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Validation of a whole blood coagulometer sensitive to the direct oral anticoagulants

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Readily available and rapid turn-around, bedside assays to measure the effect of the direct oral anticoagulants (DOACs) are not available. This study evaluates a point-of-care (PoC) coagulometer to assess the anticoagulant effects of the DOACs and low molecular weight heparin. Studies were done in fresh spiked blood from healthy volunteers. PoC coagulometer baseline clotting times were half that of the manual whole blood clotting time (WBCT, legacy method) and exhibited a sensitivity to DOAC anticoagulation approximately twice that of WBCT. All %CV values for both methods were < 10% with most being < 5% indicating acceptable precision of both methods. R^2 values for both clotting time and percent rise from baseline were > 0.98 indicating a strong correlation between the two methods. Replicate measurements for all subjects showed a maximum upper %CV value of 5.56% and a maximum upper value of all absolute %Differences of 5.5%, with both criteria meeting predefined acceptance criteria. The dose–response curves for all subjects were linear across the concentration ranges tested. The Perosphere Technology PoC coagulometer detects a range of therapeutic levels of the DOACs apixaban, rivaroxaban, edoxaban, and dabigatran, as well as the low molecular weight heparin, enoxaparin, with high precision and sensitivity.

Keywords Anticoagulation, Point-of-care, Coagulometer, Direct oral anticoagulants, Coagulation assay

With the advent of the direct oral anticoagulants (DOAC), routine monitoring of DOAC anticoagulant activity is no longer required. There are, however, clinical situations where there is a need to know the impact of a DOAC on coagulation so as to make appropriate clinical decisions and to measure the response to efforts to reverse anticoagulation[1]. These situations include some or all of the following conditions: 1) acute major bleeding, 2) the need for emergent surgery or invasive procedures and trauma with potential for major bleeding, 3) the need to manage stroke therapy, 4) situations where a patient has compromised renal or hepatic function, 5) extremes of body weight, 6) concomitant drug use, 7) or where there are questions of non-compliance. In urgent situations a widely available, rapid turnaround assay would be most clinically helpful. Sensitive and responsive pharmacokinetic assays for measuring DOAC concentration do exist, but these assays are not widely available in the U.S., are laboratory based, and when available, not always on a 24-h basis. Neither are they rapid turn-around assays to make them useful in emergent situations or for assessing coagulation after reversal of therapy[2].

A point-of-care (PoC) coagulometer was recently developed that is based on measuring native (no reagent added) whole blood clotting time, inspired by the traditional manual whole blood clotting time (WBCT)[3] and for use in assessing impaired coagulation induced by the DOACs as well as in other conditions. The aims of these studies are to validate the instrument compared to the legacy manual whole blood clotting time (Study 1) and to characterize the sensitivity of the coagulometer to DOAC-induced anticoagulation spanning a wide range of DOAC concentrations; to assess reproducibility of measurements for each drug and concentration level; and to verify linearity of dose–response curves over the tested ranges (Study 2).

Methods

Point-of-care coagulometer

Perosphere Technologies' PoC coagulometer is a handheld, battery-operated device that uses disposable, microfluidic cuvettes made of silicon and glass for clotting time measurements (Fig. 1)[3]. Test strips contain no chemical or biological reagents and clotting is initiated through contact of a blood sample with the glass surfaces inside a cuvette. The coagulometer employs an optical measurement scheme based on transmitted near infrared

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Fig. 1. Perosphere Technologies' PoC coagulometer is a handheld, battery operated instrument that performs individual coagulation tests on fresh or citrated whole blood. It monitors broad-spectrum activity of anticoagulants and reversal agents, across drug classes, at a patient's bedside, with clotting times reported in seconds. (Reprinted with Permission from Perosphere Technologies, Inc.)

(IR) light to detect fibrin assembly, the final step in the clotting cascade. Following a venous blood draw, 14 µL of fresh whole blood is transferred into an inserted cuvette. The coagulometer automatically detects blood entrance by capillary action and starts recording the intensity of IR light transmitted across cuvette and blood sample at 0.5 s intervals. An algorithm automatically detects peak fibrin formation and computes a clotting time associated with the peak in the IR signal corresponding to peak fibrin clot formation. The instrument performs numerous tests to confirm its proper function and to monitor the quality of the blood sample and collected data.

Manual whole blood clotting time

The PoC coagulometer's method of coagulation initiation using glass contact without added reagents most closely mirrors that of the historical manual whole blood clotting time[4], thus the WBCT was used as the predicate assay for comparative purposes even though it has been abandoned as a practical assessment of blood clotting. Notably,—studies have shown that other bedside assays such as the activated clotting time or various visco-elastic assays did not have discriminatory sensitivity at the low end of DOAC concentration[5,6].

The manual WBCT technique used historically[4] with various alterations through the years was performed as follows. Immediately after a venous blood draw, three glass tubes were filled each with approximately 0.3 mL of a fresh whole blood sample and immediately placed in a water bath at 37 °C when a stopwatch is started. The first tube was then manually titled every 30 s, and the blood sample visually inspected for clot formation. Tubes two and three remained stationary. Tilting the tube provides minimal agitation and additional exposure to the negatively charged glass surface, stimulating further activation of factor XII via kallikrein. Once clot formation was confirmed, the second tube was titled every 30 s until a clot was detected, while leaving the third tube stationary. Finally, the third tube was tilted every 30 s, and upon clot detection, the evaluation timepoint was logged as the whole blood clotting time.

Subjects, data collection and monitoring

All measurements were performed in vitro in whole blood from healthy volunteers under IRB oversight (Advarra, Columbia, MD USA) in accordance with applicable guidelines and regulations[7]. An IRB approved informed consent was obtained from all participants and the research was performed in accordance with the Declaration of Helsinki (2013)[8]. Samples of whole blood were collected into plastic syringes and aliquoted into BD Vacutainer™ Plastic Blood Collection Tubes with no Additives already containing various concentrations of apixaban, rivaroxaban, and edoxaban, as well as enoxaparin (enoxaparin only tested in Study 1; dabigatran not tested in Study 1) (see Supplement Table 1 for information regarding anticoagulants). Studies were conducted at a single site (Perosphere Technologies laboratory) using a single cuvette lot. Data collection was monitored and maintained by an independent, third party (Discovery Statistics, Laguna Niguel, CA). Guidance documents used for Study 1 included *CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, 3rd Edition, 2014* and *CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition, 2013*. Guidance documents for Study 2 included *The Clinical and Laboratory Standards Institute (CLSI) document H57-A, Protocol for the Evaluation, Validation, and Implementation of Coagulometers; Approved Guideline; ISO 5725–2:1994 – Accuracy (Trueness and Precision) of Measurement Methods and Results – Part 2: Basic Method for the Determination of Repeatability and Reproducibility of a Standard Measurement Method;* and *CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2003.* All data management and analysis conformed to U.S Food and Drug Administration CFR Part 11 (Subpart B, Sec. 11.10).

Subject ID	Anticoagulant	Set of Concentrations	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1, 2	Apixaban	0, 30, 75, 150, 300, 400 ng/mL	C1	C2	C3	C4	C5	C6
3, 4	Edoxaban	0, 30, 75, 150, 300, 400 ng/mL	C1	C2	C3	C4	C5	C6
5, 6	Rivaroxaban	0, 30, 75, 150, 300, 450 ng/mL	C1	C2	C3	C4	C5	C6
7, 8	Enoxaparin	0, 1, 2, 3, 4, 5 µg/mL	C1	C2	C3	C4	C5	C6

Table 1. Study 1 procedure. Blood samples were drawn from eight volunteers (two volunteers for each anticoagulant) on six different days for each of the volunteers. On each of the six days, a volunteer's blood sample was spiked with the same anticoagulant at a different, single concentration. Each value from the respective anticoagulant set of concentrations was randomly assigned to one of the daily concentrations C1-C6. On each day, a single, spiked blood sample was split and used for clotting time measurements on five coagulometers in parallel by one operator and for manual WBCT measurements by five different operators in parallel, respectively.

Study 1: comparison of PoC coagulometer to manual WBCT

Table 1 illustrates how the tests were sequenced in eight volunteers (two volunteers for each anticoagulant). On each of the six days, a volunteer's blood sample was spiked with the same anticoagulant at a different, single concentration. On each day, a single, spiked blood sample was split and used for clotting time measurements on five coagulometers in parallel by one operator and for manual WBCT measurements by five different operators in parallel, respectively. Given the labile nature of un-anticoagulated venous whole blood, in vitro spiking of anticoagulants was accomplished by addition of a fixed volume of fresh whole blood to a plastic vacutainer pre-filled with a solution of anticoagulant in saline at a specific concentration to achieve the resultant selected concentration. This approach minimizes handling and manipulation of the blood in vitro, and also minimizes the time it takes to load blood onto multiple devices after a single venous draw (< 30–45 s) (see Supplement, Fig. 1 and Table 2 demonstrated that freshly drawn whole blood at room temperature was stable for up to 8 min for WBCT measurements)).

Study 1: endpoints

Endpoints included the clotting times for each methodology and the percent rise of clotting time from baseline for each concentration of anticoagulant-spiked sample.

Study 1: statistical analysis

Statistical methods included: 1) descriptive statistics to assess the central tendency and variability of the data using mean, SD and %CV for each group of replicates (n = 5) for each anticoagulant; 2) R, correlation coefficient to assess the relationship of two methods.

Study 2: analytical measurement range and linearity

Fresh venous whole blood samples were spiked, as in Study 1, with pre-defined concentrations of apixaban, rivaroxaban, edoxaban, and dabigatran and measured on the PoC coagulometer. Clotting times for all blood samples from each volunteer were measured on a total of 24 Perosphere Technologies' PoC Coagulometers in parallel, with varying numbers of replicate measurements per drug concentration. Samples tested contained final concentrations of either 0 (sham), 30, 75, 150, 300 and 400 (apixaban, edoxaban) or 450 (rivaroxaban and dabigatran) ng/mL of a DOAC, in randomized order. Tested drug concentrations spanned the estimated clinical range for each drug with the respective lowest and highest concentrations representing estimates of medical decision points. The lowest concentration of 30 ng/mL for all DOACs represents the concentration at and above which anticoagulant antidote administration might be considered for patients requiring an urgent intervention associated with a high risk of bleeding[9–11]. The highest concentrations of 450 ng/mL for rivaroxaban and dabigatran and 400 ng/mL for apixaban and edoxaban, respectively, represent values above the 95th percentile of plasma peak concentrations of patients on the respective anticoagulants[12]. The same intermediate concentrations of 75 ng/mL, 150 ng/mL, and 300 ng/mL, respectively, were tested for all anticoagulants. Studies assessed the sensitivity of the PoC coagulometer to the various anticoagulant concentrations and determined repeatability of measurements as well as linearity of the dose–response curves within the tested ranges.

Study 2: endpoints

The primary endpoints were (1) clotting times for individual spiked blood samples; and 2) dose–response curves from two subjects for each of the four anticoagulants. Secondary endpoints were: (1) SD and %CV values for each group of replicates as a measure of repeatability for each drug concentration of each dose–response curve; 2) Results of 1st, 2nd and 3rd order polynomial regression analyses for each dose–response curve, with estimates for polynomial coefficients, P-values indicating statistical significance of coefficients and an overall error estimate for each polynomial model; (3) Differences between the linear and best fit higher order polynomial models as a measure for the non-linearity of each dose–response curve.

A tertiary endpoint was determination of the linear range within the estimated therapeutic range for each of the dose–response curves, based on pre-defined acceptance criteria for repeatability and linearity. A dose–response curve was considered linear if the repeatability for each data point of the curve, calculated as %CV value of all replicate measurements of the corresponding single blood sample, was < 10%, and the absolute values of the differences between the linear and best fit higher order polynomial models were < 10%.

Study 2: statistical analysis

Statistical methods included descriptive statistics to assess the central tendency and variability of the data, least-squares polynomial regressions, and Kroll et al. approach to analyze if the observations of a method are linear over a range of concentrations[13]. The following analyses were performed for each subject data set: Calculation of mean, standard deviation SD, %CV and standard error values for each group of replicate measurements for each anticoagulant concentration, 1st-, 2nd-, and 3rd-order polynomial regression analyses with models following the format $y = b_0 + b_1x$, $y = b_0 + b_1x + b_2x^2$, and $y = b_0 + b_1x + b_2x^2 + b_3x^3$, respectively, yielding 1) Coefficient values with corresponding standard errors, t-values and p-values; 2)The overall standard error $S_{y.x}$ as a measure for non-linearity for each regression analysis. A significance level $\alpha = 0.05$ was used for the regression analyses. Differences were then calculated between the linear model and best fit non-linear model for each anticoagulant concentration and expressed as %Difference from the clotting times predicted by the linear model for the respective concentrations. A subject data set was considered linear over the concentration range if both pre-defined acceptance criteria as defined were met (%CV values for each concentration < 10% and absolute %Difference values for each concentration < 10%). All regression analyses were performed using the statistical software JMP, Version 14.2.0 (SAS Institute Inc., Cary, NC).

Results: study 1

Accuracy and precision

Table 2 summarizes the precision of results of this study for the lowest and highest concentration of anticoagulant (data for each individual for each dose level can be found in Supplement Table 3). Coagulometer mean baseline clotting times across all subjects are in the range of 221.2—281 s (normal range), while mean baseline clotting times for manual WBCT are approximately twice as high, in the range of 456 – 498 s. Maximum percent clotting time rises at maximum anticoagulant concentrations relative to the respective baselines across all subjects are for the coagulometer in the range of 51.8 – 134.8%, while the corresponding values for manual WBCT are approximately half as high, in the range of 35.8 – 75.9%. All %CV values for both methods are within the target range of %CV < 10%, with most of the values being in the range of %CV < 5%, indicating uniformly acceptable repeatability of both methods. For both clotting time and percent rise of clotting time from baseline, R > 0.98 indicates a strong correlation between the two methods for each individual subject of each anticoagulant.

Dose–response curves (i.e. clotting times) of coagulometer and manual WBCT plotted against each other show a linear relationship and the two methods are strongly correlated as seen in Supplement Fig. 2.

Results: study 2

Analytic measurement range and linearity

Table 3 summarizes the range of %CV for replicate measurements for all subjects (individual subject results are in Supplement Table 4) as well as the ranges of absolute %Difference values, corresponding to differences between regression models. The maximum upper value of all %CV ranges is 8.25%, with all subject data sets thereby meeting the first pre-defined acceptance criterion. The maximum upper value of all absolute %Difference ranges is 6.3%, with all subject data sets thereby meeting the second pre-defined acceptance criterion as well. For both dabigatran subjects, the PoC coagulometer yielded dose–response curves that were linear from the 5th percentile of Ctrough plasma concentration to 95th percentile of Cmax plasma concentration of dabigatran.

Anticoagulant	Subject	PoC Coagulometer				Manual WBCT				Correlation coefficient R of clotting time	Correlation coefficient R of percent rise of clotting time
		Baseline mean (sec)	%Rise of clotting time from the baseline			Baseline mean (sec)	%Rise of clotting time from the baseline				
			Baseline range (sec)	Lowest concentration tested	Highest concentration tested		Baseline range (sec)	Lowest concentration tested	Highest concentration tested		
Apixaban	108,110	250.8	244–266	1.7	69.3	486	480–510	0	35.8	0.9925	0.9932
	108,115	232.8	230–237	10.3	51.8	456	450–480	5.3	36.8	0.9977	0.9976
Edoxaban	108,114	281	278–285	6	70.7	498	480–510	0	44.6	0.9944	0.9945
	108,117	249.2	237–256	10.5	81.6	468	450–480	5.1	42.3	0.9954	0.9954
Rivaroxaban	108,111	237.4	236–242	6.1	83.3	474	450–480	1.3	45.6	0.9986	0.9986
	108,119	262.6	254–272	8.4	87.1	492	480–510	1.2	42.7	0.9962	0.996
Enoxaparin	108,112	221.2	216–226	21.8	131.5	474	450–510	8.9	75.9	0.9869	0.9871
	108,116	269.8	265–278	21	134.9	492	480–510	8.5	57.3	0.9956	0.9957

Table 2. Study 1 accuracy and precision. Reported for each subject are mean baseline clotting time; the percent rise of clotting time at the lowest and highest anticoagulant concentrations tested, relative to baseline clotting time; and the correlation coefficient of clotting time and percent rise of clotting time between the Perosphere Technologies' PoC coagulometer and manual WBCT method. (See Supplement for results of testing for each subject at each anticoagulant concentration).

The dose–response curves (Fig. 2) for all subjects are therefore considered linear across the tested concentration ranges. Additionally, each lowest anticoagulant dose of 30 ng/mL for each subject produced a clotting time higher than baseline, and each other dose produced a clotting time higher than the time for the respective neighboring lower dose.

Discussion

A major difference between the WBCT and most other tests of blood clotting is that it is performed with whole blood (includes platelets, RBCs, and WBCs) without added reagents to stimulate clotting, whereas almost all other assays are done in plasma (blood anticoagulated with sodium citrate or some other anticoagulant and then centrifuged to eliminate platelets, RBCs, and WBCs) and with added reagents to initiate clotting. As shown in Study 1, Perosphere Technologies' PoC coagulometer demonstrated better sensitivity to all tested anticoagulant concentrations compared with manual WBCT.The PoC coagulometer produced baseline clotting times that were approximately half as long as the corresponding times obtained with the manual WBCT but showed sensitivities to anticoagulants that were approximately twice as high, compared to manual WBCT. Despite these differences, strong correlation and high agreement between measurement results of the two methods was observed. The manual WBCT is a relatively subjective method that requires careful training of operators for generation of consistent results, while the Perosphere Technologies' PoC coagulometer is designed to be a more sensitive method with reduction of pre-analytical factors through automation of test performance, data collection and analysis.

Although the assay methodology is unable to distinguish between different causes of impaired coagulation, such as acquired or rare genetic coagulopathies (e.g. hemophilias), suspecting or knowing that a patient is on an anticoagulant(s), especially a DOAC, suggests that the Perosphere Technologies' PoC coagulometer could be used to measure the anticoagulant effects of the DOACs and low molecular weight heparin with high precision.

The second study showed that the PoC coagulometer has a linear response to each of the anticoagulants tested. PoC coagulometer clotting times exhibited linearity across the measuring range, and sensitivity to even the lowest DOAC concentrations tested as well as a high level of precision with notably low %CVs. Each lowest anticoagulant dose of 30 ng/mL for each subject produced a clotting time higher than baseline, and each other dose produced a clotting time higher than the time for the respective neighboring lower dose. As a pharmacodynamic assay, this PoC test clearly distinguishes threshold, medical decision levels of the DOACs tested, an analysis of great importance in the bleeding patient or when deciding to administer a reversal agent.

Whether or not the DOACs need to be monitored and the advantages and disadvantages of the various assays is controversial [2,14–18]. The available routine assays, such as the PT or aPTT, which are widely available and have moderately rapid turnaround, have limitations in their sensitivity and linearity with DOAC drug concentration or effect[5,19,20]. The more precise commercially available anti-Xa assays, which report a drug concentration rather than a clotting time, are more complex to perform, have limited availability in the U.S., are not compatible with approved reversal agents or replacement therapies, and are not rapidly available at the bedside [18]. Given these limitations, some consensus guidelines recommend against waiting for laboratory results when rapid decision making is needed in response to a patient with major bleeding or needing urgent surgery[18,21]. Having a rapid turnaround, sensitive biomarker, especially at the lower end of drug concentration (e.g., 30 – 50 ng/ml) where decisions about reversal of therapy, safety for emergent surgery, or appropriate stroke therapy must be made, would be immensely desirable.

Another consideration in developing a coagulation assay is one that reflects drug effect (pharmacodynamic behavior) as opposed to one that measures drug level (pharmacokinetic behavior). Although there is great overlap in these parameters they are not always the same. This is illustrated best with the vitamin K antagonists (VKA) where the PT is a good measure of drug effect and predictor of bleeding risk but correlates poorly with VKA drug concentration. The DOACs are direct acting anticoagulants and drug concentration and drug effect are similar, but the PT and aPTT are poor predictors of drug level or bleeding risk and the more specific anti-Xa coagulation assay has many limitations as mentioned earlier. How well the manual WBCT or the PoC coagulometer clotting time predict bleeding risk when prolonged is also yet to be established, but the ability to indicate drug levels that are below what is considered therapeutic (i.e., likely an insignificant contributor to active bleeding or bleeding risk), or within or above therapeutic (i.e., likely a contributor to active bleeding or bleeding risk) would be immensely helpful to the surgeon preparing for urgent surgery in a patient taking a DOAC or to the emergency physician treating an actively bleeding patient; and, the ability to easily perform the test at the bedside with a result in minutes would potentially be a great advantage over the delay and lack of availability of a standard anti-Xa assay.

The principal limitation of these studies is that in vitro spiked whole blood was used to assess the sensitivity, precision, and linearity of the coagulometer. Although comprehensive, these studies represent an initial validation. Clinical trials, using samples from a significantly larger population of patients taking DOACs are currently ongoing at multiple sites, alongside calibrated anti-FXa measurement. From these studies, additional precision characteristics including inter-laboratory variability of the PoC coagulometer will be obtained. The effects of additional factors, such as factor deficiencies, neutropenia, anemia, thrombocytosis and -penia or hematocrit, on WBCT measurements are similarly being evaluated. Besides, additional interference studies will be carried out to evaluate the effects of other anticoagulants such as heparin, LMWHs, fondaparinux, danaparoid, hirudins or argatroban on DOAC measurements. Additionally, the potential of using fingerstick blood samples for DOAC measurements will also be explored and validated.

In conclusion, the Perosphere Technologies PoC coagulometer is able to detect a wide range of concentrations of the DOACs apixaban, rivaroxaban, edoxaban, and dabigatran, as well as the LMWH, enoxaparin, with high sensitivity and precision. It requires only a small sample of blood and provides results within minutes at the bedside. Further clinical and laboratory studies are underway to confirm these results in subjects receiving a

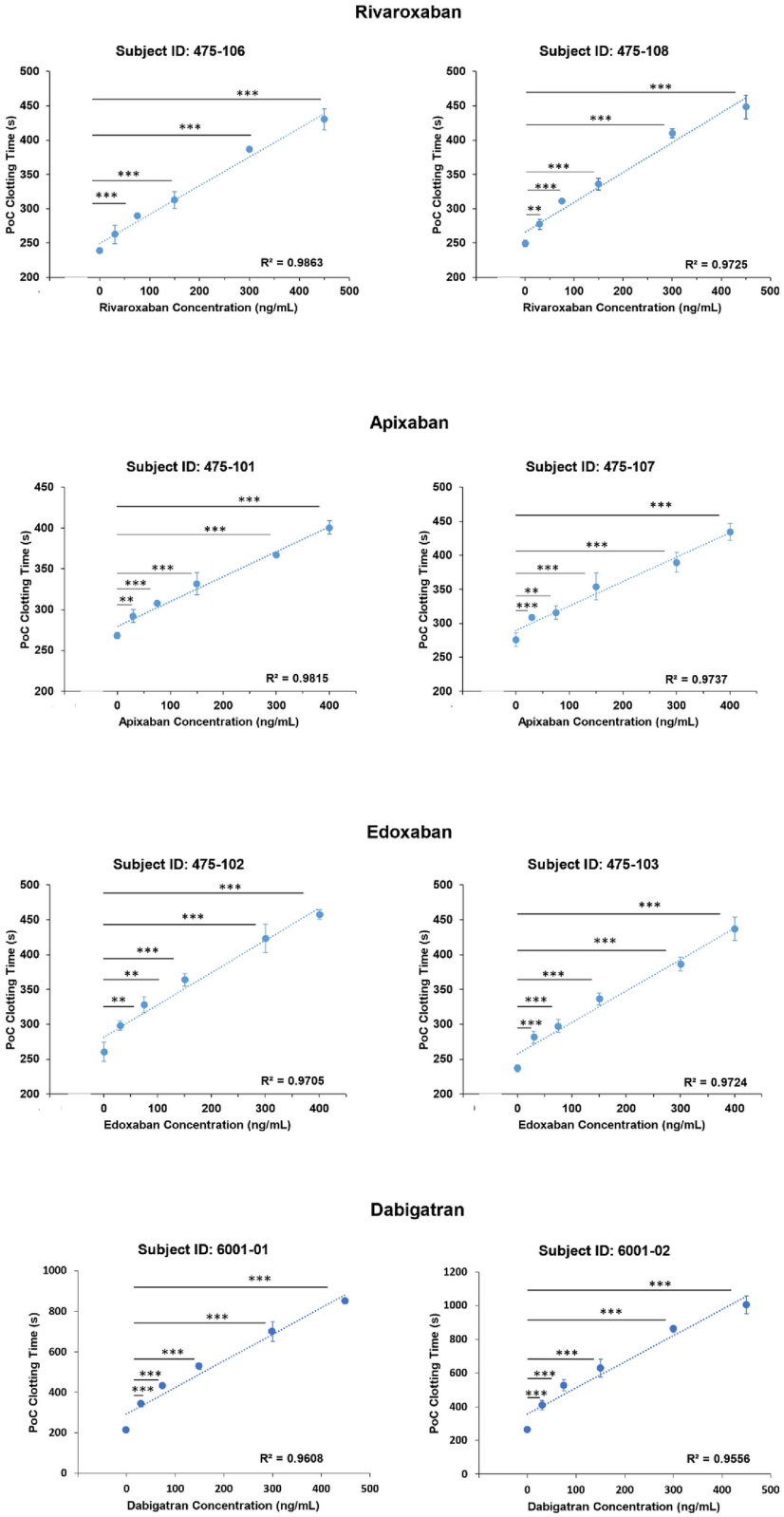

Fig. 2. Study 2 individual plots for each DOAC (2 subjects each for apixaban, rivaroxaban edoxaban and dabigatran). Plots are of PoC clotting times (mean ± SD) vs. DOAC concentration with linear regression model and corresponding R^2 values. One-way ANOVA, Tukey HSD post-hoc using JMP Version 14.2.0 (SAS Institute Inc, Cary NC). Significance level was set at $\alpha = 0.05$. A p-value $< \alpha$ indicates a statistically significant difference between the tested group means.

Anticoagulant	Subject ID	Range of %CV Values	Range of Absolute %Difference Values	Dose–Response Curve Linear / Not Linear Across Range
Rivaroxaban	475,106	0.69–4.97	0.7–2.9	Linear
Rivaroxaban	475,108	0.64–3.84	1.1–4.4	Linear
Apixaban	475,101	0.85–4.14	0.1–3.1	Linear
Apixaban	475,107	0.76–5.56	0.0–3.5	Linear
Edoxaban	475,102	1.49–5.21	1.3–3.9	Linear
Edoxaban	475,103	2.23–3.84	0.1–5.5	Linear
Dabigatran	6001–01	0.46–6.98	0.0–4.5	Linear
Dabigatran	6001–01	2.27–8.25	0.2–6.3	Linear

Table 3. Study 2 summary of linearity analyses of dose–response curves for all subjects. Listed are ranges of %CV values as repeatability measures and ranges of absolute %Difference values as linearity measures, with an overall assessment of linearity across the respective dose ranges.

DOAC and in the clinical setting of patients taking a DOAC who have acute bleeding or are in need of an urgent intervention.

Data availability

For original data, please contact Sasha Bakhru, PhD at s.bakhru@perospheretech.com.

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Author contributions

SHB, SZ and JA were responsible for interpreting results of studies and drafting the manuscript. SZ, XJ, LC, DO, KK, were involved in conducting the experiments and editing manuscript. DM reviewed and edited manuscript.

SZ developed the initial concept devices for the microfluidic cuvette technology for Perosphere in the nano@Stanford labs under a facilities use agreement. This facility is supported by the National Science Foundation as part of the National Nanotechnology Coordinated Infrastructure under award ECCS-2026822.

Declarations

Competing interests
The authors declare no competing interests.

Disclosures
SHB, XJ, LC, DO, KK, and DM are employees of Perosphere Technologies and have equity interest in the company; SZ has an equity interest in Perosphere Technologies; JA is an advisor to Perosphere Technologies and has equity interest in the company; is an advisor to Alere Home Monitoring, Norgine Pharmaceuticals and Covis Pharmaceuticals.

Additional information
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