


# Comparison of five specific assays for determination of dabigatran plasma concentrations in patients enrolled in the START-Laboratory Register

M. Cini<sup>1</sup>  | C. Legnani<sup>1</sup> | B. Cosmi<sup>1</sup> | S. Testa<sup>2</sup> | C. Dellanoce<sup>2</sup> | O. Paoletti<sup>2</sup> | R. Marcucci<sup>3</sup> | D. Poli<sup>3</sup> | R. Paniccia<sup>4</sup> | V. Pengo<sup>5</sup> | A. Tripodi<sup>6,7</sup> | G. Palareti<sup>8</sup> |  
for the START-Laboratory Register

<sup>1</sup>Department of Angiology and Blood Coagulation, S. Orsola-Malpighi University Hospital, Bologna, Italy

<sup>2</sup>Haemostasis and Thrombosis Center, Department of Laboratory Medicine, AO Istituti Ospitalieri, Cremona, Italy

<sup>3</sup>Thrombosis Center, Department of Experimental and clinical Medicine, University of Florence, Florence, Italy

<sup>4</sup>Center for Bleeding Disorders, Careggi University Hospital, Florence, Italy

<sup>5</sup>Department of Cardiothoracic and Vascular Sciences, University Hospital of Padua, Padua, Italy

<sup>6</sup>Department of Clinical Sciences and Community Health, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Università degli Studi di Milano, Milan, Italy

<sup>7</sup>IRCCS Maggiore Hospital Foundation, Milan, Italy

<sup>8</sup>Cardiovascular Diseases, University of Bologna, Coordinator of the START-Register, Bologna, Italy

## Correspondence

Michela Cini, Department of Angiology and Blood Coagulation, S. Orsola-Malpighi University Hospital, Bologna, Italy.  
Emails: michela.cini@aosp.bo.it; michela.cini76@gmail.com

## Abstract

**Introduction:** Several specific assays are commercially available to determine dabigatran anticoagulant activity. Aims of this multicenter and multiplatform study were to compare five methods for dabigatran measurement and investigate their performances in the low concentration range.

**Methods:** Dabigatran levels were analyzed in 295 plasma samples from patients enrolled in the START-Laboratory Register by the following methods using dedicated calibrators and controls: STA-ECA II (Diagnostica Stago), standard and low range Hemoclot Thrombin Inhibitors (Hyphen BioMed), Direct Thrombin Inhibitor Assay (Instrumentation Laboratory), Direct Thrombin Inhibitor Assay (Siemens), Technoclot DTI (Technoclone).

**Results:** Methods showed variable agreement with the Hemoclot Thrombin Inhibitors assay used as reference test, with modest under- or overestimations (Bland-Altman bias from -17.3 to 4.0 ng/mL). Limits of detection and quantification varied depending on the assay (4-52 and 7-82 ng/mL, respectively). Between-run precision and accuracy were good for all methods for both quality control levels. Assay's repeatability assessed at very low dabigatran concentrations (from 10 to 60 ng/mL) was also acceptable, variability generally increased at lower drug levels.

**Conclusion:** The five dabigatran-specific assays evaluated in this study provided reliable assessment of dabigatran plasma levels, although showing different performances.

## KEYWORDS

chromogenic assay, clotting assay, dabigatran, direct thrombin inhibitor, laboratory testing

## 1 | INTRODUCTION

The direct thrombin inhibitor dabigatran has been the first direct oral anticoagulant (DOAC) approved for prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation and for prevention or treatment of venous thromboembolism.<sup>1</sup> Although dabigatran

was developed for fixed-dose administration in patients with standard conditions without the need of routine laboratory monitoring and dose adjustment, there is now the widespread awareness that some clinical situations, such as adverse (thrombotic/hemorrhagic) events or emergency invasive procedures and others, might require the measurement of the drug plasma levels.<sup>2,3</sup>

The global coagulation test activated partial thromboplastin time (aPTT) was initially indicated as the method to measure the degree of anticoagulation in patients treated with dabigatran due to its wide availability and low cost. However, recent studies showed that aPTT has a low sensitivity to dabigatran levels and different responsiveness depending on commercial reagents. Furthermore, normal results are not always associated with the absence or minimal residual concentration of the drug.<sup>4,5</sup> Over the last years, several manufacturers developed specific functional tests for dabigatran plasma levels assessment, including thrombin-based clotting assays and ecarin clotting time or chromogenic assays. These methods showed good agreement with liquid chromatography-tandem mass spectrometry (LC-MS/MS) that is considered as the gold standard.<sup>6,7</sup> Accordingly, the most recent guidelines of the British Committee for Standards in Haematology (BCSH) and the guidelines of the International Society on Thrombosis and Haemostasis (ISTH) recommended the use of dedicated assays calibrated with drug-specific calibrators, and stated that aPTT should not be used to determine dabigatran plasma concentration.<sup>3,8</sup>

Aim of this study was to evaluate five commercially available specific assays for the measurement of dabigatran in plasma from a relatively large number of patients taking dabigatran. We also investigated assay's performances in the low dabigatran concentration range.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

Plasma samples were collected from patients engaged in the START-Register (Survey on anTicoagulated pAtients RegisTer) ([www.start-register.org](http://www.start-register.org); <http://www.clinicaltrials.gov> Unique identifier: NCT02219984), an observational multicenter multiplatform study in patients treated with DOACs (rivaroxaban, dabigatran, or apixaban) at the time of the study. Four Italian anticoagulation clinics (Bologna, Cremona, Florence, and Padua) affiliated with the Italian Federation of Anticoagulation Clinics (FCSA) joined the study by enrolling patients and collecting plasma; dabigatran testing was performed in three clinics (Bologna, Cremona, and Florence). The design of the START-Register has been detailed elsewhere.<sup>5,9</sup>

For this study, only patients enrolled in the START-Register from January 1<sup>st</sup>, 2014 to December 31<sup>st</sup>, 2014 and treated with dabigatran were included, after giving their informed consent. During the first month of treatment, trough (12 hours after the last dose intake) and peak blood samples (2 hours after drug ingestion) were taken. Blood was collected from the antecubital vein into 0.109 M trisodium citrate; platelet-poor plasma was prepared by centrifugation at 2000 g for 20 minutes at controlled room temperature. Each plasma sample was then aliquoted into coded plastic tubes, snap frozen, and stored at -80°C for further analysis. The entire process from blood drawing to plasma storage was carried out within 1 hour. After the end of the enrollment, frozen samples were centralized at the laboratory of the Department of Angiology and Blood Coagulation of the University Hospital S.Orsola-Malpighi, Bologna, and then one

**TABLE 1** List of assays used for dabigatran measurement with respective reagents, platforms, calibrators, and controls

Assay	ECA-STA	HTI-HY	DTI-IL	DTI-SI	DTI-TC
Reagent	STA-ECA II (Diagnostica Stago)	Hemoclot Thrombin Inhibitors (Hyphen BioMed)	Direct Thrombin Inhibitor Assay (Instrumentation Laboratory)	Direct Thrombin Inhibitor Assay (Siemens Healthcare Diagnostics)	Technoclot DTI (Technoclone GmbH)
Instrument	STA-R (Diagnostica Stago)	CS-2100 (Sysmex)	ACL TOP (Instrumentation Laboratory)	BCS (Siemens Healthcare Diagnostics)	ACL TOP (Instrumentation Laboratory)
Calibrators	STA-dabigatran calibrators (Diagnostica Stago)	Biophen dabigatran calibrators (normal and low range) (Hyphen BioMed)	Dabigatran calibrators (Instrumentation Laboratory)	Biophen dabigatran calibrators (Hyphen BioMed)	Technoview dabigatran calibrator set (Technoclone GmbH)
Controls	STA-dabigatran controls (Diagnostica Stago)	Biophen dabigatran controls (normal and low range) (Hyphen BioMed)	Dabigatran controls (Instrumentation Laboratory)	Biophen dabigatran controls (Hyphen BioMed)	Technoview dabigatran controls (Technoclone GmbH)
Clinic	Cremona	Bologna	Florence	Bologna	Bologna

Diagnostica Stago, Asnières sur Seine, France; Hyphen BioMed, Neuville-sur-Oise, France; Sysmex Europe GmbH, Norderstedt, Germany; Instrumentation Laboratory, Bedford, MA, USA; Siemens Healthcare Diagnostics, Marburg, Germany; Technoclone GmbH, Wien, Austria.

aliquot of each sample was distributed to the collaborative clinics for dabigatran measurement that was completed by 2015.

## 2.2 | Assays for dabigatran measurement

Dabigatran anticoagulant activity, expressed as drug concentration-equivalent (ng/mL), was measured by the combination of reagent/platform/calibrators and controls previously agreed in the study design as reported in Table 1; each assay was performed in a single center.

Assays were calibrated in duplicate before the beginning of the study according to manufacturer's indications using reference lyophilized standards; calibration ranges and equations of calibration curves are described in Table S1. For HTI-HY assay, manufacturers also provided low calibrators, and the calibration curve in the low range was set up in duplicate (HTI-HY low range curve); results <50 ng/mL with HTI-HY were repeated using the low range protocol, as indicated by the manufacturer. For all methods, results higher than the upper end of the calibration curve were diluted and retested.

On each working session, quality control (QC) samples were tested in single determination before the patient samples; if they fell out of the acceptance range, a new calibration curve was carried out. Tested samples were checked for hemolysis, clots, or lipemia.

## 2.3 | Statistical analysis

Data are presented as mean and standard deviation (SD) or median and range (min-max); coefficient of variation (CV%) was calculated as  $(SD/mean) \times 100$ . Accuracy and between-run precision were assessed by measuring drug concentrations for QC levels on consecutive working days. Accuracy was evaluated as follows: accuracy % = (measured concentration of QC sample/target value declared by the manufacturer)  $\times 100$ . Accuracy was considered acceptable if ranging from 85% to 115%. Precision expressed as CV was acceptable if <15%.<sup>10</sup>

To estimate the assay's repeatability at dabigatran concentrations near the lower end of the calibration range, two lyophilized dabigatran calibrators (Hyphen BioMed) with declared concentrations of 255

ng/mL and 30 ng/mL and a locally prepared pooled normal plasma were mixed to obtain a set of samples (from A to D) with the following final dabigatran concentrations: 60, 30, 20, and 10 ng/mL. They were run 10 times with each method in the same working day, and the CV was calculated.

The limit of detection (LOD) and the limit of quantification (LOQ) were evaluated repeating a pooled normal plasma 10 times with each assay. Raw data (expressed as OD/min or seconds) were reported on the X-axis of the calibration curves to obtain Y0 values, and LOD or LOQ was computed as follows:  $LOD = Y0 + 3SD$  and  $LOQ = Y0 + 10SD$  (for chromogenic assays  $LOD = Y0 - 3SD$  and  $LOQ = Y0 - 10SD$ ).

Differences between trough and peak values obtained with different assays were compared by the nonparametric Friedman test, and the Dunn's multiple comparison test was used as post test; *P* values equal or less than .05 were considered as statistically significant.

The Bland-Altman plots of [difference (A-B) vs average] and [% difference (A-B)/average vs average] were employed to investigate the agreement between methods using HTI-HY as the reference assay. For both analyses, bias and 95% limits of agreement (LoA) were calculated. A paired two-sided *t* test was also performed to evaluate the bias between assays.<sup>10,11</sup> Statistical analysis was carried out using the GraphPad Prism Software (San Diego, CA, USA).

## 3 | RESULTS

### 3.1 | Assay's precision and accuracy

All assays showed acceptable between-run precision and accuracy for the QC levels tested (total CVs from 3.3% to 11.2%; total accuracy from 91% to 111%) (Table 2). Table 3 reports the assay's repeatability evaluated at very low dabigatran concentrations (samples A, B, C, D with theoretical levels of 60, 30, 20, and 10 ng/mL, respectively). CVs were acceptable (<15%) for all samples when tested by ECA-STA and DTI-IL, and slightly higher (around 20%) for all levels using DTI-TC. CV particularly increased when dosing the lowest dabigatran concentration (sample D) with DTI-SI, while low range HTI-HY allowed a better repeatability at low levels (samples C, D) compared to the standard HTI-HY assay.

**TABLE 2** Results of quality control (QC) samples tested by different dabigatran assays. For description of QC samples see Table 1

Assay	QC level 1			QC level 2		
	Mean measured value (target value), ng/mL	Between-run, CV %	Accuracy, %	Mean measured value (target value), ng/mL	Between-run, CV %	Accuracy, %
ECA-STA	63.7 (59.5)	8.3	107	219.9 (213.0)	4.1	103
HTI-HY	120.7 (112.0)	3.7	108	298.2 (290.0)	3.3	103
HTI-HY (low range curve)	32.1 (30.0)	9.1	107	90.0 (81.0)	5.6	111
DTI-IL	54.3 (50.0)	6.1	109	231.5 (234.0)	4.4	99
DTI-SI	123.2 (112.0)	5.3	110	275.6 (290.0)	5.0	95
DTI-TC	133.8 (141.3)	10.0	95	288.4 (315.6)	11.2	91

CV, coefficient of variation.

Assay	Sample and theoretical concentration, ng/mL	Measured concentration mean (SD), ng/mL	Within-run CV, %
ECA-STA	Sample A, 60	47.9 (1.4)	2.9
	Sample B, 30	21.8 (1.5)	6.9
	Sample C, 20	17.0 (1.1)	6.5
	Sample D, 10	13.2 (1.5)	11.4
HTI-HY	Sample A, 60	58.2 (4.5)	7.8
	Sample B, 30	29.9 (2.6)	8.6
	Sample C, 20	19.6 (4.3)	21.7
	Sample D, 10	7.2 (5.2)	74.2
HTI-HY (low range curve)	Sample A, 60	71.2 (6.3)	8.8
	Sample B, 30	35.6 (3.4)	9.6
	Sample C, 20	25.2 (4.5)	17.9
	Sample D, 10	12.2 (2.9)	23.8
DTI-IL	Sample A, 60	75.8 (4.2)	5.5
	Sample B, 30	43.6 (1.7)	3.9
	Sample C, 20	30.3 (2.3)	7.6
	Sample D, 10	12.6 (1.4)	11.1
DTI-SI	Sample A, 60	71.5 (3.9)	5.5
	Sample B, 30	32.1 (4.4)	13.7
	Sample C, 20	19.9 (2.7)	13.6
	Sample D, 10	7.1 (6.0)	84.5
DTI-TC	Sample A, 60	61.7 (12.1)	19.6
	Sample B, 30	33.6 (6.0)	17.9
	Sample C, 20	27.9 (3.4)	12.2
	Sample D, 10	21.5 (4.5)	20.9

SD, standard deviation; CV, coefficient of variation.

### 3.2 | Limits of detection (LOD) and quantification (LOQ)

As shown in Table 4, LOD and LOQ values considerably varied between methods, ranging from 4 to 52 ng/mL and from 7 to 82 ng/mL, respectively. Data lower than LOQ for each assay were excluded from the analysis.

### 3.3 | Evaluation of five assays for dabigatran testing

A total of 254 patients were enrolled in the study by the 4 collaborative clinics. A total of 544 plasma samples (309 trough samples and 235 peak samples) were collected and tested for dabigatran concentration; occasionally, plasma volume was insufficient, and not all methods could be tested. We included in the analysis only samples for which all tests have been performed (295 plasma samples: 140 trough samples and 155 peak samples).

As explained in the Material and Methods section, dabigatran concentrations <50 ng/mL with HTI-HY were repeated using HTI-HY low range protocol. Figure 1 reports the comparison between standard and low range HTI-HY assays, evaluated by Bland-Altman analysis: the mean difference of results was -7.3 ng/mL

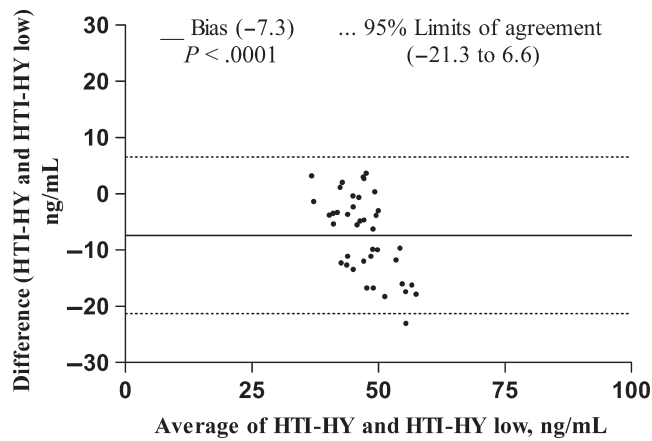
**TABLE 3** Repeatability of five assays for dabigatran measurement at very low dabigatran concentrations expressed as coefficient of variation. Samples from A to D were prepared mixing dabigatran calibrators with pooled normal plasma, and the correspondent theoretical concentration was calculated

**TABLE 4** Limits of detection (LOD) and quantification (LOQ) of different assays for dabigatran determination

Assay	LOD, ng/mL	LOQ, ng/mL
ECA-STA	12	27
HTI-HY	7	36
HTI-HY (low range curve)	10	28
DTI-IL	4	7
DTI-SI	14	82
DTI-TC	52	64

( $P < .0001$ ; 95% LoA from -21.3 to 6.6 ng/mL), suggesting that HTI-HY low range assay was able to detect lower dabigatran concentrations.

In our analysis, HTI-HY values <50 ng/mL were replaced with corresponding results obtained with HTI-HY low range assay. Trough and peak mean dabigatran concentrations measured with the five methods are in Table 5. Differences were significant for trough and peak values ( $P < .0001$ ;  $P$  values for Dunn's multiple comparison are shown in Table S2).



**FIGURE 1** Bland-Altman analysis for comparison of HTI-HY and HTI-HY low range at dabigatran concentrations <50 ng/mL as determined by HTI-HY. The plot of (difference [A-B] vs average) where A is the result of HTI-HY and B the result of HTI-HY low range is shown

The agreement between the reference assay (HTI-HY) and other methods was evaluated by the Bland-Altman analysis, where trough and peak values were assessed together (Figure 2). The mean difference of results between HTI-HY and ECA-STA was the closest to 0 ng/mL and not statistically significant ( $-2.4$  ng/mL,  $P = .19$ ). The bias between HTI-HY and other assays suggested a modest but significant over- ( $-9.2$  ng/mL for DTI-IL;  $-17.3$  ng/mL for DTI-SI;  $P < .0001$ ) or underestimation ( $4.0$  ng/mL,  $P = .025$  for DTI-TC) of dabigatran concentrations. The 95% limits of agreement were similar for all methods and suggested a wide range of differences in concentrations between HTI-HY and the other evaluated methods, with the 95% LoA lower end variable from  $-84.7$  to  $-56.1$  ng/mL and the 95% LoA upper end variable from  $36.8$  to  $66.3$  ng/mL. Moreover, Bland-Altman analysis calculated as % differences between HTI-HY and other assays has been assessed, and results are reported in Table S3.

Close scrutiny of the plots showed that the scatter of differences increased for high dabigatran values (approximately above 200 ng/mL). To better evaluate the agreement between methods at different dabigatran concentrations, data were stratified into 3 groups according to HTI-HY results (group 1 < 100 ng/mL; group 2 = 100-200 ng/mL; group 3 > 200 ng/mL), and Bland-Altman analysis was repeated. As shown in Table 6, ECA-STA confirmed the lowest deviation

of results compared to HTI-HY in all subgroups, as indicated by the mean of differences that was very close to 0 ng/mL and not statistically significant, especially for group 2 (bias  $-0.7$  ng/mL) and group 3 (bias  $0.6$  ng/mL). DTI-IL and DTI-SI slightly overestimated dabigatran concentrations compared to HTI-HY in all groups (bias from  $-5.0$  to  $-30.9$  ng/mL). Results of DTI-TC were slightly higher than HTI-HY at low concentrations (<100 ng/mL, bias =  $-22.5$  ng/mL), and lower in the other groups, especially for >200 ng/mL (bias =  $26.2$  ng/mL). The 95% limits of agreement were generally higher for dabigatran concentrations above 200 ng/mL (95% LoA lower end from  $-122.1$  to  $-41.0$  ng/mL and 95% LoA upper end from  $58.6$  to  $104.5$  ng/mL) compared to group 1 (95% LoA lower end from  $-76.6$  to  $-52.6$  ng/mL and 95% LoA upper end from  $14.6$  to  $33.6$  ng/mL). Similar results were obtained by Bland-Altman analysis calculated as % differences between HTI-HY and other assays in each subgroup, as reported in Table S4.

## 4 | DISCUSSION

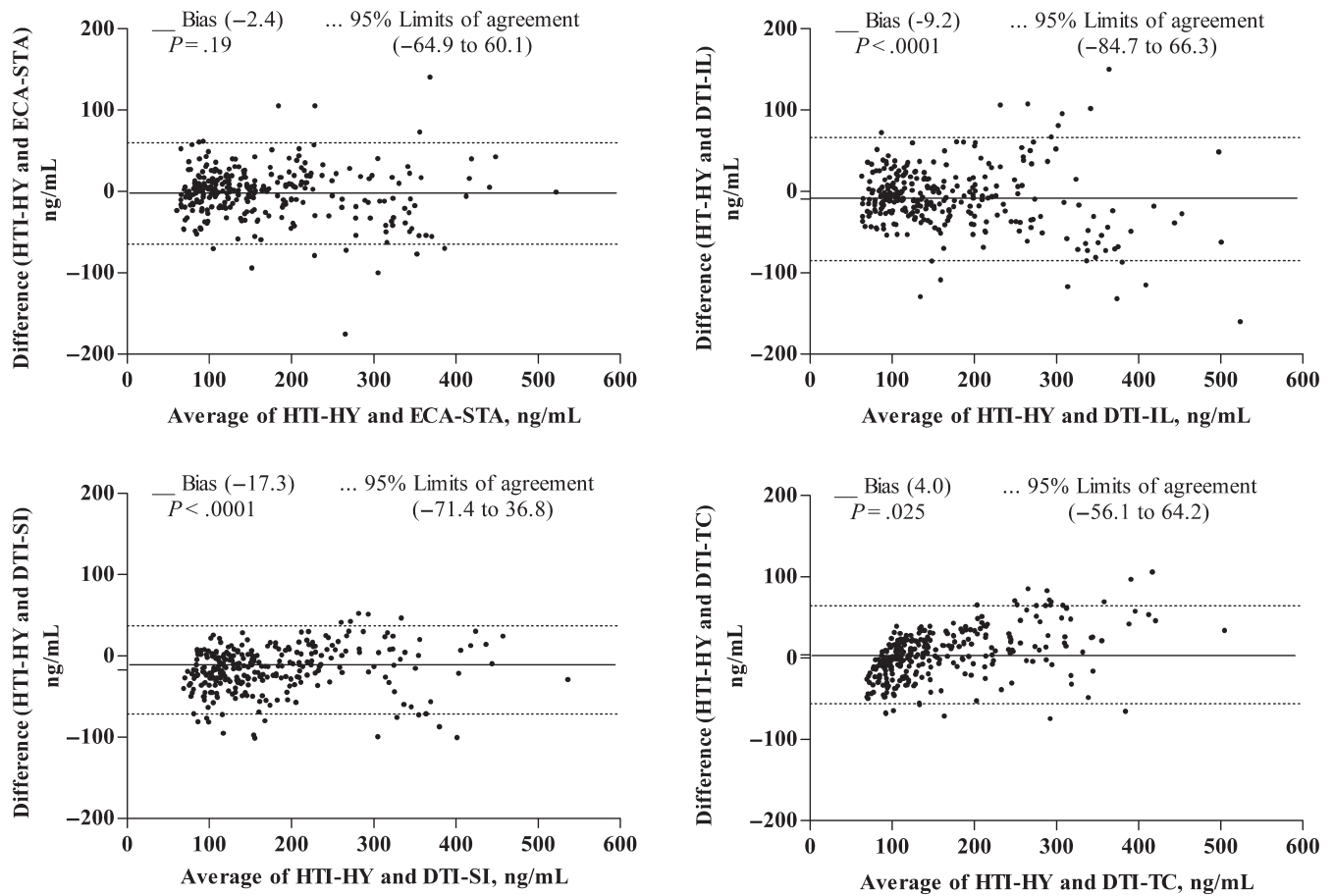
In the era of DOAC, the role of the clinical laboratory for the control of anticoagulation has proved essential in several clinical situations, both for emergency and for the optimal management of the therapy. Increasing evidences have highlighted limitations of the routine global test aPTT for dabigatran measurement, mostly with some reagents, whereas specific chromogenic or clotting-based assays have proven to be accurate and responsive.<sup>3</sup>

In the present study, we evaluated several methods that are currently available from different manufacturers for dabigatran measurement (see Table 1). Some are clotting assays (Technoclot DTI [Technoclone], Direct Thrombin Inhibitor Assay [Instrumentation Laboratory], Hemoclot Thrombin Inhibitors [Hyphen BioMed]), and others are chromogenic assays (Direct Thrombin Inhibitor Assay [Siemens], STA-ECAII [Diagnostica Stago]).

We did not use LC-MS/MS as gold standard to compare methods because it was not possible to carry out the assay in the laboratories taking part in the protocol. We adopted the HTI-HY as reference test, owing to the well-established good correlation with liquid chromatography reported in literature.<sup>12</sup> Different authors demonstrated a small interindividual variability of HTI-HY and a good agreement with LC-MS/MS measurements, and suggested that the

**TABLE 5** Dabigatran plasma levels measured by five different assays for trough and peak samples

	ECA-STA	HTI-HY	DTI-IL	DTI-SI	DTI-TC	P
Trough samples						
Median, ng/mL	112.7	117.5	115.4	133.0	118.8	<.0001
Range, ng/mL	(40.2-358.9)	(46.0-393.0)	(52.0-440.8)	(82.1-415.3)	(82.1-332.2)	
N	140	140	140	140	140	
Peak samples						
Median, ng/mL	184.3	177.1	188.8	198.5	173.7	<.0001
Range, ng/mL	(57.9-523.7)	(46.0-522.3)	(65.7-605.0)	(87.0-552.2)	(83.4-489.0)	
N	155	155	155	155	155	



**FIGURE 2** Bland-Altman analysis calculated as (difference [A-B] vs average) where A is HTI-HY used as reference method and B is one of the other assays for dabigatran measurement [Correction added on 22 January 2018, after initial online publication. The Figure 2 graphs have been published with points]

Hemoclot assay can accurately estimate dabigatran concentrations if LC-MS/MS is not available. Although precision and accuracy of Hemoclot for dabigatran concentrations <50 ng/mL proved to be inferior to the gold standard LC-MS/MS,<sup>13-17</sup> other data showed that the use of specific low calibrators could improve the performance of Hemoclot assay for levels below 50 ng/mL.<sup>6,7</sup>

In our study, dabigatran levels <50 ng/mL were tested by HTI-HY low range protocol that showed better precision and lower LOD and LOQ compared to standard HTI-HY assay. Our results suggested that the agreement between HTI-HY and the other evaluated assays was overall good. The ECA-STA was the test with less systematic difference compared to HTI-HY, also after stratification of dabigatran levels: deviation of results was very close to 0 ng/mL for each concentration range, as shown by the Bland-Altman analysis (see Figure 2 and Table 6). For the other methods, we observed a modest over (DTI-IL and DTI-SI) or under-estimation (DTI-TC); for some the agreement improved in a specific concentration range (eg, in the 100-200 ng/mL range for DTI-IL and DTI-TC or above 200 ng/mL for DTI-SI).

All the evaluated assays showed good between-run precision and accuracy for the QC levels tested (see Table 2). The assay's repeatability at the lower end of the calibration range was assessed testing samples with dabigatran concentrations from 10 to 60 ng/mL, and it was

acceptable; as expected, assay's variability increased with decreasing dabigatran levels as shown by CVs (see Table 3).

The calculated LOD and LOQ were quite different among the evaluated assays: one method allowed accurate detection of very low values (DTI-IL), while others had slightly higher LOD/LOQ (ECA-STA and HTI-HY) or even more (DTI-SI and DTI-TC).

All the methods were easy to set up, fully automated and robust as shown by the validity over time of the calibrations. They also proved suitable to be applied in emergency because results were available within few minutes.

The main limitation of the study is the lack of comparison between specific assays and the gold standard LC-MS/MS; nevertheless, the use of Hemoclot as reference test in our analysis is due to the good correlation of Hemoclot with liquid chromatography reported in literature, as described above.<sup>12</sup> Moreover, in our study, we did not compare results of the same assay performed in different laboratories, as our aim was to evaluate the performance of specific tests removing inter-laboratories variability, which has been discussed by other authors.<sup>9</sup> At last, we calculated the mean bias between reference (Hemoclot) and test methods using the average concentrations of reference and test method as "true" concentrations; this approach results in smaller bias than using dabigatran levels determined by Hemoclot as reference.



**TABLE 6** Results of the Bland-Altman analysis calculated as (difference [HTI-HY–other assay] vs average) in 3 different groups of dabigatran concentrations according to HTI-HY measurement

Assay compared to HTI-HY	Bland-Altman analysis	Group 1 HTI-HY <100 ng/mL n = 70	Group 2 HTI-HY = 100-200 ng/mL n = 141	Group 3 HTI-HY > 200 ng/mL n = 84
ECA-STA	Bias, ng/mL	-9.5	-0.7	0.6
	P	.0005	.77	.89
	95% LoA, ng/mL	-52.6-33.6	-58.0-56.5	-80.3-81.5
DTI-IL	Bias, ng/mL	-15.8	-6.2	-8.8
	P	<.0001	.01	.17
	95% LoA, ng/mL	-64.4-32.9	-61.6-49.3	-122.1-104.5
DTI-SI	Bias, ng/mL	-30.9	-17.9	-5.0
	P	<.0001	<.0001	.16
	95% LoA, ng/mL	-76.6-14.8	-62.6-26.9	-68.6-58.6
DTI-TC	Bias, ng/mL	-22.5	4.0	26.2
	P	<.0001	.03	<.0001
	95% LoA, ng/mL	-59.7-14.6	-37.9-45.9	-41.0-93.4

LoA, limits of agreement.

Strengths of the study are the relatively large sample size, its multicenter and multiplatform nature, the inclusion of five specific assays for dabigatran measurement that are currently widely used in coagulation laboratories and the use of low range Hemoclot calibrators. In conclusion, the five specific assays evaluated in this study for patients on dabigatran proved to be simple and accurate for the drug measurement across a wide range of concentrations, although with different performances. The assay's performances in the low range were improved by the use of specific low calibrators when available, but it showed different variability among commercial assays and, consequently, influenced the detection of low drug levels. Therefore, clinical laboratories should know and carefully consider the assay performances when choosing the method to employ for dabigatran measurement.

#### ACKNOWLEDGEMENTS

The authors would like to thank Diagnostica Stago, Dasit (for Hyphen BioMed reagents), Instrumentation Laboratory, Siemens Healthcare Diagnostics and Technoclone GmbH for supplying reagents needed for this study.

#### AUTHOR CONTRIBUTIONS

CL, GP, AT, and ST designed the study. OP, VP, DP, BC, and RM enrolled patients and collected plasmas. MC, RP, and CD performed laboratory measurements. MC and CL analyzed the data. MC wrote the manuscript. AT and CL revised the manuscript. All the authors revised and accepted the final version of the manuscript.

#### ORCID

M. Cini <http://orcid.org/0000-0001-9681-6159>

#### REFERENCES

- Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2009;361:1139-1151.
- Tripodi A. The laboratory and the direct oral anticoagulants. *Blood*. 2013;121:4032-4035.
- Kitchen S, Gray E, Mackie I, Baglin T, Makris M. Measurement of non-coumarin anticoagulants and their effects on tests of Haemostasis: Guidance from the British Committee for Standards in Haematology. *Br J Haematol*. 2014;166:830-841.
- Douxflis J, Mullier F, Loosen C, Chatelain C, Chatelain B, Dogne JM. Assessment of the impact of rivaroxaban on coagulation assays: laboratory recommendations for the monitoring of rivaroxaban and review of the literature. *Thromb Res*. 2012;130:956-966.
- Testa S, Legnani C, Tripodi A, et al. Poor comparability of coagulation screening test with specific measurement in patients receiving direct oral anticoagulants: results from a multicenter/multiplatform study. *J Thromb Haemost*. 2016;14:2194-2201.
- Douxflis J, Lessire S, Dincq AS, et al. Estimation of dabigatran plasma concentrations in the perioperative setting. An ex vivo study using dedicated coagulation assays. *Thromb Haemost*. 2015;113:862-869.
- Amiral J, Dunois C, Amiral C, Seghatchian J. An update on laboratory measurements of Dabigatran: smart specific and calibrated dedicated assays for measuring anti-IIa activity in plasma. *Transfus Apher Sci*. 2016;54:428-437.
- Baglin T, Hillarp A, Tripodi A, Elalamy I, Buller H, Ageno W. Measuring oral direct inhibitors (ODIs) of thrombin and factor Xa: a recommendation from the Subcommittee on Control of Anticoagulation of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost*. 2013;11:756-760.
- Testa S, Tripodi A, Legnani C, et al. Plasma levels of direct oral anticoagulants in real life patients with atrial fibrillation: results observed in four anticoagulation clinics. *Throm Res*. 2016;137:178-183.
- Schmitz EM, Boonen K, van den Heuvel DJ, et al. Determination of dabigatran, rivaroxaban and apixaban by ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulants. *J Thromb Haemost*. 2014;12:1636-1646.

11. Konigsbrugge O, Quehenberger P, Belik S, et al. Anti-coagulation assessment with prothrombin time and anti-Xa assays in real-world patients on treatment with rivaroxaban. *Ann Hematol*. 2015;94:1463-1471.
12. Douxfils J, Mullier F, Robert S, Chatelain C, Chatelain B, Dogne JM. Impact of dabigatran on a large panel of routine or specific coagulation assays. Laboratory recommendations for monitoring of dabigatran etexilate. *Thromb Haemost*. 2012;107:985-997.
13. Douxfils J, Dogne JM, Mullier F, et al. Comparison of calibrated dilute thrombin time and aPTT tests with LC-MS/MS for the therapeutic monitoring of patients treated with dabigatran etexilate. *Thromb Haemost*. 2013;110:543-549.
14. Antovic JP, Skeppholm M, Eintrei J, et al. Evaluation of coagulation assays versus LC-MS/MS for determinations of dabigatran concentrations in plasma. *Eur J Clin Pharmacol*. 2013;69:1875-1881.
15. Bozic-Mijovski M, Malmstrom RE, Malovrh P, et al. Diluted thrombin time reliably measures low to intermediate plasma dabigatran concentrations. *Ann Clin Biochem*. 2016;53(Pt 4):446-451.
16. Schmohl M, Gansser D, Moschetti V, Stangier J. Measurement of dabigatran plasma concentrations by calibrated thrombin clotting time in comparison to LC-MS/MS in human volunteers on dialysis. *Thromb Res*. 2015;135:532-536.
17. Skeppholm M, Hjemdahl P, Antovic JP, et al. On the monitoring of dabigatran treatment in "real life" patients with atrial fibrillation. *Thromb Res*. 2014;134:783-789.

#### SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Cini M, Legnani C, Cosmi B, et al. Comparison of five specific assays for determination of dabigatran plasma concentrations in patients enrolled in the START-Laboratory Register. *Int J Lab Hem*. 2018;00:1-8. <https://doi.org/10.1111/ijlh.12772>