

# 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
- ▶ laboratory guidance
- ▶ recommendations

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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.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup> 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.<sup>2,4</sup> Dabigatran competitively and irreversibly inhibits free and fibrin-bound thrombin by binding to the thrombin active site.<sup>2,4-7</sup> Usually given twice daily, dabigatran dosing

(75,110 or 150 mg) is based on indication, patient's age, and patient's renal function.<sup>2,8</sup> 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<sup>2-4,8,9</sup> (►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.<sup>10</sup>

### 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.<sup>11</sup> Rivaroxaban also inhibits FXa bound to the clot, in a concentration-dependent mechanism.<sup>12</sup> Rivaroxaban is a competitive inhibitor of FXa, with high selectivity of more than 10,000-fold over other serine proteases.<sup>13</sup> Rivaroxaban is absorbed rapidly, reaching peak plasma concentrations in 2 hours<sup>14</sup> (►Table 1) and maximum inhibition of FXa activity between 1 and 4 hours after dosing.<sup>14,15</sup> 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.<sup>14</sup> Rivaroxaban is a substrate of the efflux transporter P-gp and is metabolized by the CYP3A4 isoenzyme.<sup>16</sup>

### Apixaban (Eliquis, Bristol-Meyers Squibb)

Apixaban is a direct, reversible inhibitor of FXa administered orally twice daily as active drug.<sup>2</sup> In humans, eight metabolites have been identified, none of which appear to be active.<sup>17</sup> 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.<sup>18</sup>

**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 <sup>a</sup>	12–14 h	5–13 h	12 h	10–14 h

Abbreviation: Tmax, time to peak drug concentration after dose.

<sup>a</sup>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.<sup>19</sup>

**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.<sup>20,21</sup> Edoxaban inhibits free FXa, as well as that within the prothrombinase complex. The recommended dose varies by indication and renal function<sup>21-29</sup> (►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.<sup>20,21</sup> The absolute bioavailability is approximately 62%.<sup>20,21</sup> 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%).<sup>20,21</sup> 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.<sup>20,21</sup>

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.<sup>3,30</sup> 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.<sup>31</sup> 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.<sup>32</sup> Serum measurements

**Table 2** Expected peak and trough DOAC concentrations in patients treated for stroke prevention in NVAf or treatment of PE/VTE<sup>1,4,14,15,19,26-28</sup>

Indication	Dabigatran		Rivaroxaban		Apixaban		Edoxaban	
	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 <sup>a</sup> (117-275)	175 <sup>a</sup> (117-275)	249 <sup>b</sup> (184-343)	270 <sup>b</sup> (189-419)	171 <sup>c</sup> (91-321)	132 <sup>c</sup> (59-302)	170 <sup>d</sup> (125-245)	234 <sup>e</sup> (149-317)
Trough concentration, ng/mL	91 <sup>a</sup> (61-143)	60 <sup>a</sup> (39-95)	44 <sup>b</sup> (12-137)	26 <sup>b</sup> (6-87)	103 <sup>c</sup> (41-230)	63 <sup>c</sup> (22-177)	36 <sup>e</sup> (19-62)	19 <sup>e</sup> (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.

<sup>a</sup>Mean (25th-75th percentile).

<sup>b</sup>Mean (5th-95th percentile).

<sup>c</sup>Median (5th-95th percentile).

<sup>d</sup>Median (1.5 x IQR).

<sup>e</sup>Median (IQR).

tended to be higher than plasma measurements when chromogenic anti-FXa methods are used,<sup>32</sup> 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.<sup>33,34</sup> 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).<sup>33,34</sup> However, there is a 4-hour stability for dabigatran when using the thrombin time test.<sup>35</sup> 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.<sup>33</sup> 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,<sup>34</sup> 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.<sup>33,34</sup> Data for assessment of apixaban and dabigatran are conflicting,<sup>33,34</sup> 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.<sup>32–34</sup> Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) can use serum or plasma.<sup>32,34</sup>
- 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.<sup>35</sup>
- 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.<sup>33,34</sup>
- Data would suggest that at least three freeze–thaw cycles could be performed without significant loss of activity.<sup>33,34</sup>

## 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.<sup>36</sup> 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.<sup>7,30,37–48</sup> 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.<sup>39,40,46,47</sup> 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.<sup>2,48–50</sup> The PT may be normal (ratio <1.20) with apixaban concentrations up to 200 ng/mL.<sup>46,47,49,50</sup> The PT is more sensitive than the APTT to edoxaban, with insufficient sensitivity at low on-therapy (~30 ng/mL) drug levels.<sup>51–53</sup> The prolongation of the PT is concentration- and reagent-dependent,<sup>54,55</sup> with edoxaban concentrations required to double the PT varying from 97 to 296 ng/mL.<sup>55</sup>

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.<sup>56</sup> Although efforts to standardize PT methods by creating an ISI for rivaroxaban, analogous to the ISI for VKAs,<sup>57</sup> 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.<sup>7,30,37–45</sup> Commercial APTT reagents differ in their sensitivity, with a required dabigatran concentration of approximately and 400 ng/mL to produce a twofold prolongation in the APTT.<sup>41,52</sup> 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.<sup>46,47</sup> The APTT shows a concentration-dependent prolongation of clotting times followed by a plateau at approximately 200 ng/mL apixaban.<sup>50</sup> 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.<sup>54</sup>

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.<sup>47,52,58,59</sup> 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.<sup>60,61</sup> 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)<sup>30,40–44,50,51,53,62</sup> (– **Table 3**).

**Table 3** DOAC interference on coagulation assays<sup>3,33,40,50,51,53,62,111,123,138</sup>

	Dabigatran		Anti-Xa DOACs		Clinical impact of reported test result
	Clot-based assays	Chromogenic-based assays	Clot-based assays	Chromogenic-based assays	
Relationship between prolonged clotting time and increased drug concentration	PT/INR <sup>a,b</sup> APTT <sup>a,b</sup> Thrombin time Ecarin-based assays		PT/INR <sup>a,b,c</sup> APTT <sup>a,b,c</sup>		Diagnosis and/or Management
Relationship between DOAC presence and factitiously decreased reported result	Fibrinogen <sup>b,d</sup> Factor activity <sup>a</sup> (II, V, VII, VIII, IX, X, XI, XII)		Factor activity <sup>a,b,c</sup> (II, V, VII, VIII, IX, X, XI, XII)	Factor VIII <sup>b</sup> Factor IX	(Mis)Diagnosis and/or (Mis)Management
Relationship between DOAC presence and factitiously increased reported result	Inhibitor screen <sup>a,b</sup> Inhibitor assay <sup>a,b</sup> Lupus anticoagulant <sup>a</sup> Protein C activity <sup>a,b</sup> Protein S activity <sup>a,b</sup> APCR <sup>a,b</sup>	Antithrombin <sup>b</sup> (thrombin substrate)	Inhibitor screen <sup>a,b,c</sup> Inhibitor assay <sup>a,b,c</sup> Lupus anticoagulant <sup>a,b</sup> Protein C activity <sup>a,b</sup> Protein S activity <sup>a,b</sup> APCR <sup>a,b,c</sup>	Antithrombin <sup>b</sup> (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

<sup>a</sup>Reagent dependent.

<sup>b</sup>Concentration dependent.

<sup>c</sup>Apixaban usually not affecting result.

<sup>d</sup>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.<sup>2,35,63</sup> A normal TT suggests that little or no dabigatran is present, but a prolonged TT does not necessarily equate to a high dabigatran level.<sup>2,3,63</sup>

### 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.<sup>64,65</sup> There was a strong correlation between rivaroxaban or apixaban concentrations and LowTF-ROTEM CFT and time to maximum velocity;<sup>66</sup> yet, others report ROTEM as insensitive to detect residual rivaroxaban activity in patients.<sup>65,67</sup> An ecarin-base ROTEM has been recently reported to be sensitive to low levels of dabigatran.<sup>68</sup> These viscoelastic measurements of DOACs perform better on peak samples, lose sensitivity for trough samples, with limited findings due to small sample size.<sup>69,70</sup> 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.<sup>48,59,71–73</sup> 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.<sup>73</sup> 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);<sup>74</sup> and (5) the relatively high repeatability precision ( $\sim 13\%$  at a 2.1 INR) noted with this method.<sup>74</sup>

DOAC screening assays using urine samples have been evaluated, but no correlation was demonstrated between urine and plasma DOAC concentrations.<sup>75</sup> A commercial urinary test screening for renal function (creatinine), anti-Xa and direct thrombin inhibitor is soon to be released in Europe.<sup>76</sup> 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.<sup>2,3,7,30,37–56,58,59</sup>
- PT and APTT are not responsive to 'on-therapy' apixaban levels.<sup>49–52</sup>
- The PT and APTT should not be used to quantify DOAC concentration.<sup>2,3</sup>
- 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.<sup>3,30</sup>

- A normal TT excludes the presence of significant dabigatran concentration.<sup>2,3,30,63</sup>
- 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.<sup>64–69</sup>
- Nonspecific POCT methods may not have sufficient responsiveness to detect DOAC presence.<sup>48,59,69–73</sup>
- 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.<sup>75</sup>

### 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.<sup>77,78</sup> 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.<sup>8,79–82</sup> 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.<sup>83–88</sup>

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.<sup>89</sup> 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.<sup>78</sup> 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 pro-drug 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.<sup>8,87</sup> A similar pattern is seen with edoxaban and its M4-metabolite which is also pharmacologically active.<sup>82</sup>

Major limitations of LC-MS/MS include the absence of standardization or harmonization of mass spectrometry-based assays,<sup>90</sup> 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).<sup>91</sup> 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.<sup>8,79-82</sup>
- A suitable internal standard for each DOAC is mandatory.<sup>78</sup>
- DOAC metabolites, that are pharmacologically active, should be reported.<sup>3,8,82,87</sup>

### 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<sub>2</sub>, with equal volumes of reagent to plasma used for testing.<sup>92</sup> For high drug concentrations, the patient plasma is diluted 1:1 with normal pooled plasma (NPP). The reported imprecision is less than 5%.<sup>92</sup> Fibrinogen and prothrombin (factor II) deficiencies may impact the accuracy of the test.<sup>93,94</sup> There is reported linear relationship between dabigatran concentration and ECT results and, with use of commercial calibrators, good correlation with LC-MS/MS measurements.<sup>95</sup>

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.<sup>96</sup> When the ECA is calibrated using commercial dabigatran material, there is good correlation with LC-MS/MS,<sup>91</sup> reported LLOD ranging from 14 to 46 ng/mL, within-run imprecision of less than 5%<sup>83</sup> and between-run imprecision of 6 to 16% using quality control material.<sup>83,95,97,98</sup>

#### 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.<sup>99</sup> 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.<sup>35,97-99</sup>

#### 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.<sup>100,101</sup> 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).<sup>94,95,97</sup> In patients treated with dabigatran, a strong correlation between dTT and LC-MS/MS have been reported.<sup>84,97</sup> Commercial assays report LLOD ranges of 2 to 8 ng/mL and LLOQ ranges of 20 to 30 ng/mL.<sup>83,97,101-104</sup> 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).<sup>97</sup>

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.<sup>84,95,97,99,101,102</sup>

### 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.<sup>40,43,49,50,53,59,83,85,98,105–121</sup> *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,<sup>40</sup> while for apixaban and edoxaban, some authors reported lower thresholds (i.e. 15 and 10 ng/mL, respectively).<sup>47,53</sup> 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.<sup>119</sup> 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.<sup>110,120</sup>

*Ex vivo* studies have highlighted limitations with rivaroxaban and apixaban measurement, with an LLOQ of around 30 ng/mL for both molecules.<sup>85,88,108,120</sup> For edoxaban, an *ex vivo* study measuring the anti-Xa activity calibrated with heparin standards revealed a good correlation with LC-MS/MS measurements.<sup>20</sup> 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.<sup>53</sup>

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.<sup>88,108,121–123</sup> Results below the assay's LLOQ suggest that no or clinically insignificant concentrations of FXa DOACs are present. However, due to kit differences in chromogenic substrates, factor FXa origin, methodologies and heparin calibration, the use of heparin-calibrated C-FXa assays should be used with caution.<sup>123,124</sup> All heparin-calibrated methods may not be equally sensitive to a similar direct FXa level.<sup>124</sup>

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.<sup>40,43,49,50,53,59,83,85,88,105–118</sup>

- 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.<sup>110,120</sup>

### 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.<sup>75,76,125–127</sup> 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.<sup>128</sup> More recently, another microfluidic method has been described in stroke patients.<sup>129</sup> This POC method with sensitivity to warfarin, dabigatran and rivaroxaban, but not apixaban, employs surface acoustic waves that detect prolonged coagulation times.<sup>129</sup> The SPOCT-NOAC trial<sup>130</sup> 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<sup>131–133</sup> and laboratories,<sup>134–136</sup> 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.<sup>135,136</sup> 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.<sup>137</sup> 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.<sup>132,133,137</sup>

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<sup>131,136,137</sup> 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.<sup>131</sup> 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.<sup>131</sup> 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.<sup>131</sup> Extrapolation of data above or below a calibration curve is not recommended.<sup>131</sup> The validity of the calibration curve should be assessed by measuring samples with defined DOAC concentration limits.<sup>137</sup> 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.<sup>132,133</sup> 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.<sup>137</sup> Limits have been described as a CV of less than 15%, except at LLOQ, where the limit would be less than 20%.<sup>131</sup> 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 carry-over, if an automated analyser is used.

Linearity is determined using at least five to six samples tested over the reportable range.<sup>132-134</sup> 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.<sup>132-134</sup> Alternatively, the deviation of more than 20% from low concentrations of drug can be determined from linearity studies.<sup>131</sup> The replicate determinations for LLOQ and LLOD range from 6 to 10 samples.<sup>135,136</sup>

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,<sup>131</sup> 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,<sup>131</sup> but other statistical analyses such as bias determination<sup>136</sup> or paired *t*-test<sup>135</sup> 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  $\pm 15\%$ .<sup>137</sup> 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,  $-80^{\circ}\text{C}$ ,  $-20^{\circ}\text{C}$ , room temperature and time on-instrument studies.<sup>137</sup> 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.<sup>137</sup> 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.<sup>132,133,136</sup>

Consensus recommendations for method validation or verification of performance:

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

### 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).<sup>51,138</sup> 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 Siemens 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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