calibrated Liquid Heparin assay to rivaroxaban and apixaban using drug enriched normal pooled plasma samples. This method could potentially be used to exclude significant levels of direct Xa DOACs.

is not specific for anti-Xa DOACS and will detect all anti-Xa anticoagulants [23,31]. Furthermore the lower limits of detection for these assays varies with the calibrators used and some UFH or LMWH calibrated anti-Xa assays may underestimate the level of drug present. [Table 2] None-the-less, some heparin calibrated anti-Xa assays may have sufficient lower limits of quantitation to determine that rivaroxaban concentration is 5 ng/mL or lower and therefore can be used to evaluate patients prior to an intervention, such as surgery or the use of thrombolytics [31,52]. The use of a high phospholipid dRVVT assay calibrated with the specific drug to be measured may also be an alternative means for quantifying anti-FXa DOACs [30,48].

Anti-Xa DOAC - Interference in Special Coagulation Assays

Effect of rivaroxaban and apixaban on non-routine coagulation assays has been demonstrated in our laboratories and is also based on published literature [26,29,42,43]. The effect of edoxaban on specialty assays is not as well studied but is expected to mirror that of rivaroxaban. Rivaroxaban and edoxaban prolong the PT to a greater degree than the APTT and these agents may interfere with PT-based factor activity assays (FVII, X, V, and II) causing factitiously low activities with increasing drug concentration [29,42,43]. (Table 1) A normal plasma mixing study leads to incomplete correction and may mimic a specific inhibitor of FVII, X, V or II even though this effect is spurious. [29,43]. Apixaban has little effect on PT-based factor assays, even at a spiked dose of 400 ng/mL [DMA and RG unpublished observations, January 2015.] Clot-based protein C and protein S activity and APCR results are factitiously over-estimated in the presence of either rivaroxaban or apixaban. ([29,43], DMA and RG unpublished observations, January 2015) A FXa–based antithrombin activity assay will be over-estimated in the presence of direct Xa DOACs, but factor IIa–based assays are not affected. DOACs may factitiously and significantly decrease FVIII chromogenic activity. ([29,43], DMA and RG unpublished observations, January 2015) Direct Xa DOACS may cause false positive LA results (even when the APTT is normal) but will not affect ELISA-based antiphospholipid assays. ([43], DMA and RG unpublished observations, January 2015) This contradicts other published studies demonstrating that apixaban does not cause false positive LA results [45]. Other testing platforms, such as select chromogenic or latex immunoassays, including plasminogen activity, quantitative D-dimer, free protein S antigen, chromogenic protein C activity, von Willebrand factor antigen, and von Willebrand factor activity, are not affected by the presence of anti-Xa DOACs [29,42,43]. Laboratories without sufficient patient clinical and medication history (eg, reference laboratories) may require a pre-test algorithm to determine the suitability of samples for coagulation testing (e.g. screening chromogenic anti-Xa).

Table 2
Comparison of different chromogenic methods for the measuring anti-Xa activity of rivaroxaban using either unfractionated heparin (UFH), low molecular weight heparin (LMWH) or hybrid calibration. The LLQ (lower limit of quantitation) would determine the lowest amount rivaroxaban (as measured by tandem mass spectrometry) that could be measured, whereas the ULQ (upper limit of quantitation) would ascertain the highest amount of rivaroxaban that could be measured with the respective calibration types.

Manufacturer Reagent	Calibrator source	LLQ Anti-Xa U/mL	Lowest Rivaroxaban level with LLQ	ULQ Anti-Xa U/mL	Highest Rivaroxaban level with ULQ
Chromogenix COAMATIC	UFH	0.03	~15 ng/ML	1.0	>200 ng/mL
Siemens Berichrom®	UFH	0.03	~10 ng/mL	1.05	>250 ng/mL
Stago STA® Liquid Heparin	UFH	0	<5 ng/mL	0.60	~24 ng/mL
Chromogenix COAMATIC	LMWH	0.03	~35 ng/mL	1.00	>200 ng/mL
Stago STA® Liquid Heparin	LMWH	0	<5 ng/mL	1.82	~70 ng/mL
Stago STA® Liquid Heparin	Hybrid	0	<5 ng/mL	1.86	~80 ng/mL

Conclusion

While DOACs do not require routine laboratory monitoring, they may have significant impact on the clinical laboratory. The need to assess these drugs in emergent situations as well as their impact on routine and specialty coagulation assays has created challenges in most laboratories. Differences in the sensitivity of routine coagulation screening tests to DOACs prevent their use in reliably determining drug concentration. While commercially available, the lack of FDA approved calibrators or methods for quantifying DOACs have created a general reluctance to implement quantitative assays. Alternative methods for estimating drug concentration or the use of tests that can exclude significant amounts of DOACs, including the TCT and heparin anti-Xa chromogenic assay may be of clinical value. Use of commercial calibrators to assess reagent sensitivity to DOACs should be used with caution, especially when assessing direct FXa DOACs. While it is best to avoid performance of specialty coagulation assays for patients on DOACs, correct interpretation of results that are performed in the presence of DOACs is necessary to prevent a misleading diagnosis and subsequent intervention. For patients on DOACs, the results from coagulation assays should be used in conjunction with their clinical presentation when assessing bleeding or thrombotic risk.

Conflict of Interest Statement

None

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