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Interlaboratory Performance in Measurement of Dabigatran and Rivaroxaban

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Interlaboratory Performance in Measurement of Dabigatran and Rivaroxaban

Results of the College of American Pathologists External Quality Assessment Program

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• ***Context.***—**Assessing direct oral anticoagulant (DOAC) drug levels by reliable laboratory assays is necessary in a number of clinical scenarios.**

Objective.—**To evaluate the performance of DOAC-specific assays for various concentrations of dabigatran and rivaroxaban, assess the interlaboratory variability in measurement of these DOACs, and investigate the responsiveness of the routine clotting assays to various concentrations of these oral anticoagulants.**

Design.—**College of American Pathologists proficiency testing survey data from 2013 to 2016 were summarized and analyzed.**

Results.—**For dabigatran, the interlaboratory coefficient of variation (CV) of ecarin chromogenic assay was broad (ranging from 7.5% to 29.1%, 6.3% to 15.5%, and 6.8% to 9.0% for 100-ng/mL, 200-ng/mL, and 400-ng/mL targeted drug concentrations, respectively). The CV for diluted thrombin time for dabigatran was better overall (ranging from 11.6% to 17.2%, 9.3% to 12.3, and 7.1% to 11.2% for 100 ng/mL, 200 ng/mL, and 400 ng/mL, respectively). The rivaroxaban-calibrated anti-Xa assay CVs also showed variability (ranging from 11.5% to 22.2%, 7.2% to 10.9%, and 6.4% to 8.1% for 50-ng/mL, 200-ng/mL, and 400-ng/mL targeted drug concentrations, respectively). The prothrombin time (PT) and activated partial thromboplastin time (aPTT) showed variable dose- and reagent-dependent responsiveness to DOACs: PT was more responsive to rivaroxaban and aPTT to dabigatran. The undiluted thrombin time showed maximum prolongation across all 3 dabigatran concentrations, making it too sensitive for drug-level monitoring, but supporting its use as a qualitative screening assay.**

Conclusions.—**DOAC-specific assays performed reasonably well. While PT and aPTT cannot be used safely to determine DOAC degree of anticoagulation, a normal thrombin time excludes the presence of dabigatran.**

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In 2010, the first direct oral anticoagulant (DOAC) agent, dabigatran, was introduced into clinical practice in the United States.[1] Since then, the use of DOACs has grown exponentially in the United States and the rest of the world owing to their ease of use and favorable safety profiles.[2] DOACs approved in both the United States and Europe include direct thrombin inhibitors (DTIs; eg, dabigatran) and direct coagulation factor Xa (DXa) inhibitors (eg, rivaroxaban, apixaban, and edoxaban). The advantages of these drugs include their oral administration, their use at fixed dosage for most patients, and their predictable pharmacokinetics. Unlike traditional anticoagulant agents, such as warfarin and unfractionated heparin, DOACs do not require

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routine coagulation monitoring or dose titration. However, with their broadening and increasing use, it has become evident that assessing DOAC drug levels by reliable laboratory assays is necessary under certain clinical scenarios, which include before emergent surgery, evaluation of patients' compliance to DOAC therapy, potential overdose, unexpected bleeding or thromboembolic events while on DOAC, or assessing the efficacy of reversal agents.[3,4] Furthermore, since DOACs may interfere with many clot-based coagulation assays, causing erroneous results (eg, lupus anticoagulant, mixing studies, factor assays),[5] laboratories also need to know the impact of these oral anticoagulant medications on their assays and have methods in place to alert clinicians to the potential for inaccurate laboratory results. Although several manufacturers have developed DOAC-specific tests to measure DOAC plasma levels, only HemosIL Liquid Anti-Xa (Instrumentation Laboratory Co, Bedford, Massachusetts) for apixaban measurement has been recently US Food and Drug Administration (FDA) approved in the United States.[6]

The aims of this study were (1) to evaluate the performance of various DOAC-specific assays across different concentrations of dabigatran and rivaroxaban, (2) to assess the interlaboratory variability in measurement of these DOACs, and (3) to investigate the responsiveness of the routine clotting assays to various concentrations of these drugs. The results of 8 anticoagulant monitoring (ACM) proficiency testing surveys for dabigatran and rivaroxaban that were provided to participating laboratories from 2013 to 2016 by the College of American Pathologists (CAP) were reviewed and analyzed.

MATERIALS AND METHODS

CAP Direct Oral Anticoagulants Surveys

The CAP Proficiency Test Surveys program is the largest CLIA (Clinical Laboratory Improvement Amendments)–approved external quality assessment program in North America, and it also provides proficiency testing worldwide. Since 2013, the CAP Hemostasis and Thrombosis Committee (HaTC) started offering ACM surveys for the first FDA-approved DOACs: dabigatran and rivaroxaban.

Although most participants in the DOAC surveys were from North America, several international laboratories have subscribed for this survey since 2015. Each survey contained samples of lyophilized normal pooled plasma spiked with either dabigatran (3 samples) or rivaroxaban (3 samples) at drug concentrations determined by the Committee. The drug concentrations were verified by using STA Liquid Anti-Xa (Diagnostica Stago, Inc) for rivaroxaban and diluted thrombin time (dTT) by Hemoclot (Hyphen-BioMed) for dabigatran assays to be within ±10% of the target drug levels. The DOACs' concentrations were unknown to the participating laboratories. Laboratories performed testing according to their regular patient testing workflow and laboratory standard operating procedure. The results were submitted to the CAP, and the summarized data were reviewed by the HaTC. Results for the ACM surveys were not formally graded.

Dabigatran.—Target dabigatran concentrations of approximately 100 ng/mL, 200 ng/mL, and 400 ng/mL were selected. Drug-specific dTT and ecarin chromogenic assay (ECA) were evaluated. The dabigatran survey also included the following routine coagulation assays: prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT).

Diluted thrombin time is a modified TT method for the quantitative measurement of DTI. The assay has 2 dilution schemes to improve lower detection limits. The test plasma is first diluted 1:10 with imidazole buffer, then 1 part of the dilution is added to 2 parts of normal pooled plasma. Clotting is then initiated by adding an excess of human α-thrombin, and the clotting time is measured. The time to clot formation is directly proportional to the concentration of the DTI present in the plasma. The assays are calibrated with a specific DTI, such as dabigatran.[7,8] For the survey, the samples were sent as lyophilized specimens, and participants were asked to reconstitute the material by using 1.0 mL of distilled or deionized water.

Ecarin chromogenic assay is another method for the quantitative measurement of DTI. Ecarin (*Echis carinatus* viper venom) converts prothrombin to meizothrombin, an intermediate proteinase, whose activity can be inhibited by DTI, but not by heparin. The meizothrombin induces clotting via fibrinogen cleavage to fibrin. Prolongation in the clotting time increases in a linear fashion with increasing concentration of DTI. The ecarin chromogenic assay uses a similar approach, but the concentration of meizothrombin is measured by using a chromogenic substrate.[9–11]

Rivaroxaban.—Target rivaroxaban drug concentrations of approximately 50 ng/mL, 200 ng/mL, and 400 ng/mL were chosen. Chromogenic anti-Xa assays were used to determine rivaroxaban levels. The rivaroxaban surveys also included the following routine coagulation assays: PT and aPTT.

Drug-specific anti-Xa chromogenic assays measure the concentrations of anticoagulants that inhibit factor Xa indirectly via antithrombin (unfractionated heparin, low-molecular-weight heparin, or fondaparinux) or directly (rivaroxaban, apixaban, and edoxaban). There are several commercially available chromogenic anti-Xa assays, all of which determine the extent of factor Xa inhibition by measuring the ability of anticoagulant to cleave a chromophore from the factor Xa substrate added to the specimen.[12,13] This reaction produces a colorimetric change that can be measured by a spectrophotometer or an optical coagulation analyzer. The degree of the color change is inversely proportional to the concentration of anticoagulant in the sample. A standard curve with known amounts of anticoagulant tested (rivaroxaban) is used by the analyzer to calculate the drug concentration.[11]

Statistical Analysis

ACM survey testing challenges were sent to participating clinical laboratories twice per year. Proficiency testing results reported from 8 surveys from 2013 through 2016 (A and B mailings) were collected and analyzed in this study. Each mailing was examined separately, and observations were removed if they had no data relating to dabigatran or rivaroxaban. All analyses were conducted with SAS (Cary, North Carolina). Outliers were identified by 1 of 2 methodologies, either a 2*interquartile range (IQR) or a 2-pass 3 standard deviation (SD) scheme. Evaluation to validate the assumption of combining samples by lot was done by analysis of variation (ANOVA) at an α level of .05.

Dabigatran.—The number of participating laboratories with each dabigatran mailing slightly varied, but overall has remained the same, on average 22 laboratories per mailing. A total of 576 dabigatran assay observations were recorded. Each lot has 2 mailings by year. The distributions of all mailing results showed a Gaussian distribution. Only dTT and ECA methods were considered, based on the limited number of observations per mailing. A participating laboratory was able to submit results only for 1 method. For outliers initially, 8 observations were removed, which were reported by participants as greater than their upper level of detection. Nine values were missing or had an invalid method. Second, a total of 21 observations using the HPLC-MS method across all mailings were removed. The remaining data were put through a 2-pass 3 SD outlier screen, which removed 13 outliers of 576. An outlier screen by IQR rule was also looked at for consistency but ultimately not used.

Rivaroxaban.—The number of participants for rivaroxaban increased with each mailing (9 laboratories for 2013 ACM-A and 25 laboratories for 2016 ACM-B). A total of 456 rivaroxaban assay observations were recorded. All survey results except for the results of the 2016 B mailing showed a Gaussian distribution. Only the chromogenic anti-Xa method was considered because all other methods had a single observation per specimen per mailing. First,

Table 1. Dabigatran Concentration Statistics by Method and Lot Number						
Dabigatran Target Values	Lot	Method	N Obs	Mean	SD	CV
100 ng/mL	1[a]	Diluted thrombin time	41	109.1	18.8	17.2
		Ecarin chromogenic	9	91.2	26.6	29.1
	2[a]	Diluted thrombin time	38	100.6	14.8	14.7
		Ecarin chromogenic	11	84.5	12.2	14.5
	3	Diluted thrombin time	33	105.0	12.2	11.6
		Ecarin chromogenic	9	103.1	12.7	12.4
	4	Diluted thrombin time	33	102.8	17.2	16.7
		Ecarin chromogenic	16	106.1	7.9	7.5
200 ng/mL	5[a]	Diluted thrombin time	40	220.5	27.1	12.3
		Ecarin chromogenic	9	198.6	30.7	15.5
	6[a]	Diluted thrombin time	38	202.8	24.1	11.9
		Ecarin chromogenic	11	179.8	20.5	11.4
	7	Diluted thrombin time	34	201.0	18.7	9.3
		Ecarin chromogenic	9	189.6	11.8	6.3
	8	Diluted thrombin time	31	203.8	23.2	11.4
		Ecarin chromogenic	16	197.6	21.0	10.6
400 ng/mL	9	Diluted thrombin time	40	381.1	42.5	11.2
		Ecarin chromogenic	9	382.3	26.5	6.9
	10	Diluted thrombin time	37	384.1	41.0	10.7
		Ecarin chromogenic	11	377.1	25.6	6.8
	11	Diluted thrombin time	34	375.8	26.8	7.1
		Ecarin chromogenic	8	391.6	28.5	7.3
	12	Diluted thrombin time	31	395.2	40.9	10.4
		Ecarin chromogenic	15	394.9	35.6	9.0

Abbreviations: CV, coefficient of variation; Obs, observations; SD, standard deviation.

[a] Statistcally different between the 2 methods ($P < .05$).

there were 9 observations removed, which were reported by participants as greater than their upper level of detection or missing method. Second, a total of 21 observations using the HPLC-MS method across all mailings were removed. The remaining data were put through a 2*IQR (IQR, percentile [P] 75–P25) outlier screen, which removed 8 outliers of 456, including 2 more lot-5 outliers removed by a 1.5*IQR rule.

For the DOAC-specific assays, data were combined by lot numbers and then separated by methods. For dabigatran, the mean, SD, and coefficient of variation (CV) were determined for results from each challenge type (≈100-ng/mL, ≈ 200-ng/mL, and ≈ 400-ng/mL dabigatran) for each dabigatran assay method (dTT and ECA). Means and SDs between the 2 methods were also compared. Two-sided *t* tests were used to test for differences in the 2 method means for each dabigatran lot at an α level of .05.

For rivaroxaban, the mean, SD, and CV were determined for results from each challenge type (≈50-ng/mL, ≈200-ng/mL, and ≈400-ng/mL rivaroxaban) for chromogenic anti–factor Xa method.

Routine Coagulation Assays.—For the routine coagulation assays, participants were separated into groups reporting results using the same reagent/instrument platforms for comparison. There were a total of 9 instruments reported, but only the 4 major instrument-reagent combinations were included for analysis. The *t* tests were performed to validate the combining of both major Diagnostica Stago, Inc, instrument/reagent combinations for PT and aPTT at each drug concentration for both drugs by using a Bonferroni-corrected significance level of $P < .02$ (due to 3 drug concentration tests). Except for PT for dabigatran assay, for all other assays, Stago STA Compact and Stago STA-R/Evolution were combined. Lastly, ANOVA was used to compare the instrument reagent means for PT and aPTT at each drug concentration for both drugs by using a Bonferroni corrected significance level of $P < .02$. The performance of the following routine coagulation assays was assessed: PT, aPTT, and TT.

Although the ACM results were not graded, we assessed the interlaboratory variation with CV as previously reported (<5% = very good, 6%–10% = good, 11%–20% = intermediate, and >20% = poor interlaboratory variation).[14]

RESULTS

Dabigatran Testing

In 2013, a total of 25 laboratories participated in the dabigatran survey, and this total number of participants remained stable through 2016. The methods used for dabigatran measurement included dTT and ECA, with dTT being the most widely used method. The results are summarized in Table 1.

Plasma With Estimated Dabigatran Concentration of 100 ng/mL.—For the ECA method, the mean dabigatran concentration ranged from 84.5 ng/mL (lot 2) to 106.1 ng/mL (lot 4) with the interlaboratory CV ranging from 7.5% (lot 4) to 29.1% (lot 1). For the dTT method, the drug concentration ranged from 100.6 ng/mL (lot 2) to 109.1 ng/mL (lot 1) with CV ranging from 11.6% (lot 3) to 17.2% (lot 1). Lots 1 and 2 means between the 2 methods were statistically different ($P < .05$).

Plasma With Estimated Dabigatran Concentration of 200 ng/mL.—For the ECA method, the mean dabigatran concentration ranged from 179.8 ng/mL (lot 6) to 198.6 ng/mL (lot 5) with the interlaboratory CV ranging from 6.3% (lot 7) to 15.5% (lot 5). For the dTT method, the drug concentration ranged from 201.0 ng/mL (lot 7) to 220.5 ng/mL (lot 5) with CV ranging from 9.3% (lot 7) to 12.3% (lot 5). Lots 5 and 6 means between the 2 methods were statistically different ($P < .05$).

Table 2. Summary of Prothrombin Time (PT) Interpretations for 100-ng/mL Dabigatran Concentration by Major Reported Reagents/Instruments			
PT Instrument/Reagent[a]	Prolonged, No. (%)	Not Prolonged, No. (%)	All Responses, No.
Diagnostica Stago STA Compact[b]	18 (90.0)	2 (10.0)	**20**
Siemens BCS, BCS XP[c]	23 (79.3)	6 (20.7)	**29**
IL ACL TOP Series[d]	26 (76.5)	8 (23.5)	**34**
Diagnostica Stago STA -R/Evolution[b]	76 (98.7)	1 (1.3)	**77**
Total	**143** (89.4)	**17** (10.6)	**160**

[a] Same instrument/reagent combination.
[b] Diagnostica Stago, Inc, Parsippany, New Jersey.
[c] Siemens Healthineers, Malvern, Pennsylvania.
[d] Instrumentation Laboratory, Bedford, Massachusetts.

Plasma With Estimated Dabigatran Concentration of 400 ng/mL.—For the ECA method, the mean dabigatran concentration ranged from 377.1 ng/mL (lot 10) to 394.9 ng/mL (lot 12) with interlaboratory CV ranging from 6.8% (lot 10) to 9.0% (lot 12). For the dTT method, the drug concentration ranged from 375.8 ng/mL (lot 11) to 395.2 ng/mL (lot 12) with CV ranging from 7.1% (lot 11) to 11.2% (lot 9).

Method Comparison and Interlaboratory Variation.—The interlaboratory variation of the dabigatran assays was broad, especially at the lower drug levels, but mostly within the good to intermediate range.

Prothrombin Time.—Overall data demonstrated that PT methods were all responsive to dabigatran concentration in the 200- to 400-ng/mL range for plasma samples spiked with dabigatran (the PT was reported as prolonged by 100% of participants). For samples spiked with 100-ng/mL dabigatran, PT was reported as prolonged by 143 of 160 participants (89.4%) (Table 2). The difference in interpretations was probably driven by the reagents that were being used on these instruments. There was a closeness in responsiveness between Siemens Innovin and HemosIL RecombiPlasTin 2G reagents.

Diagnostica Stago Neoplastin Plus reagent appeared to be more sensitive overall to dabigatran across all 3 drug concentrations (Figure 1).

Activated Partial Thromboplastin Time.—Data demonstrated that aPTT methods were all responsive to dabigatran concentration in the 100- to 400-ng/mL range for plasma samples spiked with dabigatran. The aPTT was reported as prolonged by 100% of participants across all 3 concentrations. The degree of increase in clotting time was dependent on the reagent used. HemosIL SynthASil reagent appeared to be highly sensitive to dabigatran across all 3 drug concentrations (Figure 2).

Thrombin Time.—The standard (undiluted) TT assay showed maximum prolongation for all 3 dabigatran drug concentrations, indicating it was too sensitive for monitoring, but this assay can serve as a sensitive qualitative assay for determining the presence or absence of dabigatran.

Rivaroxaban Testing

In 2013, nine laboratories participated in the rivaroxaban survey. The number of participants gradually increased and in 2016 reached 28 participants. All laboratories used a chromogenic anti-Xa assay with the drug-specific rivaroxaban calibrator. Table 3 shows data analysis for each rivaroxaban concentration level by lot number.

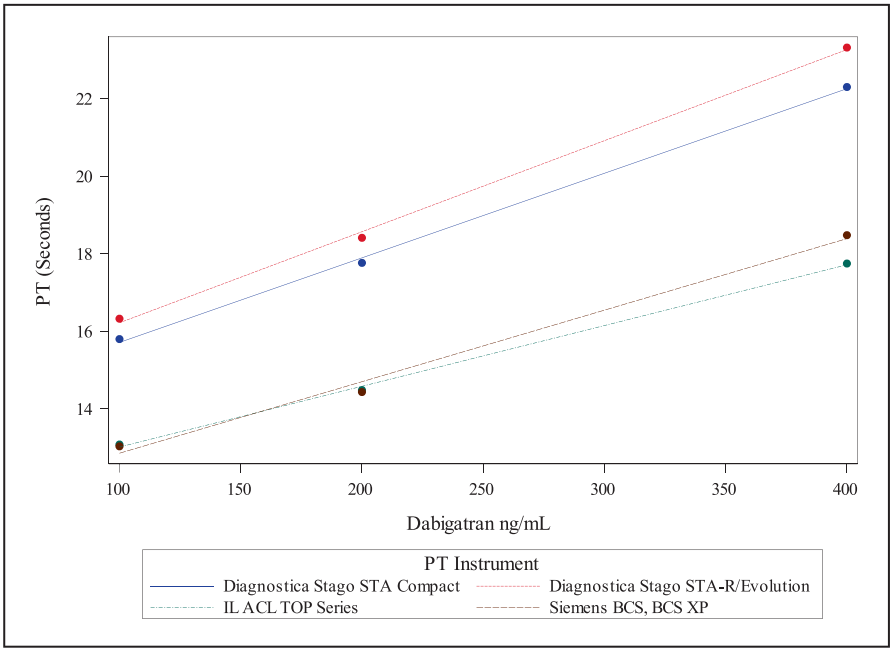

Figure 1. *Prothrombin time (PT) sensitivity to dabigatran. Mean plotted points for the 4 main instrument-reagent combinations across the 3 concentrations along with the fitted line are shown. The means were compared for each concentration; they were all significantly different for each of the drug concentrations (P <.001).*

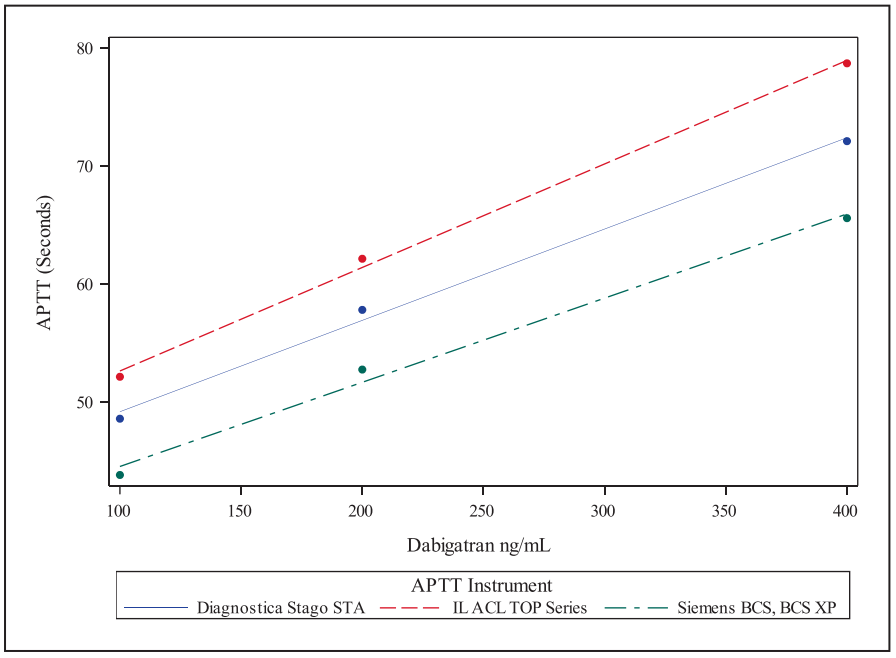

Figure 2. *Activated partial thromboplastin time (aPTT) sensitivity to dabigatran. Mean plotted points for the 3 main instrument-reagent combinations across the 3 concentrations along with the fitted line are shown. The means were compared for each concentration; they were all significantly different for each of the drug concentrations (*$P < .001$*).*

Plasma With Estimated Rivaroxaban Concentration of 50 ng/mL.—The mean rivaroxaban concentration ranged from 39.9 ng/mL (lot 4) to 54.0 ng/mL (lot 1) with the interlaboratory CV ranging from 11.5% (lot 1) to 22.2% (lot 4).

Plasma With Estimated Rivaroxaban Concentration of 200 ng/mL.—The mean rivaroxaban concentration ranged from 184.5 ng/mL (lot 6) to 250.4 ng/mL (lot 7) with the interlaboratory CV ranging from 7.2% (lot 6) to 10.9% (lot 8).

Plasma With Estimated Rivaroxaban Concentration of 400 ng/mL.—The mean rivaroxaban concentration ranged from 387.5 ng/mL (lot 13) to 440.3 ng/mL (lot 11) with the interlaboratory CV ranging from 6.4% (lot 10) to 8.1% (lot 11).

Interlaboratory Variation.—Applying previously described criteria,[14] interlaboratory variation of rivaroxaban assay for drug concentration 50 ng/mL was intermediate to poor, while the variation was good for drug concentrations 200 ng/mL and 400 ng/mL.

Prothrombin Time.—At the low concentration of rivaroxaban, 49.6% of participants (61 of 123) reported normal PT results (Table 4). The difference in interpretations was probably driven by the reagents that were used on these instruments. Prothrombin time reagents were responsive to rivaroxaban concentration in the 200- to 400-ng/mL range. The PT was reported as prolonged by 98.7% of participants for 200 ng/mL and by 100% of participants for 400 ng/mL. Diagnostica Stago Neoplastin Plus CI reagent appeared to be the most sensitive to rivaroxaban across all 3 drug concentrations (Figure 3).

Activated Partial Thromboplastin Time.—The aPTT appeared to be less sensitive to rivaroxaban. For the low concentration of rivaroxaban, 67.5% of participants (83 of 123) reported aPTT as normal (Table 5). The difference in interpretations was probably driven by the reagents that were used on these instruments. The aPTT reagents were more responsive to rivaroxaban concentration in the 200- to 400-ng/mL range. The aPTT was reported as prolonged by 92% of participants for 200 ng/mL and by 94% of participants for 400 ng/mL. HemosIL SynthASil reagent appeared to be the most sensitive to rivaroxaban across all 3 drug concentrations (Figure 4).

Table 3. Rivaroxaban Concentration Statistics by Lot Number

Rivaroxaban Target Values	Lot	N Obs	Mean	SD	CV
50 ng/mL	1	19	54.0	6.2	11.5
	3	41	43.3	9.5	22.0
	4	49	39.9	8.9	22.2
	5	26	45.3	9.5	20.9
200 ng/mL	6	20	184.5	13.2	7.2
	7	15	250.4	19.0	7.6
	8	40	191.8	20.9	10.9
	9	75	198.0	19.8	10.0
400 ng/mL	10	20	389.5	25.0	6.4
	11	14	440.3	35.8	8.1
	12	61	403.2	29.4	7.3
	13	54	387.5	25.4	6.6

Abbreviations: CV, coefficient of variation; Obs, observations; SD, standard deviation.

DISCUSSION

Although clinicians should not monitor DOAC levels to adjust their dosing, sometimes quantitative DOAC levels are medically necessary, specifically for the emergent and nonemergent scenarios, as previously described.[3,4] Despite noninferior efficacy when compared with vitamin K antagonists and their other advantages, DOACs still have 1% to 3% annual risk of major bleeding and 1% to 2% annual risk of thromboembolic events. Several studies demonstrated that there is a dose-response relation between DOAC concentrations and those adverse events. Thromboembolic events, as well as strokes, mainly occurred in patients with the lowest trough levels, whereas high trough levels were associated with a higher risk of major bleeding. The recently published opinion article by Toorop et al[15] summarizes the current available studies that show

Table 4. Summary of Prothrombin Time (PT) Interpretations for 50-ng/mL Rivaroxaban Concentrations by Major Reported Reagents/Instruments

PT Instrument/Reagent[a]	Not Prolonged, No. (%)	Prolonged, No. (%)	All Responses, No.
Diagnostica Stago STA Compact[b]	13 (72.2)	5 (27.8)	**18**
Diagnostica Stago STA -R/Evolution[b]	22 (36.7)	38 (63.3)	**60**
IL ACL TOP Series[c]	10 (45.5)	12 (54.5)	**22**
Siemens BCS, BCS XP[d]	16 (69.6)	7 (30.4)	**23**
Total	**61** (49.6)	**62** (50.4)	**123**

[a] Same instrument/reagent combination.
[b] Diagnostica Stago, Inc, Parsippany, New Jersey.
[c] Instrumentation Laboratory, Bedford, Massachusetts.
[d] Siemens Healthineers, Malvern, Pennsylvania.

this association and suggest that patients could benefit from tailored DOAC therapy.

Currently, idarucizumab and andexanet alfa are the only FDA-approved DOAC reversal agents that can reverse the anticoagulant effects of dabigatran and DXa inhibitors (rivaroxaban and apixaban), respectively. They were approved for DOAC-treated patients requiring emergency surgery or urgent procedures (idarucizumab) or with life-threatening or uncontrolled bleeding (idarucizumab and andexanet alfa).[16,17] For both antidotes, dosing is based on prior exposure rather than on DOAC levels. Incomplete reversal, thrombotic events, and even death are important potential postreversal complications.[18] The estimated cost of reversal of DOAC effect for idarucizumab and andexanet alfa is $3500 and $58 000 per reversal, respectively.[19]

Therefore, for patients with life-threatening bleeding and potential or unknown exposure to DOACs, rapid pretreatment identification of drug type and level may be clinically beneficial. Andexanet does not facilitate DXa inhibitor clearance. It is a recombinant modified factor Xa molecule that binds and sequesters the DXa inhibitors. The reversal of anticoagulation persists for about an hour after the infusion is completed.[20,21] Patients may have rebound anti-Xa activity since andexanet quickly dissociates from DXa inhibitors, which can result in a rebound increase in anti-Xa activity from the unbound drugs. If a patient continues to bleed after reversal agent administration or conventional screening assay results remain abnormal, suggesting supratherapeutic drug level, postreversal DOAC assessment may be warranted.

As of now, there are no studies identifying DOACs' clinically relevant threshold concentration associated with hemostasis impairment. Expert consensus opinion has empirically set thresholds from the aggregate knowledge of trough levels; however, no validation studies have been performed. The Scientific Standardization Committee of the International Society on Thrombosis and Hemostasis recommends that patients who require urgent surgical intervention need antidote administration if their residual DOAC drug concentration is greater than 30 ng/mL. In patients with serious bleeding, antidote administration should be considered if the drug concentration exceeds 50 ng/mL.[22]

As DOAC use continues to increase, it may be advisable for laboratories to have available quantitative assays for DOAC measurements. For accurate assessment of DOAC concentrations, laboratories must use drug-specific assays with the appropriate methods for the expected DOAC plasma level (eg, methods detecting low plasma DOAC concentrations in the perioperative setting).[23] In this

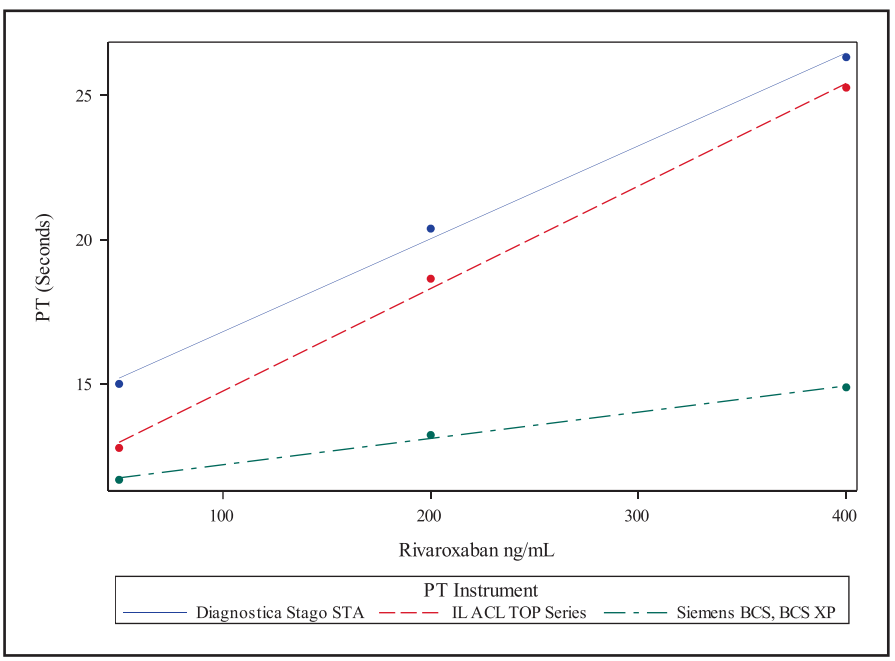

Figure 3. *Prothrombin time (PT) sensitivity to rivaroxaban. Mean plotted points for the 3 main instrument-reagent combinations across the 3 concentrations along with the fitted line are shown. The means were compared for each concentration; they were all significantly different for each of the drug concentrations (P < .001).*

Table 5. Summary of aPTT Interpretations for 50-ng/mL Rivaroxaban Concentrations by Major Reported Reagents/Instruments			
aPTT Instrument/Reagent[a]	Not Prolonged, No. (%)	Prolonged, No. (%)	All Responses, No.
Diagnostica Stago STA Compact[b]	10 (55.6)	8 (44.4)	**18**
Diagnostica Stago STA - R/Evolution[b]	42 (70.0)	18 (30.0)	**60**
IL ACL TOP Series[c]	10 (45.5)	12 (54.5)	**22**
Siemens BCS, BCS XP[d]	21 (91.3)	2 (8.7)	**23**
Total	**83**	**40**	**123**

Abbreviation: aPTT, activated partial thromboplastin time.

[a] Same instrument/reagent combination.
[b] Diagnostica Stago, Inc, Parsippany, New Jersey.
[c] Instrumentation Laboratory, Bedford, Massachusetts.
[d] Siemens Healthineers, Malvern, Pennsylvania.

retrospective study, we evaluated the performance of drug-specific assays for various concentrations of dabigatran and rivaroxaban, as well as the responsiveness of the routine clotting time assays to various concentrations of these drugs.

The Performance of Direct Oral Anticoagulant Assays

Dabigatran.—When 150-mg dabigatran etexilate is taken twice daily, the peak plasma concentration of dabigatran is estimated at 175 ng/mL with a range of 117 to 275 ng/mL (IQR), and the trough concentration (12 hours after drug intake) is about 91.0 ng/mL with a range of 61.0 to 143 ng/mL (IQR).[3] Dabigatran results were variable among the laboratories. dTT is the most commonly used method. Because of the small number of participants, comparison between the different reagent groups was not possible. Mean values for the group correlated well with plasma specimens spiked with 100 ng /mL, 200 ng/mL, and 400 ng/mL of dabigatran. Interlaboratory variation of dabigatran assay was broad but overall intermediate (11%–20%). The highest difference between the methodologies' (dTT versus ECA) CV variation was observed for the plasma samples spiked with 100 ng/mL of dabigatran (Table 1). This likely reflects differences in methodology, instrumentation, calibration, workflow, or survey sample matrix effect. This discrepancy could have an impact on clinical management decisions. As shown in Table 1, for the lot-4 specimens, the mean dTT was 102.8 ng/mL (SD, ±17.2 ng/mL). The observed results ranged in some laboratories from 59.0 ng/mL (close to trough level) to 145.0 ng/mL (close to peak level) when testing the same specimen. Recently published data from the international External Quality Control for Assays and Tests (ECAT) Foundation that encompass similar time frames (2013–2017) showed slightly lower CV at 10% for the dTT method. Contrary to our study, their highest difference between the methodologies was observed for the samples with the dabigatran concentration of 343 ng/mL. Owing to the higher number of participants, they were able to evaluate the data per reagent group if more than 10 participants were present in the survey from 2017.[24]

Another ECAT international dabigatran and rivaroxaban survey with a large number of international participants and a small percentage of North American laboratories (11 of 123) assessed the interlaboratory variation by using the same definition[14] and showed similar findings[25]: intermediate CV for the 2 most commonly used dabigatran assays (dTT, Hemoclot; and anti-IIa assay, Biophen DTI). Owing to the large number of participants, they were able to sort the data by reagents and calibrators. There was no clear

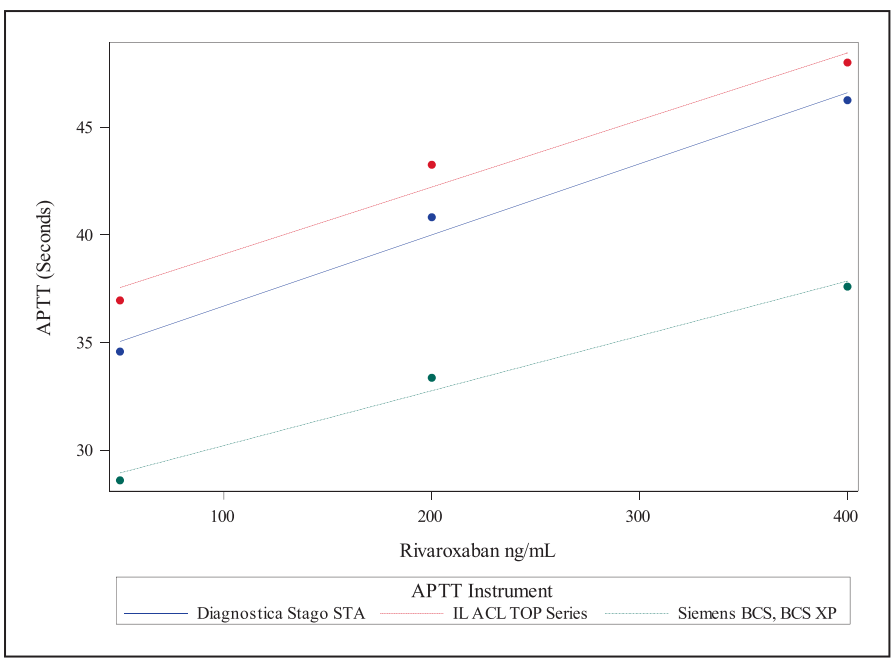

Figure 4. *Activated partial thromboplastin time (aPTT) sensitivity to rivaroxaban. Mean plotted points for the 3 main instrument-reagent combinations across the 3 concentrations along with the fitted line are shown. The means were compared for each concentration; they were all significantly different for each of the drug concentrations (*P < .001*).*

difference among the 5 different reagents according to their results. Most laboratories used the same reagent-calibrator combination. Unlike our study, they only evaluated 2 specimens that were sent out to participants in 2013.[25]

Rivaroxaban.—For the FDA-approved rivaroxaban once-daily doses of 10 mg, 15 mg, and 20 mg, peak rivaroxaban levels (2–4 hours after a dose) were as follows: 10-mg dose: 91 to 196 ng/mL, 15-mg dose: 178 to 313 ng/mL, and 20-mg dose: 189 to 419 ng/mL for deep venous thrombosis treatment, and 184 to 343 ng/mL for stroke prevention (5th–95th percentile range of the median estimated values). Trough levels (20–28 hours after a dose), according to the same study, were as follows: 10-mg dose: 1 to 38 ng/mL, 15-mg dose: 18 to 136 ng/mL, and 20-mg dose: 6 to 87 ng/mL for deep venous thrombosis treatment, and 12 to 137 ng/mL for stroke prevention (5th–95th percentile range).[26]

Rivaroxaban assays calibrated with rivaroxaban demonstrate intermediate to poor interlaboratory variation for the rivaroxaban concentration of 50 ng/mL (>20%), which likely has a clinical impact. As shown in Table 3, for the lot-5 specimens, the mean for the rivaroxaban assay was 45.3 ng/mL (SD, ±9.5 ng/mL). The observed results ranged from 21.0 ng/mL to 63.0 ng/mL, which were both in the trough range for the 15-mg and 20-mg once a day rivaroxaban concentrations for patients treated for prevention or treatment of venous thromboembolism (6–87 ng/mL, 5th–95th percentile).[27] Based on our results, it appears that some methods may underestimate this level of rivaroxaban concentration, thus falsely giving reassuring measurements for clinicians in the setting of preoperative workup or assessment for potential antidote administration that may have catastrophic consequences (eg, neuraxial anesthesia). The lower limits of drug detection vary by commercial kits but are generally about 30 ng/mL, which is also in the trough range.

Interlaboratory CV was good (6%–10%) for the rivaroxaban concentrations of 200 ng/mL and 400 ng/mL. Because of the small number of participants in our surveys, comparison between the different reagent groups was impossible. The previously mentioned first ECAT study's lowest rivaroxaban concentration of 85 ng/mL had an overall CV of 13%, which differed significantly between different methodologies (up to 20% difference), with the lowest CV observed for Stago and HemosIL Liquid Anti-Xa assays.[24] The second ECAT study assessed 2 rivaroxaban concentrations (100 ng/mL and 300 ng/mL) and showed that 5 of the 6 most commonly used reagents had intermediate interlaboratory precision (11%–20%) for a 100-ng/mL drug concentration.[25] These results suggest that interlaboratory variability for dabigatran and rivaroxaban measurement could be improved. The potential hurdles include the lack of an international standard that can be used to align the calibrators and the lack of FDA-approved kits or assays.

The Influence of Direct Oral Anticoagulants on Routine Coagulation Assays

Dabigatran.—Dabigatran prolongation of the PT and aPTT was both drug concentration and clotting time reagent dependent, with aPTT being more responsive than PT. Among aPTT reagents used in the survey, HemosIL SynthASil was the most sensitive to dabigatran. Prothrombin time or aPTT cannot be used safely to determine the degree of anticoagulation with dabigatran. Nationwide external quality assessment Belgian and Swedish surveys showed similar findings.[28,29] Variability of the PT and aPTT to dabigatran and other DTIs has been observed with a relatively poor correlation with the degree of anticoagulation and drug concentration.[30] The undiluted TT showed maximum prolongation across all 3 dabigatran concentrations, which made it too sensitive for drug-level monitoring. However, a normal TT result virtually excludes the possibility of the presence of dabigatran in a plasma sample, supporting its potential role as a qualitative screening assay.

Rivaroxaban.—Prothrombin time was more responsive to rivaroxaban. Among the most commonly used PT reagents in the survey, Diagnostica Stago Neoplastin Plus was the most sensitive to rivaroxaban. Even so, a normal PT result still cannot exclude the presence of rivaroxaban. Similar to dabigatran, neither PT nor aPTT can be used safely to determine the rivaroxaban anticoagulation level.

A recently published International Council for Standardization in Hematology document for DOAC management addresses all phases of laboratory DOAC measurements.[27] To ensure a high quality of DOAC testing, international DOAC standards and well-standardized assay procedures need to be established. Laboratories also need to use various quality improvement processes, such as internal quality control and external quality assessment. Despite a relatively small number of survey participants, CAP ACM survey results provided a glimpse of the current status of the DOAC laboratory testing in the United States.

In 2020 only 25 North American and 6 international laboratories participated in the dabigatran survey. Owing to decreased dabigatran use, its testing is trending down, as is reflected in the ACM participants' number. At the same time, the number of rivaroxaban survey participants has trended up since 2014 and reached 43 North American and 11 international laboratories in the survey last year. It is worth noting that despite the apparent clinical need for DOAC laboratory testing and a growing number of rivaroxaban participants, DOAC measurement in clinical laboratories is not widely available. Per the American Hospital Association, which conducts an annual survey of hospitals in the United States, as of 2018, there are 6146 hospitals.[31] The likely reason is the lack, up until 2020, of FDA-approved DOAC assays. As we mentioned earlier, HemosIL Liquid Anti-Xa for apixaban measurement has been recently FDA approved in the United States.[6]

There are several limitations of this study. The first is the small sample size of participating laboratories, which likely reflects the still-limited availability of DOAC testing in clinical laboratories across the country. Because of the small sample size, we also were not able to separate data per reagent group for dabigatran and rivaroxaban assays. Additionally, the nature of proficiency testing material may be a limitation, as the sample matrix used in proficiency testing samples may be different from real patient samples. The third limitation is that data are not applicable across all the medications for the DXa inhibitor class of drugs (eg, apixaban and edoxaban). Literature suggests that, as is seen in this study, the impact for each reagent is different, and our data for rivaroxaban should not be applied across all medications in this class (eg, apixaban). Data on apixaban were not available in our 2013–2016 surveys, while both apixaban and edoxaban assays' performance was assessed in the international ECAT survey.[15] Despite these limitations, to our knowledge, this is the only study with 4 consecutive years of data from clinical laboratories in North America. Several other external quality assessment programs have

published reports on dabigatran and rivaroxaban with mostly European and other international participants.[15,25,28,29,32] Our report represents the current status of North American interlaboratory performance in the measurement of dabigatran and rivaroxaban, demonstrates the responsiveness of routine clotting tests, and also identifies potential improvement opportunities for such assays.

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