International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

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Abstract

This guidance document was prepared on behalf of the International Council for Standardization in Haematology (ICSH) for providing haemostasis-related guidance documents for clinical laboratories. This inaugural coagulation ICSH document was developed by an ad hoc committee, comprised of international clinical and laboratory direct acting oral anticoagulant (DOAC) experts. The committee developed consensus recommendations for laboratory measurement of DOACs (dabigatran, rivaroxaban, apixaban and edoxaban), which would be germane for laboratories assessing DOAC anticoagulation. This guidance document addresses all phases of laboratory DOAC measurements, including pre-analytical (e.g. preferred time sample collection, preferred sample type, sample stability), analytical (gold standard method, screening and quantifying methods) and post analytical (e.g. reporting units, quality assurance). The committee addressed the use and limitations of screening tests such as prothrombin time, activated partial thromboplastin time as well as viscoelastic measurements of clotting blood and point of care methods. Additionally, the committee provided recommendations for the proper validation or verification of performance of laboratory assays prior to implementation for clinical use, and external quality assurance to provide continuous assessment of testing and reporting method.

Keywords
- direct oral anticoagulants
- laboratory measurement
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Background

In 2008, the European Medicines Agency (EMA), an oversight agency for the European Union, approved the use of dabigatran etexilate (Pradaxa, Boehringer Ingelheim), an oral direct thrombin (factor [F]IIa) inhibitor, for thromboprophylaxis in patients after knee and hip replacement surgery.[1] Since then, dabigatran and the direct factor Xa (FXa) inhibitors rivaroxaban (Xarelto, Bayer Pharma AG and Janssen Pharmaceuticals), apixaban (Eliquis, Bristol-Meyers Squibb and Pfizer) and edoxaban (Savaysa in the United States, Lixiana in Europe, Canada and Japan, Daiichi Sankyo) have been approved by the EMA and other regulatory agencies. Betrixaban (Bevyxxa, Portola Pharmaceuticals, Inc.), another anti-Xa direct acting oral anticoagulant (DOAC), was recently approved for VTE prophylaxis in the United States. However, as of this writing, there has been limited published data on the effect of this DOAC on laboratory assays.

Each DOAC has been reported to have predictable pharmacokinetic and pharmacodynamic responses, with no known dietary effect on efficacy, although food enhances the absorption of rivaroxaban.[2] Unlike VKAs, DOACs do not require routine laboratory monitoring of anticoagulant activity, but emergent and nonemergent circumstances in which DOAC assessment may be required have been described.[3] This publication serves as a technical International Council for Standardization in Haematology (ICSH) guidance document for laboratories that intend to assess (screen or quantify) DOAC anticoagulation. The recommendations provided are based on (1) information from peer-reviewed publications about laboratory measurement of DOACs, (2) contributing author's personal experience/expert opinion and (3) good laboratory practice. This document will primarily address the laboratory assessment of dabigatran, rivaroxaban, apixaban and edoxaban. Consensus recommendations indicate agreement by *all* contributing authors.

Dabigatran Etexilate (Pradaxa, Boehringer Ingelheim)

Dabigatran is formed when the oral prodrug, dabigatran etexilate, is hydrolyzed by esterases in the gut, liver, and blood.[2,4] Dabigatran competitively and irreversibly inhibits free and fibrin-bound thrombin by binding to the thrombin active site.[2,4–7] Usually given twice daily, dabigatran dosing (75,110 or 150 mg) is based on indication, patient's age, and patient's renal function.[2,8] There is low bioavailability (3–7%), with 35% protein binding and 80% renal clearance. The time to reach maximum concentration is usually 1.25 to 3 hours after dose, with a half-life of approximately 12 to 14 hours in patients with normal renal function[2–4,8,9] (►**Table 1**) Dabigatran is a substrate of efflux transporter P-glycoprotein (P-gp) (encoded by ABCB1) but is not metabolized by the cytochrome P450 isoenzymes.[10]

Rivaroxaban (Xarelto, Bayer Pharma AG and Janssen Pharmaceuticals)

Rivaroxaban is an oral, direct FXa inhibitor, inhibiting both free FXa and that bound to prothrombinase complex, thereby preventing thrombus extension.[11] Rivaroxaban also inhibits FXa bound to the clot, in a concentration-dependent mechanism.[12] Rivaroxaban is a competitive inhibitor of FXa, with high selectivity of more than 10,000-fold over other serine proteases.[13] Rivaroxaban is absorbed rapidly, reaching peak plasma concentrations in 2 hours[14] (►**Table 1**) and maximum inhibition of FXa activity between 1 and 4 hours after dosing.[14,15] The half-life of rivaroxaban is 5 to 13 hours with high bioavailability (80–100%) in the nourished state; however, rivaroxaban displays dissolution-limited absorption with decreased bioavailability in the fasting state. Plasma protein binding is approximately 92 to 95% with albumin as the main binding component.[14] Rivaroxaban is a substrate of the efflux transporter P-gp and is metabolized by the CYP3A4 isoenzyme.[16]

Apixaban (Eliquis, Bristol-Meyers Squibb)

Apixaban is a direct, reversible inhibitor of FXa administered orally twice daily as active drug.[2] In humans, eight metabolites have been identified, none of which appear to be active.[17] Apixaban exhibits a half-life of approximately 12 hours, has a high affinity for FXa and inhibits free FXa, FXa in the prothrombinase complex and FXa bound to platelets (►**Table 1**). Absorption of apixaban is approximately 50%. Following oral administration, peak plasma concentrations are observed about 3 to 4 hours post dosing. Apixaban is 87% bound to plasma proteins and is predominantly eliminated via the faecal route (56%), with 25 to 29% of the recovered dose eliminated via renal excretion.[18]

Table 1 DOAC characteristics

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Mechanism of action	Direct, reversible inhibitor of free and clot-bound thrombin	Direct, reversible inhibitors of free and prothrombinase bound factor Xa		
Bioavailability	3–7%	80–100%	50%	62%
Protein binding	35%	92–95%	87%	55%
Primary clearance	80% renal	67% renal	56% faecal	50% renal
Tmax	1.5–3 h	2–3 h	3–4 h	1–2 h
Half-life[a]	12–14 h	5–13 h	12 h	10–14 h

Abbreviation: Tmax, time to peak drug concentration after dose.
[a]Half-life varies with renal function, with increasing half-life with increased renal impairment.

Apixaban is a substrate of the efflux transporter P-gp and is metabolized by the CYP3A4-isoenzyme.[19]

Edoxaban (Savaysa in the United States, Lixiana in Europe, Canada and Japan, Daiichi Sankyo)

Edoxaban is a highly selective, direct and reversible inhibitor of FXa.[20,21] Edoxaban inhibits free FXa, as well as that within the prothrombinase complex. The recommended dose varies by indication and renal function[21–29] (►**Table 1**). Edoxaban is absorbed rapidly with peak plasma concentrations within 1 to 2 hours and exhibits a half-life of 10 to 14 hours.[20,21] The absolute bioavailability is approximately 62%.[20,21] In vitro plasma protein binding is approximately 55%. Unchanged edoxaban is the predominant form in plasma and this compound is metabolized via hydrolysis (mediated by carboxylesterase 1), conjugation or oxidation by CYP3A4/5 (< 10%).[20,21] Edoxaban has three active metabolites; the predominant metabolite (M-4), formed by hydrolysis, is active. Edoxaban is a substrate for the efflux transporter P-gp.[20,21]

Consensus DOAC measurement recommendations: general patient considerations.

- If nonemergent testing is necessary, recommend trough drug level assessment (►**Table 2**).
- Recommend DOAC levels be reported in ng/mL units.
- Recommend a comment with each reported DOAC result to indicate lack of DOAC 'therapeutic ranges', but cite expected trough levels (correlating with dose) for DOAC-treated patients from published studies (►**Table 2**).

Laboratory Assessment of DOACs

The optimal laboratory method to measure a DOAC depends on whether the test(s) are used for qualitative (presence or absence) or quantitative (ng/mL) purpose, and the required turn-around-time (TAT) for result.[3,30] Automated coagulation analysers have the capacity to quantify or screen for DOACs; however, both laboratory staff and treating clinicians require a thorough understanding of the limitations of the available assays, especially those used for qualitative purposes. Timely evaluation is critical in several scenarios such as life-threatening bleeding or acute stroke management.[31] As centralized hospital laboratories may take up to 1 hour to provide the results of routine coagulation parameters and possibly longer for DOAC concentration, appropriately validated point-of-care testing (POCT) methods should be considered if laboratory result TAT is not suitable for clinical urgency (see 'Other Screening Assays' section).

Consensus DOAC measurement recommendations: general laboratory considerations.

- Proper validation of any method used to quantify DOACs is required prior to clinical use of these assays (see the following sections).
- Recommend laboratories perform Internal Quality Control (IQC) at least once daily during testing performance, or at the minimum frequency required by regulatory agencies.
- Recommend enrollment in established External Quality Assurance program (EQA) (see the following section).

Sample Requirement for DOAC Assessment

Most data generated for functional qualitative or quantitative DOAC assessment have used sodium citrate samples, but comparisons of serum samples to plasma samples have been reported for rivaroxaban and apixaban.[32] Serum measurements

Table 2 Expected peak and trough DOAC concentrations in patients treated for stroke prevention in NVAF or treatment of PE/VTE[1,4,14,15,19,26–28]

	Dabigatran		Rivaroxaban		Apixaban		Edoxaban	
Indication	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE	Stroke prevention in NVAF	Treatment PE/VTE
Dose	150 mg bid	150 mg bid	20 mg qd	20 mg qd	5 mg bid	5 mg bid	60 mg qd	60 mg qd
Peak concentration, ng/mL	175[a] (117–275)	175[a] (117–275)	249[b] (184–343)	270[b] (189–419)	171[c] (91–321)	132[c] (59–302)	170[d] (125–245)	234[e] (149–317)
Trough concentration, ng/mL	91[a] (61–143)	60[a] (39–95)	44[b] (12–137)	26[b] (6–87)	103[c] (41–230)	63[c] (22–177)	36[e] (19–62)	19[e] (10–39)

Abbreviations: bid, twice daily; IQR, interquartile range; NVAF, non-valvular atrial fibrillation; PE, pulmonary embolism; qd, once daily; VTE, venous thromboembolism.

Notes: Other approved indications for DOACs include secondary prevention of PE/VTE, and post hip and knee replacement, which may have alternative dosing strategies. Additionally, changes in doses may occur after initiation phase of DOAC treatment. Consultation of regional DOAC labeling information is required before interpreting or using these peak and trough DOAC concentration data.

[a]Mean (25th–75th percentile).
[b]Mean (5th–95th percentile).
[c]Median (5th–95th percentile).
[d]Median (1.5 x IQR).
[e]Median (IQR).

tended to be higher than plasma measurements when chromogenic anti-FXa methods are used,[32] although this was also influenced by the drug concentration and reagents used. Mass spectrometry assays have included serum, lithium heparin and EDTA anticoagulated samples. Urinary assessment of DOACs has also been described and will be detailed later.

Stability data generated for both functional assays (e.g. dilute thrombin time [dTT] or chromogenic anti-FXa) and mass spectrophotometry methods have been published using both contrived (in vitro DOAC spiked) and patient samples.[33,34] For dabigatran, the stability in plasma at room temperature is 24 hours, without improved stability at refrigerated temperature (5°C), but at 14 months when maintained at < 20°C (personal communication via email from Joann van Ryn, Scientist, Boehringer Ingelheim, July 2017).[33,34] However, there is a 4-hour stability for dabigatran when using the thrombin time test.[35] For rivaroxaban and apixaban, the stability of DOAC in plasma has been shown to be at least 8 hours at room temperature, 48 hours at 5°C and at least 30 days when maintained at < 20°C.[33] Edoxaban demonstrated an 18% reduction in measurement when maintained at room temperature for 24 hours, but is stable up to 2 weeks at refrigerated temperatures when assessed by mass spectrometry,[34] but it is unclear whether this refrigerated stability also applies to functional anti-FXa assays.

Multiple freeze–thaw cycles of DOAC containing plasma have also been described, with three cycles demonstrating no-effect on the measurement of rivaroxaban and edoxaban using chromogenic anti-Xa or mass spectrometry methods.[33,34] Data for assessment of apixaban and dabigatran are conflicting,[33,34] although closer scrutiny would suggest no clinically significant differences with three thaw cycles.

Consensus sample recommendations for DOAC assessment:

- Plasma prepared from 3.2% sodium citrate can be used for quantitative and qualitative clot-based and chromogenic assays.[32–34] Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) can use serum or plasma.[32,34]
- Citrated whole blood samples should be processed within 4 hours of collection.
- Plasma samples for dabigatran that cannot be tested within 24 hours of collection should be frozen (stability of 14 months or greater if maintained at −20°C or colder) using monitored freezers or dry ice (personal communication via email from Joann van Ryn, Scientist, Boehringer Ingelheim, July 2017).
- For thrombin time testing (dabigatran), plasma samples are stable for 4 hours at room temperature.[35]
- Plasma samples for anti-FXa DOACs that cannot be tested within 8 hours of collection should be refrigerated (stability of 48 hours) or frozen (stability of 30 days or greater if maintained at −20°C or colder) using monitored freezers or dry ice.[33,34]
- Data would suggest that at least three freeze–thaw cycles could be performed without significant loss of activity.[33,34]

Qualitative Assays for DOAC

Due to their direct anti-FIIa or anti-FXa activity, DOACs can interfere with most clot-based haemostasis tests. Numerous studies performed using either spiked normal plasmas or ex vivo patient or healthy volunteer plasmas have shown that the DOAC effect on clotting assays depends on the reagent as well as drug, with wide inter-individual variability. Early recommendations suggesting that laboratories could locally assess DOAC sensitivity to PT and activated partial thromboplastin time (APTT) reagents using commercial calibrators and controls may not be optimal, as these materials are not optimized for clot-based screening assays.[36] This practice may overestimate reagent sensitivity to DOACs due to matrix variations (e.g. biased result due to components other than targeted analyte, calibration material may have other than 3.2% citrate concentration) and thus provide false assertions that a normal PT and/or APTT infers DOAC absence. Coagulation inhibitors or endogenous changes in coagulation factor levels can also affect the PT and APTT, and therefore lack specificity for the measurement of DOAC anticoagulation.

Of note, special consideration is required for DOAC-treated patients who may be bridged with unfractionated heparin (UFH) or low-molecular-weight heparin in acute situations. Depending on renal function, in the first 24 to 36 hours, there may be an additive effect on screening tests, and in these circumstances, the laboratory should be able to provide alternative strategies (e.g. anti-Xa for dabigatran-treated patients and thrombin time for UFH-treated anti-Xa DOAC patients) for assessing heparin anticoagulation, if required.

Prothrombin Time

Dabigatran and rivaroxaban prolong the PT in a concentration-dependent manner with a wide variability among reagents. The PT is less responsive to dabigatran than to rivaroxaban, regardless of the thromboplastin used.[7,30,37–48] The rivaroxaban concentrations required to double PT vary from 66 to 750 ng/mL. The PT ratios corresponding to 120 ng/mL rivaroxaban vary from 1.15 to 1.56, while those corresponding to 200 ng/mL of dabigatran vary from 1.31 to 1.88, depending on the reagent used.[39,40,46,47] The apixaban concentrations required to double the PT range from 480 ng/mL with the most sensitive reagent to over 1000 ng/ mL with other reagents.[2,48–50] The PT may be normal (ratio <1.20) with apixaban concentrations up to 200 ng/mL.[46,47,49,50] The PT is more sensitive than the APTT to edoxaban, with insufficient sensitivity at low on-therapy ($\sim$30 ng/mL) drug levels.[51–53] The prolongation of the PT is concentration- and reagent-dependent,[54,55] with edoxaban concentrations required to double the PT varying from 97 to 296 ng/mL.[55]

As international normalized ratio (INR) and the international sensitivity index (ISI) are based on VKA sensitivity, the PT should not be expressed as INR in patients treated with DOACs.[56] Although efforts to standardize PT methods by creating an ISI for rivaroxaban, analogous to the ISI for VKAs,[57] have been published, this practice has not been widely embraced and has not been demonstrated to be applicable to apixaban or edoxaban PT measurements.

Activated Partial Thromboplastin Time

The APTT is prolonged in a nonlinear manner with increasing concentrations of dabigatran and rivaroxaban, with a lower sensitivity to rivaroxaban than with dabigatran.[7,30,37–45] Commercial APTT reagents differ in their sensitivity, with a required dabigatran concentration of approximatively and 400 ng/mL to produce a twofold prolongation in the APTT.[41,52] The APTT ratios corresponding to 100 ng/mL dabigatran vary from 1.43 to 1.71 and those corresponding to 200 ng/mL from 1.67 to 1.97.[46,47] The APTT shows a concentration-dependent prolongation of clotting times followed by a plateau at approximately 200 ng/mL apixaban.[50] After a single 60 mg dose of edoxaban, the mean peak (1.5 hours after dose) APTT modestly increased from pretreatment APTT of 32.3 to 41.1 seconds.[54]

When combined, a normal PT and APTT measured with responsive reagents may exclude dabigatran concentrations above 50 ng/mL but fails to detect the presence of rivaroxaban at concentrations of 50 ng/mL, and apixaban of up to 200 ng/mL in a substantial number of patients.[47,52,58,59] Overall, the low sensitivity and specificity of the PT and APTT to DOACs suggests that the ability of these tests to quantify DOAC concentration is poor and reagent dependent.

Additional note on DOACs and other haemostasis assays: The knowledge of the impact that DOACs have on coagulation testing is vital to avoid misinterpretation of laboratory test results that may result in mismanagement, especially in bleeding patients.[60,61] DOACs are known to impact PT and APTT, tests that are modified PT and APTTs (e.g. factor assays, factor inhibitor assays, clot-based protein C or protein S), other clot based (e.g. dilute Russell's viper venom time) and chromogenic assays (e.g. antithrombin)[30,40–44,50,51,53,62] (►**Table 3**).

Table 3 DOAC interference on coagulation assays[3,33,40,50,51,53,62,111,123,138]

	Dabigatran		Anti-Xa DOACs		
	Clot-based assays	**Chromogenic-based assays**	**Clot-based assays**	**Chromogenic-based assays**	**Clinical impact of reported test result**
Relationship between prolonged clotting time and increased drug concentration	PT/INR[a,b] APTT[a,b] Thrombin time Ecarin-based assays		PT/INR[a,b,c] APTT[a,b,c]		Diagnosis and/or Management
Relationship between DOAC presence and factitiously decreased reported result	Fibrinogen[b,d] Factor activity[a] (II, V, VII, VIII, IX, X, XI, XII)		Factor activity[a,b,c] (II, V, VII, VIII, IX, X, XI, XII)	Factor VIII[b] Factor IX	(Mis)Diagnosis and/or (Mis)Management
Relationship between DOAC presence and factitiously increased reported result	Inhibitor screen[a,b] Inhibitor assay[a,b] Lupus anticoagulant[a] Protein C activity[a,b] Protein S activity[a,b] APCR[a,b]	Antithrombin[b] (thrombin substrate)	Inhibitor screen[a,b,c] Inhibitor assay[a,b,c] Lupus anticoagulant[a,b] Protein C activity[a,b] Protein S activity[a,b] APCR[a,b,c]	Antithrombin[b] (factor Xa substrate) UFH, LMWH or heparinoids/ pentasaccharide	(Mis)Diagnosis and/or (Mis)Management
No effect	Reptilase time	Antithrombin (factor Xa substrate) Protein C activity (chromogenic) Plasminogen activity Alpha-2-antiplasmin Factor XIII activity FVIII activity	Fibrinogen Thrombin time Reptilase time Ecarin-based assays	Antithrombin (thrombin substrate) Protein C activity (chromogenic) Free protein S antigen Plasminogen activity Alpha-2-antiplasmin Factor XIII activity	None—desired testing, when clinically necessary or relevant

[a]Reagent dependent.
[b]Concentration dependent.
[c]Apixaban usually not affecting result.
[d]For fibrinogen—if measured using the Clauss method, most reagents will not be affected. For PT-derived measurements, results are more likely to be factitiously increased.

Other Screening Assays

The TT is highly sensitive to dabigatran but is not affected by direct anti-Xa inhibitors. Dabigatran concentrations lower than 30 ng/mL lead to significant prolongation of the TT and concentrations of 50 ng/mL or greater typically produce a TT greater than the upper limit of measurement, depending on the reagent used.[2,35,63] A normal TT suggests that little or no dabigatran is present, but a prolonged TT does not necessarily equate to a high dabigatran level.[2,3,63]

Point-of-Care Tests

Viscoelastic measurements of clotting blood including the thromboelastograph (TEG) and rotational thromboelastogram (ROTEM) demonstrate that R times and clot formation times (CFT) correlated with dabigatran and rivaroxaban concentration.[64,65] There was a strong correlation between rivaroxaban or apixaban concentrations and LowTF-ROTEM CFT and time to maximum velocity;[66] yet, others report ROTEM as insensitive to detect residual rivaroxaban activity in patients.[65,67] An ecarin-base ROTEM has been recently reported to be sensitive to low levels of dabigatran.[68] These viscoelastic measurements of DOACs perform better on peak samples, lose sensitivity for trough samples, with limited findings due to small sample size.[69,70] POCT methods (PT, APTT, activated clotting time [ACT]) for assessing DOACs have mostly been shown to have poor correlation, poor sensitivity or overlap between normal range and 'on-therapy' DOAC levels.[48,59,71–73] A study using the Hemochron Signature POCT reported an INR cut-off for rivaroxaban of $\leq$1.0 and $\leq$1.1, respectively, equating to $<$30 and $<$100 ng/mL of drug, and for dabigatran of $\leq$1.1 and $\leq$1.2, respectively, equating to $<$30 and $<$50 ng/mL of drug.[73] Items of concern with these reported cut-offs include (1) the requirement to know which DOAC is under investigation; (2) lack of method utility in apixaban-treated patients; (3) the fact that dedicated study personnel performed the POCT testing, which may not reflect real patient practice; (4) described INR cut-offs are below the normal published reference range (INR: 0.8–1.3);[74] and (5) the relatively high repeatability precision ($\sim$13% at a 2.1 INR) noted with this method.[74]

DOAC screening assays using urine samples have been evaluated, but no correlation was demonstrated between urine and plasma DOAC concentrations.[75] A commercial urinary test screening for renal function (creatinine), anti-Xa and direct thrombin inhibitor is soon to be released in Europe.[76] Utilization of heparin-calibrated anti-FXa testing to potentially screen for the presence of anti-FXa DOACs will be discussed later.

Consensus screening test recommendations:

- The PT and/or APTT may not be reliable to detect the presence of 'on-therapy' concentrations of *all* DOACs.[2,3,7,30,37–56,58,59]
- PT and APTT are not responsive to 'on-therapy' apixaban levels.[49–52]
- The PT and APTT should not be used to quantify DOAC concentration.[2,3]
- In a patient with known DOAC exposure, a prolonged PT or APTT should be considered secondary to drug effect until proven otherwise, and in emergent or life-threatening conditions, tests for quantifying DOAC should be performed to aid in patient management.[3,30]
- A normal TT excludes the presence of significant dabigatran concentration.[2,3,30,63]
- At the time of writing this article, there is not enough clear data to support the use of TEG or ROTEM for detecting DOAC anticoagulant activity.[64–69]
- Nonspecific POCT methods may not have sufficient responsiveness to detect DOAC presence.[48,59,69–73]
- Urine DOAC screening tests may provide a rapid assessment (qualitative and semiquantitative) of recent DOAC exposure, but may not reflect circulating drug presence or concentration.[75]

Quantitative Assays for DOAC Measurement

The most accurate means of assessing DOAC exposure is by measuring concentration using LC-MS/MS or drug-calibrated clot-based or chromogenic methods. The availability and complexity associated with LC-MS/MS testing may limit its widespread use, whereas drug-calibrated clot-based or chromogenic methods can be adapted to automated coagulation analysers.

Mass Spectrometry Measurement of DOACs

The routine use of LC-MS/MS for the measurement of prescribed drugs in clinical laboratories has increased over the past 15 years and can be used to measure all DOACs.[77,78] Due to its high degree of specificity, sensitivity, selectivity and reproducibility, LC-MS/MS is considered the gold standard method for the measurement of DOACs and is often used in clinical development to evaluate DOAC pharmacokinetics.[8,79–82] The lower limit of detection (LLOD) and quantitation (LLOQ) for DOACs using LC-MS/MS has been reported to lie between 0.025 and 3 ng/mL, depending on the method and the drug. The reportable range of quantitation has been described to be between 5 and 500 ng/mL, which is suitable for expected peak and trough concentrations in most patients (►**Table 2**). The intra- and inter-assay precisions have been reported to be below 6 and 10%, respectively.[83–88]

Several factors limit the widespread use of mass spectrometry in the clinical setting, including labour-intensive sample preparation steps, complexity of the technique and instrument availability.[89] Additional assay challenges include matrix effect, co-elution of other compounds (drugs or xenobiotics), internal standard preparation and inadvertent detection or inability to detect drug metabolites (see below). These assays are mostly considered 'in-house' or laboratory-developed tests (LDTs), which may have additional method validation requirements as mandated by regional authorities (e.g. EMA, FDA).

With LC-MS/MS testing, the presence of phospholipids (PL), salts or molecules (e.g. such as surface-active compounds that can interfere with the droplet formation process in the ion sources) can cause a matrix effect. Plasma sample preparation (vs. serum) requires the removal of proteins, using protein precipitation with or without phospholipid

removal. Solid-phase extraction (SPE) can provide a 'clean' sample for LC-MS/MS testing, and liquid–liquid extraction (LLE) methods for sample preparation can also be used.

In LC-MS/MS analysis, an internal standard is mandatory to compensate for variability of the response due to the ionization process and to the recovery during the sample preparation procedure.[78] For DOACs, stable, isotope-labeled, standard versions are commercially available from several manufacturers. Additional considerations when developing an assay include (1) whether the drug must be metabolized to be functional, (2) possible interference by drug metabolites, (3) either co-elution of isotopomeric analytes or analytes that undergo in-source fragmentation to yield an isotopomers, (4) conversion of a metabolite to the parent drug during sample processing. Active metabolites should also be measured and reported. Dabigatran etexilate is a prodrug that must be metabolized to dabigatran to be fully functional. Furthermore, dabigatran exists in a free form and also conjugated to glucuronide. Dabigatran glucuronide adds approximately 20% anticoagulant activity. Alkaline hydrolysis of the sample prior to analysis splits the conjugate allowing measurement of total dabigatran.[8,87] A similar pattern is seen with edoxaban and its M4-metabolite which is also pharmacologically active.[82]

Major limitations of LC-MS/MS include the absence of standardization or harmonization of mass spectrometry–based assays,[90] and the lack of a universal calibration material or international reference standard. Significant variability between laboratories can be attributed to calibrators (matrix-based vs. solvent-based), calibrator source, sample preparation and the MS ion monitoring (ions selected in selected reaction monitoring or the use of high-resolution accurate mass spectrometry).[91] Commercially available, high-quality reference materials, traceable to an international standard, are urgently needed for each DOAC to improve LC-MS/MS performance.

Consensus LC-MS/MS recommendations:

- LC-MS/MS should be considered the gold standard test for measuring DOAC concentration.[8,79–82]
- A suitable internal standard for each DOAC is mandatory.[78]
- DOAC metabolites, that are pharmacologically active, should be reported.[3,8,82,87]

Other Methods for Quantifying Anti-FIIa (Dabigatran) DOAC

Published methods for measuring DTIs include the ecarin clotting time (ECT), chromogenic ecarin assay (ECA), chromogenic anti-FIIa (C-FIIa) assay, dTT and, to a lesser extent, the dilute Russell's viper venom time. Each of these methods can potentially be used for quantifying dabigatran, when calibrated appropriately.

Ecarin-Based Methods

Ecarin is a metalloprotease from saw-scaled viper, *Echis carinatus*, that converts prothrombin to meizothrombin, which can be inhibited by DTIs, but not heparin. The ECT reagent contains (~5 ecarin units/mL) ecarin, buffer (HEPES or Tris) and $CaCl_2$, with equal volumes of reagent to plasma used for testing.[92] For high drug concentrations, the patient plasma is diluted 1:1 with normal pooled plasma (NPP). The reported imprecision is less than 5%.[92] Fibrinogen and prothrombin (factor II) deficiencies may impact the accuracy of the test.[93,94] There is reported linear relationship between dabigatran concentration and ECT results and, with use of commercial calibrators, good correlation with LC-MS/MS measurements.[95]

The ECA pre-dilutes the patient sample with a buffer containing prothrombin to alleviate the prothrombin factor limitation as reported with ECT. As the ECA is not a clot-based assay, fibrinogen to fibrin formation is not measured, and thus, fibrinogen levels do not influence this assay. An equal volume of a substrate specific for thrombin cleavage is added to the diluted patient sample and incubated at 37°C. An equal volume of ecarin is then added and the reaction is read either kinetically or over a fixed period of time.[96] When the ECA is calibrated using commercial dabigatran material, there is good correlation with LC-MS/MS,[91] reported LLOD ranging from 14 to 46 ng/mL, within-run imprecision of less than 5%[83] and between-run imprecision of 6 to 16% using quality control material.[83,95,97,98]

Chromogenic Anti-FIIa Assay

Several commercial kits are available for measuring dabigatran using chromogenic anti-FIIa assay (C-FIIa) methods. Similar to ECA, a substrate specific for thrombin is added to a neat or diluted plasma samples and incubated for a period of time (~2 minutes). A thrombin reagent is then added and the test is read either kinetically or the reaction stopped using an acid or alkaline solution. The kits may contain a heparin neutralizing agent that can be used in patients who are on transitional therapy.[99] When the drug is calibrated, the C-FIIa demonstrates good correlation with LC-MS/MS ($R^2 = 0.96$ for samples containing <150 ng/mL dabigatran), with between-run imprecision of less than 5%, and LLOD of approximately 15 ng/mL, which can be further reduced with test modifications.[35,97–99]

Dilute Thrombin Time

First descriptions used one part plasma to three parts NPP. The final concentration of thrombin used was 0.75 NIH U/mL.[100,101] Equal volumes of diluted sample and thrombin are added, and clotting time recorded. When used in conjunction with drug calibrators, there is a linear relationship between clotting time and drug concentration. Commercial kits are available using same sample dilution with NPP (usually 1:8).[94,95,97] In patients treated with dabigatran, a strong correlation between dTT and LC-MS/MS have been reported.[84,97] Commercial assays report LLOD ranges of 2 to 8 ng/mL and LLOQ ranges of 20 to 30 ng/mL.[83,97,101–104] Both LLOD and LLOQ can be improved with the use of a lower sample dilution with NPP and use of specific calibrators and controls (e.g. 1:2).[97]

Consensus anti-FIIa (dabigatran) DOAC test recommendations:

- Demonstrated to be comparable to LC-MS/MS, drug-calibrated DTT, ECA, ECT and anti-FIIa chromogenic methods

are recommended as suitable methods to provide rapid quantitation of dabigatran.[84,95,97,99,101,102]

Other Methods for Quantifying Anti-Xa DOACs

Chromogenic Anti-Xa Assay

Chromogenic anti-Xa assay (C-FXa) assays have been used in the clinical laboratory for several decades as a means for assessing heparin anticoagulation. These assays are based on p-nitroaniline release from a specific chromogenic FXa substrate. The optical density generated per minute (OD/min) is inversely proportional to the amount of direct FXa inhibitor in the sample. Several in vitro and ex vivo studies have shown that C-FXa assays are very sensitive to the presence of direct FXa inhibitors.[40,43,49,50,53,59,83,85,98,105–121] In vitro studies have shown that, for rivaroxaban, plasma samples with suspected levels less than 30 ng/mL may not be adequately assessed by C-FXa assays due to limited LLOQ,[40] while for apixaban and edoxaban, some authors reported lower thresholds (i.e. 15 and 10 ng/mL, respectively).[47,53] For rivaroxaban, an adapted procedure may be used to enhance sensitivity (i.e. the Biophen Direct Factor Xa Inhibitors LOW, Hyphen BioMed, France) to lower concentrations of drug, but may result in a decrease of the range of measurement.[119] Thus, the assay sensitivity and LLOD/LLOQ threshold depends on the methodology and the C-FXa assay used, highlighting the importance of using a validated platform to assess the measurement of direct FXa inhibitors (►**Table 2**). Antithrombin supplementation of C-FXa kits leads to overestimation of direct FXa inhibitors and should be avoided.[110,120]

Ex vivo studies have highlighted limitations with rivaroxaban and apixaban measurement, with an LLOQ of around 30 ng/mL for both molecules.[85,88,108,120] For edoxaban, an ex vivo study measuring the anti-Xa activity calibrated with heparin standards revealed a good correlation with LC-MS/MS measurements.[20] However, if comparison with LC-MS/MS is required, the potential anti-Xa activity contribution of the M4 metabolite should be taken into account, since it is pharmacodynamically active and will interfere with the test, giving an elevated edoxaban concentrations in comparison to the LC-MS/MS measurement.[53]

A C-FXa assay calibrated with heparin standards can be used to inform on the relative presence of direct FXa inhibitors but is associated with a more limited range of linearity and quantitation.[88,108,121–123] Results below the assay's LLOQ suggest that no or clinically insignificant concentrations of FXa DOACS are present. However, due to kit difference in chromogenic substrates, factor FXa origin, methodologies and heparin calibration, the use of heparin-calibrated C-FXa assays should be used with caution.[123,124] All heparin-calibrated methods may not be equally sensitive to a similar direct FXa level.[124]

Consensus anti-FXa DOAC test recommendations:

- Demonstrated to be comparable to LC-MS/MS; drug-calibrated anti-FXa is recommended as suitable methods to provide rapid quantitation of anti-Xa DOACs.[40,43,49,50,53,59,83,85,88,105–118]
- Antithrombin supplement anti-FXa methods should not be used for DOAC assessment, as these methods tend to overestimate drug concentration and are not validated by the manufacturers.[110,120]

Point-of-Care Testing Assays and in Development Assays

Unless institutions have the capacity to rapidly report (<30 minutes) DOAC concentrations using aforementioned calibrated laboratory assays on a daily or on-demand basis, specific whole-blood POC assays for DOAC quantification are urgently needed but not yet available. Harenberg et al have described results of a POCT qualitative and semiquantitative assay using urine samples of patients treated with dabigatran, rivaroxaban or apixaban.[75,76,125–127] A miniaturized microfluidic coagulation test has been described for anti-FXa measurements; although specific for heparin anticoagulation, it may offer similar application for use in DOAC anticoagulation.[128] More recently, another microfluidic method has been described in stroke patients.[129] This POC method with sensitivity to warfarin, dabigatran and rivaroxaban, but not apixaban, employs surface acoustic waves that detect prolonged coagulation times.[129] The SPOCT-NOAC trial[130] is an ongoing investigator-initiated prospective trial which aims to test the correlation between the Cascade Abrazo POCT device (Helena Laboratories, United States) and plasma levels of apixaban, dabigatran and rivaroxaban, and to determine the diagnostic accuracy of POCT to rule out or detect relevant levels of DOACs in patient samples.

Quantifying DOACs: Assay Validation or Verification of Performance

Prior to offering a test for clinical use, the assay must be either verified or validated in the laboratory in which it is to be performed. Guidance documents have been published for industry[131–133] and laboratories,[134–136] although challenges to performing all studies due to limited resources (financial and staff) are acknowledged. An assay validation is required when the method is a standard (agency approved) method that is either modified or used outside the scope of the test, or a non-standardized test, or a LDT or research use only (RUO) assay. A verification of standardized assays (rather than full validation) may be a suitable approach for the laboratory to document it has achieved the reported testing performance.[135,136] The validation methods typically include accuracy (or trueness), precision (repeatability and intermediate [inter-assay] precision), specificity (selectivity), LLOD (DOAC level that is significantly different than zero), LLOQ (lowest DOAC measurement that meets acceptable performance criteria), linearity, range (reportable range) and stability.[137] Verification of a standardized method typically includes precision, accuracy and possibly linearity. Accuracy may be inferred if the precision, linearity and specificity criteria have been established.[132,133,137]

A validation or verification of performance plan (protocol) must be developed and approved by the laboratory director

(or delegate as appropriate). The plan should include processes to be performed (precision, linearity, etc.), the sequence of analysis, type of validation samples to be used, the number of runs to be performed over a specified (minimum) number of days and the quality control that will be utilized. The plan should describe the statistical analysis and acceptance criteria. A summary report, to include the validation data, intended use and reporting format of the DOAC assay, must be approved by the laboratory director (or designate), prior to clinical use[131,136,137] and each laboratory must maintain validation or verification documentation.

Validation Samples

The materials used as validators can include a variety of sources, such as de-identified patient samples, ex vivo drug-spiked plasma, quality control materials or calibrators. It is critical that the validation samples are of like-matrix to the patient samples that will ultimately be tested in the assay.[131] Contrived, ex vivo DOAC samples must verify that the native drug is the active metabolite. For mass spectrometry assays, the measurement of active metabolites in addition to the parent compound must be considered. Validator samples should be representative of the samples that will be tested and should fall on the calibration curve, typically with one in the upper third of the curve, one in the mid portion and one on the lower third of the curve.[131] If calibrators are used as validation samples, the lot used as validator material should either be from a different manufacturer source or a different lot than that used to calibrate the assay.

For chromogenic or clot-based quantitative DOAC assays, the first step in the process is the assay calibration and the criteria required for acceptable calibration curve. The calibration curve should cover the expected DOAC concentration and a calibrator sample near or at the LLOQ.[131] Extrapolation of data above or below a calibration curve is not recommended.[131] The validity of the calibration curve should be assessed by measuring samples with defined DOAC concentration limits.[137] If the calibration curve and DOAC sample concentration steps are acceptable, within-run precision (repeatability) should be assessed. Different recommendations for intra-assay precision include a minimum of nine determinations covering the range (e.g. three replicates of three concentrations), or at least six determinations at a single level.[132,133] For between-run precision, it has been recommended that LLOQ, low, mid and high validator samples from at least three runs are analysed on two different days.[137] Limits have been described as a CV of less than 15%, except at LLOQ, where the limit would be less than 20%.[131] Verification of performance repeatability limits should approximate manufacturer package insert or published data. Unacceptable within-run precision may suggest problems with instrument assay protocol definitions or possible reagent or sample carryover, if an automated analyser is used.

Linearity is determined using at least five to six samples tested over the reportable range.[132–134] LLOQ and LLOD using standard deviation calculations have been described using samples with no drug (blank), blank samples compared with low concentrations of drug (signal/noise), or calibration intercepts.[132–134] Alternatively, the deviation of more than 20% from low concentrations of drug can be determined from linearity studies.[131] The replicate determinations for LLOQ and LLOD range from 6 to 10 samples.[135,136]

Accuracy, or trueness, is a measure of the closeness of the DOAC result obtained to the true measured (assayed or reported) value. A minimum of three levels of validator samples are required for accuracy studies,[131] and these should include samples that fall on the lower one-third as well as upper one-third of the calibration curve. Validation samples should not fall outside of the standard curve. The acceptable limits for accuracy vary, but have been reported to be within 15% of measured value or within 20% of LLOQ,[131] but other statistical analyses such as bias determination[136] or paired *t*-test[135] have been described. Dilution integrity may be evaluated if samples with DOAC concentrations about the upper limit of the calibration curve will be diluted with the appropriate matrix to obtain measurable results. Dilution should not affect precision and accuracy and these should fall within ± 15%.[137] This study will permit extension of the reportable range.

Stability studies, if required (e.g. new methodology employed and no published references), may include freeze–thaw cycle stability, −80°C, −20°C, room temperature and time on-instrument studies.[137] Reagent or sample carryover can be evaluated during precision experiments by placing the different levels of validators in specified orders (e.g. low concentration, then high, then repeat low) of testing.[137] Robustness of the assay can be evaluated by including more than one lot of reagent (as well as commercial calibrators and controls) in the validation process.[132,133,136]

Consensus recommendations for method validation or verification of performance:

- Method validation or verification of performance is required before assays are used for clinical reporting.[131–137]
- Prior to performing method validation or verification, a plan (protocol) should be written that describes how the validation will be conducted and acceptance criteria.[131,136]
- Method validation studies should include precision, accuracy, linearity, determination of LLOQ, LLOD and reportable range and may include stability studies.[131–137]
- Method verification of performance studies should include precision, accuracy and possibly linearity.[135,136]

DOAC External Quality Assessment/ Assurance

Various processes can be utilized by laboratories to ensure the quality of testing, including internal quality control (IQC) and external quality assessment (EQA).[51,138] IQC utilizes homogeneous samples of a predetermined nature tested by the laboratory over a period of time, at a minimum of daily or whenever testing is performed, whereas EQA is a process whereby blinded samples are dispatched to laboratories and tested in the manner in which patient samples are tested and return results to the EQA provider for analysis. Several EQA programs are currently available for DOAC assessment (►**Table 4**).

Table 4 External quality assurance programs for DOAC

DOAC	Providers	Qualitative tests	Quantitative tests
Dabigatran	RCPA QAP NEQAS ECAT CAP	PT, APTT, TT	dTT/DTI, ECA, ECT, anti-IIa, LC/MS-MS
Rivaroxaban	RCPA QAP NEQAS ECAT CAP	PT, APTT	Anti-Xa, LC/MS-MS
Apixaban	RCPA QAP NEQAS ECAT CAP	None	Anti-Xa, LC/MS-MS
Edoxaban	ECAT (2019)		Anti-Xa, LC/MS-MS

Abbreviations: APTT, activated partial thromboplastin time; CAP, College of American Pathologists; DTI, direct thrombin inhibitor; dTT, dilute thrombin time; ECA, ecarin chromogenic assay; ECAT, external quality control of diagnostic assays and tests; ECT, ecarin clotting time; LC/MS-MS, tandem mass spectrometry; PT, prothrombin time; RCPA QAP, Royal College of Pathologists of Australasia Quality Assurance Program; UKNEQAS, United Kingdom National External Quality Assessment Service.

All tests used by the laboratory to assess DOACs should be covered by EQA and subject to IQC. EQA helps assess the accuracy of test systems used by laboratories, as the result submitted by laboratories can potentially be compared with some predefined 'gold standard' (or 'target') result, as well as whether the laboratory test result is within an acceptable range of 'closeness' to the target. The range of acceptability is used to determine if a given laboratory's test result is within the acceptable range, or outside this range, thereby offering a means to 'assure' the quality of test results (hence, the term 'external quality assurance' is sometimes used).

It is recognized that the EQA process represents an imperfect test assessment system. First, in the peer-comparison test system, the 'trueness' of the target result is dependent on the quality of the results submitted by participants of the EQA program, and may be skewed by outliers (possibly representing poor laboratory performance) or by dominant methodologies (e.g. popular reagent kit methods). Thus, the median is usually used in preference to the average, as this is less influenced by such factors. The predetermined range of acceptability also differs according to different EQA programs, and although usually expressed as a percentage variance (e.g. 5, 10 or 20% ranges around the median), this may be defined by statistical models or by expert committees. In addition to providing numerical data analysis, EQA providers should also be encouraging laboratories to interpret EQA test results in a manner that would reflect real patient test interpretation.

Any material generated for EQA or proficiency test purposes should undergo stability and homogeneity testing, and if available, some pre-dispatch testing by LC/MS-MS or a reference laboratory using a 'reference' quantitative method (e.g. dTT for dabigatran and drug-calibrated anti-Xa assays for anti-Xa DOACs).

Consensus DOAC EQA recommendations:

- Each laboratory must enrol in an EQA program specific for the DOAC being measured.
- EQA should be at a minimum two samples per dispatch, with at least two dispatches in a calendar year.

What is known about this topic?

- PT and APTT are not reliable to assess DOAC concentration.
- dTT, ECA, or ECT demonstrates linear relationship with dabigatran concentration.
- Drug-calibrated anti-Xa tests are comparable to tandem mass spectrometry measurements of anti-Xa DOACs.

What does this paper add?

ICSH document providing guidance to laboratory DOAC testing:

- Consensus recommendations for the pre-analytical phase of DOAC testing, including recommended timed collection (trough) and guidance for sample stability.
- Consensus recommendations for the analytical phase of DOAC testing, including method validation or verification of performance of DOAC test, identifying tandem mass spectrometry as the gold standard for DOAC measurement. Indicating that drug-calibrated dTT, ECA, ECT, anti-FIIa and anti-FXa are suitable for rapid quantitation of DOACs, and requiring that laboratories perform IQA at a minimum of once per day of testing.
- Consensus recommendations for the post-analytical phase of DOAC testing, including that DOAC results are reported in ng/mL, and the reported results are accompanied with published (trough) range of results, and requiring that laboratories that perform DOAC testing must participate in external QAP to assure continuous quality assurance.

Conflicts of Interest

RCG: Advisory board for Instrumentation Laboratory, Roche Diagnostics, NovoNordisk, and Boehringer Ingelheim; Speaker honoraria for Siemans Healthcare Diagnostics, expert testimony on rivaroxaban and dabigatran.
DMA: Advisory board for NovoNordisk, Baxalta, Bayer Healthcare; speaker honoraria from Siemens Healthcare Diagnostics, consulting for rivaroxaban litigation.
SB: Grants and research: site investigator Bayer (RASET) study, partial salary support for endowed chair funded in part by Eli Lilly, Canada.
JD: Advisory board: Bayer, travel awards and/or speaker honoraria: Daichii Sankyo; Diagnostica Stago, Roche Diagnostics.

EF: None
IG-T: Advisory boards and speaker honoraria: Bayer Healthcare, Boehringer Ingelheim, Bristol-Myers-Squibb/Pfizer.
CG: Advisory board Werfen; speaker honoraria: Roche Diagnostics, Werfen.
YK: Advisory board and consultant: Daichii Sankyo.
EL-L: Advisory boards: Bayer Healthcare, Boeh.

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2021 Update of the International Council for Standardization in Haematology Recommendations for Laboratory Measurement of Direct Oral Anticoagulants

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Abstract

In 2018, the International Council for Standardization in Haematology (ICSH) published a consensus document providing guidance for laboratories on measuring direct oral anticoagulants (DOACs). Since that publication, several significant changes related to DOACs have occurred, including the approval of a new DOAC by the Food and Drug Administration, betrixaban, and a specific DOAC reversal agent intended for use when the reversal of anticoagulation with apixaban or rivaroxaban is needed due to life-threatening or uncontrolled bleeding, andexanet alfa. In addition, this ICSH Working Party recognized areas where additional information was warranted, including patient population considerations and updates in point-of-care testing. The information in this manuscript supplements our previous ICSH DOAC laboratory guidance document. The recommendations provided are based on (1) information from peer-reviewed publications about laboratory measurement of DOACs, (2) contributing author's personal experience/expert opinion and (3) good laboratory practice.

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Introduction

In 2018, the International Council for Standardization in Haematology (ICSH) published a consensus document providing guidance for laboratories on measuring direct oral anticoagulants (DOACs).[1] Since that publication, several significant changes related to DOACs have occurred, including the approval of a specific DOAC reversal agent (intended for use when the reversal of anticoagulation with apixaban or rivaroxaban is needed due to life-threatening or uncontrolled bleeding), andexanet alfa (Andexxa in the United States and Ondexxya in the European Union) from Portola Pharmaceuticals Inc.[2,3] Betrixaban (Bevyxxa, Portola), the fourth direct factor Xa (FXa) DOAC was approved for use in the United States but has since been discontinued by the manufacturer and will not be addressed. In addition, this ICSH Working Party recognized areas where additional information was warranted, including patient population considerations and updates in point-of-care testing (POCT). The information in this manuscript supplements our previous ICSH DOAC laboratory guidance document.[1] The consensus recommendations provided are based on (1) information from peer-reviewed publications about laboratory measurement of DOACs, (2) contributing author's personal experience/expert opinion and (3) good laboratory practice.

Patient Selection for DOAC Testing

As with the first ICSH DOAC publication, whether or not patients should be tested is beyond the scope of this document.[1,4–6] However, laboratory staff should be aware of emerging publications conveying potential advantages of measuring DOAC levels (►**Table 1**). In addition to previously

Table 1 Indication for testing of direct oral anticoagulants (DOACs) according to the level of evidence for non-urgent situations

Indication	Rationale	Practical consideration	Source of information
Non-urgent situations			
Advanced age	Increased rate of bleeding events with age and increased susceptibility of bleeding events with DOAC accumulation	If done, plasma DOAC concentrations should be measured at trough, just before the next pill or capsule intake after 5 or more intakes to ensure the DOAC has reached its steady state. Plasma DOAC concentration should be in the range of concentrations observed in other populations	Post hoc analyses of safety outcome from phase 3 clinical trials and post-marketing observational studies. *NB: Data are lacking to show the benefit of adjusting the dose based on individual pharmacokinetic (PK) evaluation, but these data suggest that the optimal drug level varies with age*
Severe renal failure and dialysis dependence	Increased levels of DOAC reflected by increased C_{MAX} and AUC, especially for dabigatran, rivaroxaban and edoxaban. Apixaban seems less affected based on PK studies. Bleeding risk and bleeding-related death were increased significantly in these population compared with warfarin *NB: AHA, ACC, HRS and EHRA guidelines all refrained from supporting use of dabigatran, rivaroxaban and edoxaban in patients with chronic kidney disease (CKD) or on dialysis. Only warfarin and apixaban seems to be safer in these populations*	If done, plasma DOAC concentrations should be measured at trough, just before the next pill or capsule intake after 5 or more intakes to ensure the DOAC has reached its steady state. Plasma DOAC concentration should be in the range of concentrations observed in other populations	Post hoc analyses from phase 3 clinical trials and post-marketing observational studies *NB: Data are lacking to show the benefit of adjusting the dose based on individual PK evaluation, but these data suggest that the optimal drug level varies with renal function*
Prior interventions with high bleeding risk	To be on the safe side, intervention categorized as being at high bleeding risk should be done in patients with no or undetectable DOAC concentration. Using the PK approach would not ensure all patients will have cleared completely the DOAC as many variables could interfere with the elimination of DOACs. As some of the factors used to set up the PK approach also rely on surrogate biomarkers (e.g., serum creatinine or liver function), the most obvious and rationale solution could be the measurement of DOAC concentrations	Plasma DOAC concentration should be measured within a few hours before the intervention and planned surgical intervention should proceed when the level is considered low enough. Plasma DOAC concentration should be in the range of concentrations observed in other populations	Post-marketing observational studies *NB: there are currently no prospectively validated data with hard clinical endpoints on cut-off values of any coagulation test to guide the timing of elective or urgent surgery*

(*Continued*)

Table 1 (*Continued*)

Indication	Rationale	Practical consideration	Source of information
Non-urgent situations			
Body mass index (BMI above) 40 kg/m^2	For patient with BMI above 40 kg/m^2, if a DOAC is chosen, obtaining a peak and trough DOAC concentration estimate after at least 5 doses may be of interest to ensure the plasma concentrations are roughly within the range published for other patients *NB: It remains unclear whether adequate DOAC concentrations are achieved to be clinically effective. The majority of post hoc analyses showed reassuring data for patients up to 40 kg/m^2 but further data are needed in extreme obese*	If done, plasma DOAC concentrations should be measured at trough, just before the next pill or capsule intake and at peak after 5 or more intakes to ensure the DOAC has reached its steady state. Plasma DOAC concentration should be in the range of concentrations observed in other populations.	PK studies and expert opinion
Drug interactions	Numerous drug interactions have been described and investigated by the manufacturers, sometimes requiring dose adaptations. However, unknown drug interactions as well as multiple drug interactions may interfere with drug levels to a degree, which may have a clinical relevance. Evaluating DOAC levels in these conditions may identify drug accumulation or clearance	If done, plasma DOAC concentrations should be measured at trough, just before the next pill or capsule intake after 5 or more intakes to ensure the DOAC has reached its steady state. Plasma DOAC concentration should be in the range of concentrations observed in other populations	PK studies, case reports and post-marketing observational studies

indicated clinical situations (usually urgent situations) where DOAC measurements may be useful,[1,6] evidence is accumulating between drug exposure and clinical outcome (►**Table 2**).[7–11] Additional data, albeit low grade, may support other situations and patients who may benefit from DOAC assessment.[12,13] Included are patients with advanced age,[14,15] severe renal failure and dialysis dependence,[15,16] as well as patients with acute bleeding, to determine appropriate reversal strategies and associated dosing required.[17–19] Some have also suggested DOAC measurements in patients the day prior to undergoing interventions with high bleeding risk (e.g., complex endoscopy, spinal or epidural anesthesia, thoracic surgery, abdominal surgery, major orthopaedic surgery or neurosurgery),[5,20–23] although it should be noted that this approach of measuring DOACs is currently not supported by clinical evidence and the relevance of the

Table 2 Indication for testing of direct oral anticoagulants according to level of evidence for urgent situations

Indication	Rationale	Practical recommendation	Source of information
Urgent situations			
Acute bleeding and determination of appropriate reversal strategies	Measuring the anticoagulant effects or plasma drug levels of DOAC can help determine their contribution to bleeding or to determine when it is safe to perform an urgent or unplanned intervention. Assessing potential rebound effect after administration of reversal agents *NB: Delaying antidote administration until coagulation test results are available may be detrimental in DOAC-treated patients with life-threatening bleeding, such as intracranial bleeding or in those requiring emergency surgery for life-threatening conditions such as a ruptured aortic aneurysm*	Measurement of plasma DOAC concentration should be done as soon as possible *NB: Recommendations for antidote administration are based on plasma DOAC concentrations. In patients with serious bleeding, a DOAC concentration > 50 ng/mL is considered sufficiently high to warrant antidote administration, whereas in those requiring an urgent intervention associated with a high risk of bleeding, antidote administration should be considered if the DOAC concentration exceeds 30 ng/mL*	Case series and expert opinions. Post hoc analyses from phase 3 clinical trials and case series

current threshold is questioned.[24] Specifically, although the "Perioperative Anticoagulant Use for Surgery Evaluation" (PAUSE) study reported acceptable bleeding rates with their clinically defined anticoagulant interruption strategies and defined thresholds, that is, analyses were done for residual DOAC levels $\geq$30 ng/mL and $\geq$50 ng/mL,[25,26] it is not known what DOAC level would be considered "safe" to undergo a surgical procedure or intervention and with the vast majority of patients, a wait time period appears to be safe.[25,26] With limited data on patients with a body mass index (BMI) $>40\,kg/m^2$, DOAC pharmacokinetic and/or pharmacodynamic measurements in this population may be considered.[27,28] In addition, many elderly patients with non-valvular atrial fibrillation may acutely develop decompensated heart insufficiency with increase of liver enzymes, decreased intestinal blood flow and develop an unpredictable pharmacokinetic profile which may lead to an increased bleeding risk. DOAC measurements may be useful in detecting DOAC overexposure and bleeding risk, DOAC underexposure and thrombotic risk, and identifying previously undescribed and described drug–drug interactions, although this needs to be confirmed in larger cohorts.[29–31] It should be noted that paediatric patients may have lower DOAC levels than adults,[32] and modifications of anti-Xa methods may be required. In addition, discrete age-partitioned and age-appropriate reference intervals are likely needed for coagulation test in the paediatric population.[33]

Consensus Recommendations

- *This ICSH Working Party recognizes there are insufficient data to date for providing dose-adjustment recommendations based on DOAC levels alone. Nevertheless, DOAC measurements may identify potential excessive clearance or drug accumulation and could be used in situations where the benefit of such measurement is likely to outweigh the risk, for example, in non-urgent situations.*
- *Several categories of patients may benefit from DOAC level measurements to ensure they are within the concentration range observed in pharmacokinetic investigations during drug developments.*
- *If a DOAC measurement has been requested for urgent purpose, results should be provided within 30 minutes to aid in acute clinical decision-making.*
- *This ICSH Working Party encourages laboratories to provide DOAC measurements per clinical need. DOAC results must be used (and interpreted) in the context of patient history, DOAC type, DOAC dose, last dose and potential impact on clinical management (e.g., surgical intervention, bleeding, reversal strategies).*

DOAC and Laboratory Testing

The first ICSH laboratory DOAC guidance document already detailed test procedures or methods for quantifying DOACs such as the ecarin clotting time (ECT), dilute thrombin time (dTT) or anti-Xa measurements (►**Fig. 1**).[1] More methodological details can also be found elsewhere.[34–36] Of particular note, the ECT used in the dabigatran trials and the ECT range cited in prescriber information are based on an ECT reagent concentration of 6 IU/mL.[35]

Interference of DOAC on Coagulation Assays

It has been widely shown that DOACs may interfere with coagulation testing, even at low DOAC concentrations.[37] Thus, even trough collections aimed to minimize DOAC concentration may be inadequate to completely eliminate drug interference in certain assays.[37] To ensure an undetectable DOAC concentration, a delay of 3 days or more (depending on DOAC, renal function and clinical situations) between the last intake and testing could be necessary. A longer delay is likely necessary for lupus anticoagulant (LA) testing with dilute Russell viper venom time (dRVVT) tests, due to the interference that may still be present when DOAC concentration is below the lower limit of quantification of the anti-Xa-based method (anti-Xa). However, due to high inter-individual DOAC variability and potential thrombotic risk, a wait period of 3 days may not be a suitable alternative unless bridging therapy (e.g., low-molecular-weight heparin [LMWH]) is considered.[38] Alternatively, in vitro removal of DOAC compounds from plasma prior to coagulation testing has been reported and may be more suitable.[39–47] DOAC-Stop (adsorbing agent, Hematex Research, Hornsby, Australia) and DOAC-Remove (activated carbon, 5-Diagnostics, Basel, Switzerland), both reportedly able to neutralize all DOACs with minimal effect on haemostasis tests, have been recently commercialized.[39–47] However, care should be taken, especially in LA testing, since in the reported studies, complete reversal did not occur in every sample and reversal varies among the different DOACs.[42,43,45,48,49] Some differential effects may be observed between use of DOAC-Stop and use of DOAC-Remove since these products are not identical or necessarily interchangeable.

Additionally, a slight procoagulant effect of DOAC-Stop has been shown in thrombin generation assays (TGAs) that use an intermediate concentration of tissue factor (i.e., around 5 pM). This procoagulant effect seems to be related to slight reduction in tissue factor pathway inhibitor (TFPI).[50,51] The elimination of DOAC presence in plasma using filters like the DP-Filter (5-Diagnostics) or the DOAC-Filter (Diagnostica Stago, France) showed promising results.[39,52] However, potential unintended filtration of coagulation proteins seen with other filtering mechanisms (e.g., von Willebrand factor) may occur, but the interference of DOAC-Stop or DOAC-Remove on these other coagulation proteins has also been found (e.g., interference on TFPI), impacting mainly TGA.[51] Lastly, new products are currently under evaluation that demonstrates low to no DOAC interference for LA detection.[53,54]

Interference of DOAC on Platelet Aggregation and Fibrinolysis Assays

Sokol et al demonstrated a reduction in thrombin-induced platelet aggregation with rivaroxaban and apixaban, a result different from a previous investigation with rivaroxaban.[55,56] This requires further investigations and

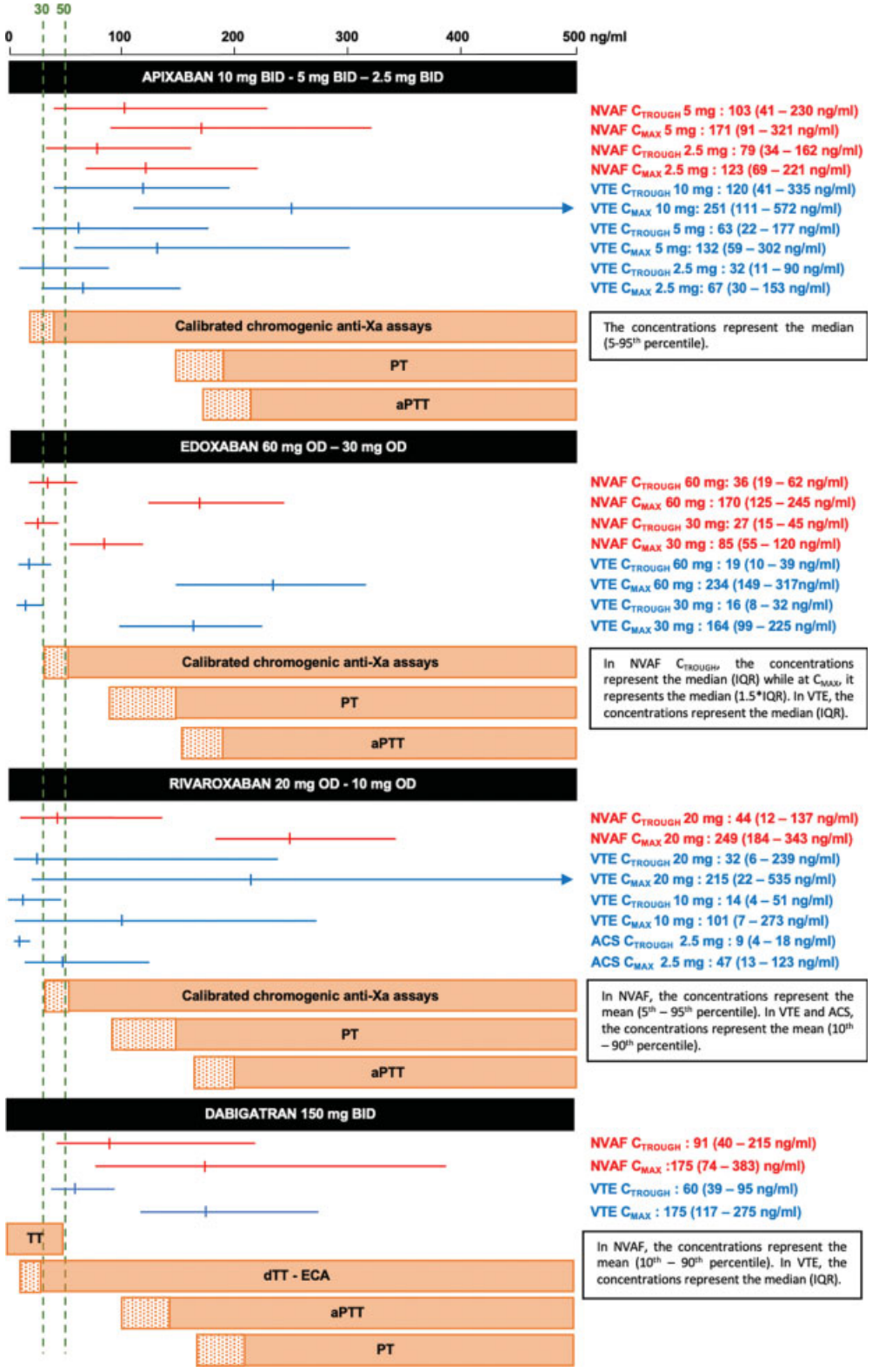

Fig. 1 Laboratory testing for direct oral anticoagulant (DOAC) and expected plasma concentrations after therapeutic doses. *Orange boxes* represent ranges of applicability of the corresponding test. *Dashed orange boxes* represent the zone in which the variability may change due to different reagent sensitivities. Note that only reagents considered as sensitive/reactive were considered. Plasma concentration ranges are extracted from the European Summary of Product Characteristics for all indications of apixaban[128] and dabigatran[129] and for VTE and ACS indications of rivaroxaban,[130] from Mueck et al for rivaroxaban in NVAF,[131] and from Ruff et al,[7] Weitz et al[132] and Verhamme et al[133] for edoxaban. (ACS, acute coronary syndrome; APTT, activated partial thromboplastin time; C_{MAX}, maximum plasma concentration during the dosing interval; C_{TROUGH}, minimum plasma concentration during the dosing interval; dTT, diluted thrombin time; ECA, ecarin chromogenic assay; IQR, interquartile range; NVAF, non-valvular atrial fibrillation; PT, prothrombin time; TT, thrombin time; VTE, venous thromboembolism.)

confirmations. As expected, a similar effect has been reported by Shimizu et al with dabigatran.[57] However, the interference with platelet aggregation is most likely an indirect effect of DOACs driven by the inhibition of thrombin generation.[58] Additionally, it has been shown that dabigatran, rivaroxaban, and apixaban enhance fibrinolysis, but this depends on the presence of thrombomodulin in the test system.[59–62] As such, caution should be used when performing and interpreting the results from any coagulation-related test from a DOAC-treated patient.[37]

Management of Heparin Bridging in DOAC Treated Patients

DOAC-treated patients may suffer an acute event that requires bridging with unfractionated heparin (UFH) or LMWH. For UFH bridging of dabigatran, only the anti-Xa activity should be considered suitable to measure UFH effect, as activated partial thromboplastin time (APTT) and (dilute) thrombin time will be prolonged by both drugs.[63] In the case of direct FXa inhibitors, alternatives to APTT or anti-Xa measurements are required, since both anticoagulant types affect these tests, leading to supra-therapeutic anti-Xa values.[64] Testing options to address this could include (1) an UFH-calibrated thrombin time test or (2) neutralizing the DOAC effect in vitro using aforementioned neutralizing products. In studies using drug-enriched plasma, DOAC-Stop extracts DOACs efficiently with no effect on heparin-type anticoagulants, but it binds argatroban and hirudin-type anticoagulants.[45] To date, data on the efficacy of UFH monitoring in the presence of such compounds or using thrombin time calibration curve are lacking.

DOAC and Thrombin Generation Assays

Global tests such as the TGA have been described as promising to assess the pharmacodynamic profile of anticoagulants.[65,66] Given the known DOAC thrombin generation profiles, the concentration thresholds proposed in the literature may provide highly different anticoagulant activities in a particular patient and TGA may be seen as another way of expressing and assessing the degree of anticoagulation in DOAC-treated patients (►**Fig. 2**).[24,66–71] The ST Genesia (Diagnostica Stago, Asnières sur Seine Cedex, France), an automated analyzer for thrombin generation testing has the potential for a wide implementation in routine laboratories. Preliminary observations showed that thrombin generation testing is affected by all anticoagulant drugs, suggesting that this assay could be useful in assessing DOAC activity, but this deserves further confirmation in larger cohorts to validate this approach since to date, the role of TGA for clinical decision-making in DOAC-treated patients is not clear.[68,70–72]

Limitations of Laboratory Testing

Previously, the ICSH DOAC Working Party provided provisional guidance for the effect of DOACs on commonly ordered coagulation assays.[1] The limitations for assessing DOAC presence, pharmacokinetics or pharmacodynamics using screening or global assays, or other coagulation tests are still present, although the use of neutralizing systems appears promising. DOAC-neutralizing systems have not been fully evaluated for all tests or test platforms and their use and interpretations must employ a degree of caution.[1] Local verification of in vitro neutralizing agents (activated charcoal or filters) to assure (1) adequate DOAC neutralization by using sensitive techniques and (2) no deleterious effect on the test method is required prior to clinical use.

Consensus Recommendations

- *Caution should be used when performing and interpreting the results from any coagulation test result from a DOAC-treated patient.*
- *In vitro use of DOAC-neutralizing agents must be used with caution and must be locally verified prior to clinical use.*
- *Select thrombophilia test methods (e.g., clot-based measurement of protein C or LA) can show interference at low DOAC concentrations. Use of DOAC-neutralizing products*

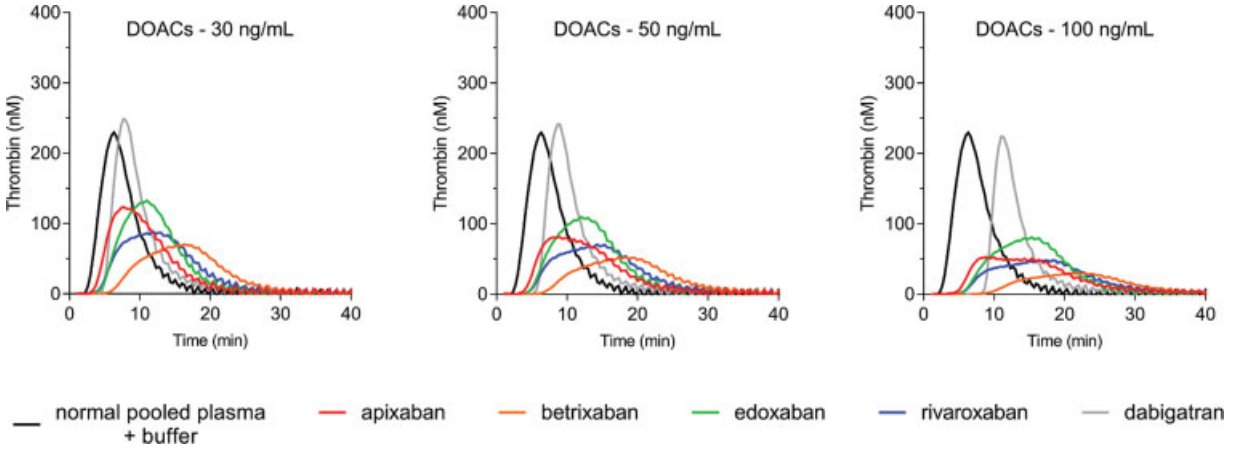

Fig. 2 The thrombogram parameters from thrombin generation test and representative changes at relevant concentrations of direct oral anticoagulants (DOACs). Note: Thrombin generation was triggered by 5-pM tissue factor with 4-μM phospholipids in absence of exogenous thrombomodulin or exogenous activated protein C.

may not completely achieve DOAC reversal; thus results from some assays may refrain factitiously elevated despite neutralization.

- *DOAC removal systems appear to be a suitable in vitro means of neutralizing DOAC from plasma to minimize drug interference in coagulation testing, although it is unclear whether these DOAC removal systems are interchangeable with DOAC neutralization products.*
- *Laboratories should have a procedure for adequately assessing and differentiating anticoagulation effect when bridging therapy is required.*
- *Although preliminary results are encouraging, there is currently no sufficient evidence to recommend TGAs to guide clinical decisions in a DOAC-treated patient.*
- *Laboratories should be aware of limitations of laboratory testing for DOAC measurements and/or effect of DOACs on coagulation assays.*

DOAC Reversal Agents

Andexanet Alfa

Since the initial publication of the guidance, a specific reversal agent, andexanet alfa, was approved in the United States for rivaroxaban and apixaban when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding, and has also been approved by the Committee for Medicinal Products for Human Use in Europe for the same application.[3,73] There are two dosing strategies (low and high dose), as a bolus followed by a continuous infusion. This reversal agent will reduce the levels of direct FXa inhibitors, as measured by calibrated chromogenic anti-Xa activity.[74] There is a transient rise in prothrombin F1 + 2 fragments and D-dimer value after andexanet alfa is given, and an increase in thrombin generation, which may be related to the observed concomitant TFPI inhibition.[2,3,75,76] Whether this observation is due to the inhibition of TFPI or whether it is clinically important is not yet known and deserves some caution.

Idarucizumab

Idarucizumab is a specific reversal agent for dabigatran and is indicated in adult dabigatran etexilate–treated patients with dabigatran etexilate when rapid reversal of its anticoagulant effects is required such as in emergency surgery or urgent procedures and in life-threatening or uncontrolled bleeding. It has been approved for the same indications in both the United States and Europe based on the results from the REVERSE-AD study, which showed the efficacy and safety of idarucizumab to reverse the anticoagulant effect of dabigatran in dabigatran-treated patients who experienced serious bleeding or required urgent invasive procedures.[77–79] Although the product has been on the market for more than 3 years, only a small case series and four large studies evaluated its safety and efficacy in a real-world setting.[80–84] No specific dabigatran monitoring is currently recommended before reversal or during follow-up according to the prescribing information as approved by the regulatory authorities.[77,78]

DOAC Reversal Agents and the Laboratory

As andexanet alfa reduces the DOAC level after bolus and/or infusion, but DOAC levels recover following cessation of infusion, it can be speculated that post-infusion coagulation tests may be affected (for rivaroxaban, the residual drug level after andexanet alfa treatment was ~40% from pre-treatment levels, a concentration that can still affect coagulation tests).[85] Evaluating post-infusion rivaroxaban or apixaban anti-Xa measurements is not supported by current Food and Drug Administration (FDA) recommendations as they indicate that the likelihood of using anti-FXa activity as a surrogate endpoint to predict a clinical benefit of haemostasis is not evident.[3] However, pre-treatment DOAC measurements may be warranted[86] to determine whether the low- or high-dose regimen should be used, as well as providing the potential to avoid unnecessary patient exposure to reversal antidotes.[87] However, this cannot be detrimental to the patients and should not delay the administration of reversal agents, especially in DOAC-treated patients with life-threatening bleeding, such as intracranial bleeding or in those requiring emergency surgery for life-threatening conditions such as a ruptured aortic aneurysm. In such context, rapid POC device with appropriate clinical performance is highly needed to guide the best strategy for patient's management.

It should be noted that the current dosing recommendations of andexanet alfa are based on both the dose and the time since the last intake of apixaban and rivaroxaban.[2,3] However, in an unconscious patient, such information cannot always be obtained. The plasma concentration of apixaban or rivaroxaban could be of interest in this context, but the definition of specific thresholds based on these plasma concentrations at the time of the admission is not yet available. Otherwise, specific tests are required in the unconscious patient to discriminate between the type of anticoagulant (IIa or Xa inhibitor) and could be useful to follow the efficacy of andexanet alfa administration. Several POC devices are currently under investigation that may prove useful in this setting (see the section on POC device below). Importantly, commercially available anti-FXa assays measure FXa inhibitors using drug-specific calibrators and controls. However, there are limitations when these assays are used for measuring DOAC concentration in andexanet alfa patient samples. One of the limitations is the large sample dilution in the assay set-up, which causes dissociation of the inhibitor from the andexanet alfa-inhibitor complex (due to the reversible binding equilibrium of the andexanet alfa inhibitor), resulting in an erroneous elevation of the anti-FXa activity following andexanet alfa administration. Therefore, some anti-Xa assays may have to be modified to be utilized if chromogenic anti-Xa assays are used to evaluate the degree of reversal of andexanet alfa.[88]

For dabigatran reversal, a single dose of idarucizumab (Praxbind, Boehringer Ingelheim) will bind up to 1,000 ng/mL of the drug, but there appears to be a rebound or dissociation effect after 12 to 24 hours. As such, measurements of dabigatran may predict the need for secondary dosing of this reversal agent.[19] In a retrospective study, it has been shown that the

assessment of dabigatran levels before introducing the reversal therapy could predict the haemostasis effectiveness and the potential rebound in dabigatran levels after idarucizumab injection and that specific dabigatran threshold (i.e., 264 ng/mL as reported in this study) may be of interest to predict haemostatic ineffectiveness, dabigatran rebound, and outcomes after reversal.[80] Idarucizumab has no known impact on coagulation parameters by itself.

Other agents that have been used for DOAC reversal include three- or four-factor prothrombin complex concentrates (PCCs) or activated PCCs.[89–94] These non-specific reversal agents are expected to have an impact on coagulation screening tests but not on anti-Xa- or anti-IIa-based assays, but data are currently limited with DOAC reversal strategies. The amount of PCC needed to stop DOAC-induced bleeding may depend on the residual DOAC concentration at the time PCC is administered.[95] However, although clinical bleeding may be sufficiently controlled with a single dose of PCC, the impact of DOAC on some laboratory tests may not be completely abolished as the relationship between residual DOAC level as measured by laboratory testing and the risk of uncontrolled bleeding is currently unclear.[18]

Consensus Recommendations

- *For andexanet alfa, due to its pharmacodynamic profile, the use of anti-Xa techniques for the evaluation of post-infusion rivaroxaban or apixaban anti-Xa activity is not supported.*
- *Post–andexanet alfa treatment, testing of apixaban and rivaroxaban concentrations is affected by anti-Xa methods that use high sample pre-dilutions causing factitiously elevated FXa DOAC results.*
- *For idarucizumab, measurements of dabigatran may predict the need for secondary dosing of this reversal agent since the presence of idarucizumab does not seem to interfere with dabigatran.*
- *It is currently unclear how to best assess the reversal efficacy of specific antidotes (i.e., andexanet alfa or idarucizumab) using laboratory tests and requires further investigation.*
- *PCC administration should not be monitored by measurement of DOAC concentrations that will not be modified.*
- *Assessment of DOAC reversal by global or specialized laboratory assays is method dependent and may be misleading.*

DOAC Point-of-Care Testing

The widespread use of DOACs and the need for urgent determination in aforementioned specific clinical situations have spurred several investigators and manufacturers to pursue POCT technologies for measuring (or quantifying) DOAC effect.[96–98] Included are microfluidic technologies[98–101] and surface acoustic wave (SAW) technologies.[102] Although the preliminary findings are promising, shortcomings include use of an animal model,[101] or in vitro enriched DOAC blood,[99] data from a small series of patients[99,100] and only a limited number of DOACs assessed.[99,101,102] In addition, these methods appear to be several years from actual clinical implementation, as none have undergone the rigors of in vitro device (IVD) clinical trials.

The TEG 6s NOAC assay is a cartridge currently undergoing clinical trials which can be used for qualitative DOAC assessment.[103–105] The four-channels, single-use NOAC cartridge contains kaolin in channel 1, ecarin in channel 2, FXa in channel 3, and abciximab in channel 4, with channels 2 and 3 providing differentiation in DOAC effect of prolonged clotting times. In a small series of patients receiving dabigatran, rivaroxaban, or apixaban, the receiver operating characteristic (ROC)analysis yielded a sensitivity of 94 and 92% for channel 2 (dabigatran) and channel 3 (direct FXa inhibitors), respectively.[105] Since the last publication,[1] Harenberg et al published recommendations regarding the use of a urine dipstick device which was shown to be sensitive and specific to determine the presence of both FXa and factor IIa inhibitors in urine samples. The evaluation of the DOAC dipstick test in emergency medicine and other patient groups is currently ongoing. This device allows qualitative determination of direct thrombin or FXa inhibitors and may aid in generating algorithms for clinical decision-making in a bleeding patient or for a patient requiring urgent surgical intervention in conjunction with laboratory plasma-based assays.[106,107] However, cautious and informed use of this urine DOAC screening method is required, as there is no direct relationship between plasma and urine DOAC concentrations despite the excellent sensitivity and specificity of the device. In any case, if DOAC is detected in the urine by the dipstick device, it should be confirmed with more specific testing to confirm the presence of DOAC in the blood.

Although not specifically a POCT, dried blood spot (DBS) technology may be a suitable alternative to traditional blood collection for non-emergent assessment of DOACs.[98,108] This method would allow for at-home collection using finger stick blood collection onto filter paper, which is then sent via postal service to a laboratory that can provide a quantitative DOAC level determination using tandem mass spectrometry. However, it must be emphasized that mass spectrometry testing using DBS must also be validated using DBS-collected samples. In addition, the haematocrit level of blood may cause systematic bias in analyte measurement in DBS samples, and it is also a practical challenge to train and ensure appropriate DBS collection procedures being performed by in-home patients since inappropriate DBS collection can cause significant variability in assay measurement. However, volumetric absorptive microsampling (VAMS), a recent microsampling technique used to obtain dried specimens of blood, promises to bring some significant advantages over DBS, related to sampling volume accuracy, haematocrit (HCT) dependence, pre-treatment and automation.[109] We also must emphasize that the lack of availability of liquid chromatography with tandem mass spectrometry (LC-MS)/MS in smaller laboratories, long turnaround time, cost and labour-intensive sample preparation restrict the use of this strategy in most laboratories. However, if the testing is not urgent, the VAMS collection device can be sent to a reference laboratory which can provide standardized and validated DOAC analyses overcoming the potential geographical limitations.[110]

Consensus Recommendations

- *Tests and technologies of various POCT devices may provide totally different type of results.*
- *Global coagulation POCT like SAWs and thromboelastometry are promising for identifying the drug on board, but their usefulness to evaluate the degree of anticoagulation is still unclear and further investigations are warranted.*
- *Rapid urine testing may rapidly identify the DOAC type taken, which may assist clinical decision-making.*
- *DBS and VAMS technology may be of interest to perform pharmacokinetic investigations without suffering from geographical limitations and rapid access to specialized laboratories.*

External Quality Control

Most international external quality control (EQA) programmes now have established EQA exercises for DOACs and demonstrate a wide implementation of specific DOAC testing in certain regions of the world.[111–118] Nevertheless, in regions where the regulatory authorities have refused the approval of these specific kits, access to drug measurements may be limited to specialized laboratories. In addition, only few undertake the in-house validation of these techniques refraining the clinicians to ask for these specific drug measurements. This is detrimental to the patients, especially knowing the limitations of routine coagulation tests for DOAC testing which are used instead. These routine tests showed a poor analytical and clinical performance in the different clinical settings where DOAC measurement may be beneficial.[6,119]

Some international EQA programmes have also undertaken and published studies looking at DOAC interference in haemostasis tests.[111–118] Some have also undertaken studies looking at neutralizing the interference of DOACs in haemostasis tests.[120] Although differences were seen between the various methodologies, reliable and reproducible DOAC levels were measured overall. A 5-year overview of experience for the quality performance of DOACs over a large concentration range showed a good correlation between the different methodologies. Although no international calibration standards were available, the overall coefficients of variation (CVs) were small for dabigatran, rivaroxaban and apixaban, and were also comparable to the CVs (range: 3–14%) for the international normalized ratio derived from the same years.[113]

The outcome for the various methodologies in the EQA surveys could be used to establish clinical decision rules adapted for specific reagents. This is especially relevant in the ranges approaching clinical decision limits. Laboratories are strongly encouraged to participate in EQA programmes that adequately address the pharmacodynamics and pharmacokinetics of DOACs, as well as the identification of DOAC sources of interference in other coagulation assays.

Consensus Recommendations

- *Laboratories are strongly encouraged to participate in EQA programmes that assess DOAC effects on screening tests and quantitative measurements, as well as their interference in other coagulation assays.*
- *Collecting information on DOAC testing availability and performance around the world is necessary to help various working parties to provide guidelines.*

Future Perspectives

emergence of DOACs and their increased use as well as the introduction of anticoagulants in future will provide a challenge for clinical laboratories. It is likely that DOAC use will increase as clinical trials are currently in the process for paediatric use. Dabigatran use in paediatric patients with VTE demonstrated non-inferiority to standard treatment.[121] Rivaroxaban use in paediatric patients (Einstein-Jr clinical trial, NCT02234843) is completed and awaiting approval for use in cerebral venous thrombosis[122] and catheter-related VTE.[123] Summary of the use of rivaroxaban in the paediatric population is also available elsewhere.[124] Apixaban is being evaluated in VTE reduction in paediatric patients with congenital heart disease[125] and acute lymphoblastic.[126] Edoxaban is currently under investigation for use in paediatric patients at risk of thromboembolic complications due to heart disease (www.clinicaltrials.gov; NCT 03395639). Other DOAC clinical trials include use for VTE prevention in patients with cancer (clinicaltrials.gov; NCT03240120; NCT03692065), post-bariatric surgery (clinicaltrials.gov; NCT03522259; NCT02406885), SARS-CoV-2 infections (clinicaltrials.gov; NCT04757857; NCT04650087; NCT04542408) and others will likely increase the use of DOACs once efficacy has been established.

In addition to the increase in use of DOACs in multiple settings with unclear expected "on-therapy" ranges and drug detection requirements, other technical considerations and concerns for the clinical laboratory would be the other anticoagulants under investigation.[127] As these drugs effect in vivo anticoagulation, it is likely their ex vivo effect will also add another layer of complexity and concern for the clinical coagulation laboratory. The hope and promise of POC methods with increased sensitivity and specificity for novel anticoagulants, including DOACs, may alleviate some burden on the laboratory.

What is known about this topic?

- Direct oral anticoagulants are used worldwide for several thromboembolic indications.
- The 2018 ICSH document provided haemostasis-related guidance for clinical laboratories.
- This study addressed all phases of laboratory DOAC measurements.

What does this paper add?

- This guidance updates the 2018 edition with a particular focus on antidotes, POCT and global coagulation tests.

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Conflict of Interest
Among the authors, J.D. is the CEO and founder of QUALIblood s.a., a contract research organization manufacturing the DP-Filter, is a co-inventor of the DP-Filter (patent application number: PCT/ET2019/052903) and reports personal fees from Daiichi Sankyo, Mithra Pharmaceuticals, Stago, Roche and Roche Diagnostics outside the submitted work. E.L.-L received lecture fees and consulting fees from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb-Pfizer, Daiichi Sankyo, Portola, CSL Behring, Leo and Aspen. She received external funds for conducting a clinical contract study from Bayer and Daiichi Sankyo, for a research project that she initiated from Bayer AG, Bristol-Myers Squibb-Pfizer, Daiichi Sankyo and CSL Behring. E.J.F. and S.M.B. have no conflict of interest. I.G.T. received consulting fees from Bayer, Boehringer Ingelheim and Bristol-Myers Squibb-Pfizer. R.C.G. reports personal fees from Diagnostica Grifols, Siemens Healthcare Diagnostics and Diagnostica Stago, and has provided expert testimony on dabigatran and rivaroxaban testing.

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