

PROF. JONATHAN DOUXFILS (Orcid ID : 0000-0002-7644-5298)

MISS LAURE MORIMONT (Orcid ID : 0000-0001-9195-304X)

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**Assessment of the analytical performances and sample stability on ST Genesis system using the STG-DrugScreen
application**

Authors:

Jonathan Doux fils^{1,2}, Laure Morimont¹, Céline Bouvy², Marie de Saint-Hubert³, Bérangère Devalet⁴, Célia Devroye⁵, Anne-Sophie Dincq⁶, Jean-Michel Dogné¹, Maïté Guldenpfennig⁵, Justine Baudar⁵, Anne-Sophie Larock⁷, Sarah Lessire⁶, François Mullier⁵.

¹ University of Namur, Department of Pharmacy, NAMur Research Institute for Life Sciences, Namur Thrombosis and Hemostasis Center, Namur, Belgium

² Qualiblood s.a., Namur, Belgium

³ Université catholique de Louvain, CHU UCL Namur, Department of Geriatric, Yvoir, Belgium

⁴ Université catholique de Louvain, CHU UCL Namur, Department of Hematology, NAMur Research Institute for Life Sciences, Namur Thrombosis and Hemostasis Center, Yvoir, Belgium

⁵ Université catholique de Louvain, CHU UCL Namur, Hematology Laboratory, NAMur Research Institute for Life Sciences, Namur Thrombosis and Hemostasis Center, Yvoir, Belgium

⁶ Université catholique de Louvain, CHU UCL Namur, Department of Anesthesiology, NAMur Research Institute for Life Sciences, Namur Thrombosis and Hemostasis Center, Yvoir, Belgium

⁷ Université catholique de Louvain, CHU UCL Namur, Department of Pharmacy, NAMur Research Institute for Life Sciences, Namur Thrombosis and Hemostasis Center, Yvoir, Belgium

Corresponding author:

Prof. Jonathan Doux fils

University of Namur, Department of Pharmacy, NAMur Research Institute for Life Sciences, Namur Thrombosis and Hemostasis Center, Namur, Belgium

Qualiblood s.a., Namur, Belgium

M: jonathan.doux fils@unamur.be

T: +32 81 72 43 42

F: +32 81 72 42 99

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ESSENTIALS

- Thrombin generation testing is widely used to provide information on the coagulation phenotype of patients
- Some limitations have been highlighted such as the lack of established standardization
- This is the first study reporting precision results of the ST Genesia
- Normal range of the STG®-DrugScreen application has been assessed in healthy subjects
- The ST Genesia may facilitate the implementation of thrombin generation in the routine laboratory

ABSTRACT

Background:

Thrombin generation testing has been used to provide information on the coagulation phenotype of patients. The most used technique is the calibrated automated thrombogram (CAT) but it suffers from a lack of standardization preventing its implementation in routine. The ST Genesia is a new analyzer designed to assess thrombin generation based on the same principle as the CAT. Unlike the CAT system, the ST Genesia is a benchtop fully automated analyzer, able to perform the analyses individually and not by batch, with strict control of variables like temperature and volumes, ensuring, theoretically, a maximal reproducibility.

Objectives:

This study aimed at assessing the performance of the STG®-DrugScreen application on the ST Genesia analyzer. We also aimed at exploring stability of plasma samples after freezing and defining a reference normal range.

Results:

Results demonstrated the excellent inter-experiment precision of the ST Genesia and confirmed that the use of a reference plasma helps reducing the inter-experiments variability. Stability revealed that plasma samples are stable for at least 11 months at -70°C or below, except for those containing low molecular weight heparins which have to be tested within 6 months. Freezing had no impact on the majority of thrombin generation parameters except on time-to-peak.

Conclusions:

Our results suggest an easy implementation of thrombin generation with the use of ST Genesia in the routine laboratory. This will facilitate the design of multicentric studies and enable the establishment of reliable and evidence-based thresholds, which may improve the management of patients treated with anticoagulants.

KEYWORDS

Blood coagulation tests, Clinical laboratory techniques, Anticoagulants, Normal range, Reproducibility

INTRODUCTION

Thrombin generation has been used since the early 1950s [1]. In the beginning, whole blood or plasma was supplemented with triggers such as tissue factor (TF) or activator of the intrinsic pathway of the coagulation combined with calcium chloride to initiate coagulation. Thrombin generation was then evaluated by sampling the clotting mixture at regular intervals into a test tube containing fibrinogen. The clotting times were then recorded and the quantity of thrombin generated was derived from a calibration curve constructed with a known amount of thrombin [2]. Changes have been made and the test was improved by replacing fibrinogen by a chromogenic substrate specific for thrombin. This required the use of defibrinated plasma and a computer was used to calculate the parameters stemming from the thrombogram [3]. Finally, the chromogenic substrate was replaced by a fluorogenic substrate which permits the measurement of thrombin in samples that were not defibrinated since fluorescence is not hampered by the turbidity generated during the clotting process. These improvements have laid the groundwork for what is now known as calibrated automated thrombogram (CAT) [4]. Currently, different solutions are available on the market to measure thrombin generation [5]. These are the Innovance ETP (Siemens healthcare), the only one still based on the cleavage of a chromogenic substrate as readout, the Technothrombin TGA (Technoclone) and the Thrombinoscope CAT (Diagnostica Stago). These last two both utilize a fluorogenic substrate [5]. However, some limitations have been highlighted such as the lack of established standardization of the methods and reagents, and missing quality controls. In addition, batch-to-batch variations have been reported which further hampers study-to-study comparisons [6-8].

As a consequence, efforts have been made since the last decade to reduce the inter-laboratory variation, such as the use of a reference plasma to normalize the results [9]. Without the use of this reference plasma, CVs for the most global parameter of CAT, i.e. the endogenous thrombin potential (ETP), were often higher than 15%. This also raised the question of different local practices which may explain, in part, this high CV [10]. Even if this strategy appeared to efficiently reduce the inter-laboratory variation and showed the potential benefit of results normalization, improvements are still needed to ensure a proper standardization of the method and its implementation in the routine daily care of patient. Namely, it has been reported that standardization of the tested conditions, i.e. control of the temperature throughout the process, pre-heating of the sample and the reagents, collection tube or centrifugation, etc. may significantly impact the CAT parameters [8-11]. Thus, for its implementation in routine daily care, the CAT system had to move to a next generation of analyzers able to perform the analyses individually and not by batch, with strict control of all variables to ensure maximal reproducibility.

The newly developed ST Genesis is a fully automated system intended to measure thrombin generation. It provides capacity to continuously load patients' samples for unitary testing. This new system, has been designed to offer enhanced reproducibility compared to CAT and also to provide traceability and standardization, two criteria necessary to fulfill the requirements of an *in vitro* diagnostics format and to enter the clinical laboratory.

Numerous studies showed that thrombin generation measurement is sensitive enough to detect the presence of anticoagulants in patients' plasma and to evaluate the intensity of the treatment [12-19]. Therefore, Diagnostica Stago aimed at developing a kit for the assessment of thrombin generation in anticoagulated patients, the STG®-DrugScreen. The first aim of this study was to determine the precision of the device with this kit and the utility of the normalization of the results. Secondly, this study aimed at assessing the stability of the plasma samples over time after freezing as well as defining the normal range of the STG®-DrugScreen application in a normal healthy population.

MATERIALS & METHODS

The study aims at evaluating the precision performance of the device under final user setting and at investigating the stability of thrombin generation results on samples stored at -70°C or lower. The study was in accordance with the Declaration of Helsinki and has been approved by the Ethical Committee of the CHU UCL Namur, Yvoir, Namur, Belgium. The study took place from June 2016 to September 2017 at the CHU UCL Namur, Yvoir, Belgium. During this period, two batches of the kit were used (batch 201797, from 21 June 2016 to 3 November 2016 and batch 202028, from 4 November 2016 to 29 September 2017).

Plasma collection of patients and healthy donors' samples

Blood was taken by venipuncture in the antecubital vein and collected into 0.109 M sodium citrate (9:1 v/v) tubes (Vacuette®, Greiner, Austria) without corn trypsin inhibitor using a 21-gauge needle (Terumo). The first tube was always discarded and the first centrifugation was performed within 30 minutes. The platelet poor plasma (PPP) was obtained from the supernatant fraction of blood tubes after a double centrifugation for 15 minutes at $1,500 \times g$ at room temperature. The centrifuge brake was set to the minimum position at the end of the process. The residual platelet count was assessed every week to ensure the centrifugation procedure provides plasma with a platelet count $<10,000$ platelets/ μL . Immediately after centrifugation, PPP was aliquoted by 600 μL ($n=12$ for the stability study, $n=4$ for the normal range definition study) and frozen at $\leq -70^{\circ}\text{C}$ without any delay (except for 1 aliquot tested fresh in the stability study). Frozen PPP samples were thawed, heated to 37°C for 2-3 minutes and mixed gently just before the experiment. All tests were performed within 4 hours after thawing.

Normal and targeted population and samples

Six healthy individuals and 23 samples from patients treated with an anticoagulant have been included in the stability study (apixaban n=4; dabigatran n=3; low molecular weight heparins (LMWH) n=5; rivaroxaban n= 5; vitamin K antagonists (VKA) n=6). For patients under apixaban, dabigatran and rivaroxaban, plasma had to be taken at peak (i.e. between 30 minutes and 4 hours after drug intake) in order to obtain the highest effect on thrombin generation. For patients under LMWH, the blood had to be taken between 1 and 6 hours after the administration while for patients under VKA, the targeted INR was between 2 to 3.

For the definition of the normal range, 42 healthy individuals (mean age = 20 ± 3 years; min-max range = 18 - 32 years), not taking any antithrombotic therapy and not having any hemostasis disorders were included in the study.

Assessment of thrombin generation

Thrombin generation has been assessed on a ST Genesia analyzer using the STG®-DrugScreen application. More information about the analyzer, its methodology and the reagents are provided in **the supplementary material**.

Assessment of anticoagulant activity with specific tests

For patients on apixaban, rivaroxaban and LMWH samples, the estimated concentrations or the anti-Xa activity have been measured using the STA®-Liquid Anti-Xa (Diagnostica Stago) and the corresponding calibrators and controls according to the recommendations of the manufacturer on a STA-R Max analyzer (Diagnostica Stago). For dabigatran samples, the estimated dabigatran plasma concentrations have been measured using the STA®-ECA II (Diagnostica Stago) with the corresponding calibrators and controls. For VKA patients, the INR's have been measured with the STA®-Neoplastine®R (Diagnostica Stago) according to the recommendations of the manufacturer. For healthy individuals, the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) have been assessed using the STA®-Neoplastine®R (Diagnostica Stago) and the STA®-C.K. Prest (Diagnostica Stago) according to the recommendations of the manufacturer.

Determination of the precision of the ST Genesia in its STG®-DrugScreen application

Each day of testing (n=97), a new calibration is set and the quality controls (two levels) and the reference plasma are assessed. During the study period, two batches of reagent kits were used (batch 201797, from 21 June 2016 to 3 November 2016 and batch 202028, from 4 November 2016 to 29 September 2017). The precision has been assessed by collecting the daily values of the quality controls (two levels, i.e. the STG®-QualiTest Norm DS and the STG®-QualiTest Low

DS) and the reference plasma (i.e. the **STG®-RefPlasma DS**) over this period. Both the absolute and normalized results of the lag time, time-to-peak, Endogenous Thrombin Potential (ETP) and peak height were extracted, and the mean, standard deviation (SD) and coefficient of variation (CV) computed from those results.

Determination of the stability after freezing of the plasma samples for thrombin generation measurement

Fresh plasma from the 6 healthy individuals, 6 patients under VKA, 5 patients under LMWH, 4 patients under apixaban, 3 patients under dabigatran and 5 patients under rivaroxaban were tested at different time points to assess the stability of the different thrombin generation parameters over time. Testing have been performed:

- On fresh sample (the day of plasma collection after centrifugation)
- At day 1, month 1, month 3, month 6, month 9, month 10, month 11 (for healthy individuals only) after freezing at -70°C or lower. The maximal deviation tolerated was 7 days except for day 1 for which no deviation was tolerated.

The stability of thrombin generation results once the plasma has been frozen at -70°C or lower was assessed on the lag time, time-to-peak, ETP and peak height using the method of the rejection limit (RL). This method aims to establish the length of time during which the measurement of the thrombin generation is acceptable, when carefully following laboratory's established sample handling and storage conditions. Briefly, the percent bias (or the %RL) was plotted versus time for each time point. The regression line of this plot represents the average percent bias (proportional change) over time. For each parameter, the 100% RL is defined based on the maximal allowable error (MAE). To take into account method precision, reagents aging and sample aging, the MAE was set at 20% for the ETP and at 18% for the lag time, time-to-peak and the peak height. The RL is also adjusted according to the value of the intercept of the linear regression with the Y-axis. The maximal sample stability is defined as the intercept of the 95% confidence interval (CI) of the linear regression with the adjusted rejection limit. If there is no interception, the maximal duration limit is defined as the last point of measurement (see results section and ► **Figure 1&2** for graphical representation).

The impact of freezing was assessed comparing the results of each sample at day 0 (i.e. fresh sample) to the results obtained at day 1. To provide evidence of the normal distribution of the results, a Shapiro-Wilk test using an α -risk of 5% (two-sided confidence interval) has been performed. If the population had not a normal distribution, then the paired results were compared using a Wilcoxon matched rank signed test. If the distribution was normal, then the means were

compared with a t-test for paired samples. In both cases, a p-value below 5% leads to the conclusion of significant impact of freezing on results.

RESULTS

Thrombin generation values of the reference plasma and controls and precision of the ST-Genesis®

The within-batch variability was always below the between-batches relative differences, except for the normalized ETP of the STG®-QualiTest Low DS (► **Table 1**). The within-batch variability was always below 6%. All results are summarized in

► **Table 1**.

Impact on freezing on thrombin generation parameters

On the total cohort (n=29 samples), Shapiro-Wilk normality test showed normal distribution for the lag time and the ETP parameters while the normality failed for the peak height and the time-to-peak. This means that, according to the statistical analysis plan, a t-test for paired samples was used for the lag time and the ETP while a Wilcoxon matched rank signed test was used for the peak height and the time-to-peak. Due to too small sample size, the Wilcoxon matched rank signed test was used when the results were stratified by treatment (► **Table 2**).

Wilcoxon matched rank signed test revealed no significant difference between the two conditions on lag time, peak height, ETP while the time-to-peak was impaired (mean of difference = - 0.15 min). When results are analyzed by treatment, there is no significant difference between fresh and frozen plasma (► **Table 2**).

Stability of the plasma samples once conserved at -70°C or below

For the **lag time**, the adjusted RLs are -100% to 100%, the slope of the linear regression was equal to 0.414 (95%CI = -1.358 to 2.185). The 95% CI of the linear regression did not cross the adjusted RLs (► **Figure 1**) and the maximal storage duration was defined as the latest point assessed in this study, i.e. 11 months for healthy volunteers and 10 months for anticoagulated patients. Stratification by treatment did not provide different results (► **Table 3**). For the **time-to-peak**, the adjusted RLs were -104% to 96%, the slope of linear regression was equal to -1.596 (95%CI = -3.113 to -0.078; significant non-zero). The 95%CI of the linear regression did not cross the adjusted RLs (► **Figure 1**) and the maximal storage duration for this parameter was also defined at the latest point assessed in this study, i.e. 11 months. Again, stratification by treatment did not provide different results (► **Table 3**). For the **peak height**, the adjusted RLs were -110% to 90%, the slope of linear regression was equal to 3.749 (95%CI = -1.675 to 5.822). The 95%CI of the linear regression did not cross the adjusted RLs (► **Figure 1**) and the maximal storage duration for this parameter was also defined at the latest point

assessed in this study, i.e. 11 months. Stratification by treatment revealed that the peak height is only stable for 6 months in samples from patients with LMWH (► **Table 3 and Figure 2**). For the ETP, the adjusted RLs were -104% to 96%, the slope of linear regression was equal to 2.453 (95%CI = 0.886 to 4.019). The 95%CI of the linear regression did not cross the adjusted RLs (► **Figure 1**) and the maximal storage duration for this parameter was defined at the latest point assessed in this study, i.e. 11 months. Stratification by treatment did not provide different results (► **Table 3**).

Thrombin generation in the normal and targeted population

The reference values obtained in the **healthy population** revealed thrombin generation values close to the value of the reference plasma (► **Table 4**). Screening coagulation testing, namely the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) were normal in all healthy individuals (PT: mean = 13.7 ± 0.7 sec; min-max range = 11.9 - 15.10 sec and aPTT: mean = 31.5 ± 2.5 sec; min-max range = 27.1 - 36.7 sec). For **apixaban**, measured drug plasma levels through anti-Xa method were between 158 and 370 ng/mL, the normalized thrombin generation parameters were all impacted by the treatment (► **Table 5**). No clear correlation could be drawn from this small population (n=4) between thrombin generation parameters and the plasma level. For **dabigatran**, peak plasma levels were between 51 and 187 ng/mL, the normalized lag time and time-to-peak were impacted in all subjects while the ETP and the peak height were not different from the healthy subjects at the lowest concentration observed (i.e. 51 ng/mL) (► **Table 5**). For **LMWH**, the peak anti-Xa effect were comprised between 0.35 IU/mL and 1.37 IU/mL. All thrombin generation parameters were impacted. In the patient with the highest exposure (i.e. 1.37 IU/mL), the peak height and the ETP were highly impacted (8% and 14% the value of the reference plasma respectively) (► **Table 5**). For **rivaroxaban**, the peak plasma concentrations were between 197 and 300 ng/mL. The most impacted thrombin generation parameters were the peak height and the time-to-peak (► **Table 5**). In patient 1, the rivaroxaban concentration was 229 ng/mL, the peak height dropped to 37% with significant prolongation of the time-to-peak but the ETP was not impacted (i.e. 101% the value of the reference plasma). In patients treated with **VKA**, the INR varied between 1.86 to 3.31. The peak and the ETP were the most influenced thrombin generation parameters while lag time and time-to-peak were less influenced (► **Table 5**).

DISCUSSION

This is the first study evaluating the performances of the ST Genesis, a new fully automated benchtop analyzer for thrombin generation, in the routine setting. This instrument aims at introducing thrombin generation testing in the routine laboratory. Indeed, to date, the CAT system is probably one of the most used thrombin generation technique in research laboratories but the use of a microtiter plate, the manual placing of the sample and the reagents into the wells, added to the lack of standardized procedure and reagents have prevented its introduction into the routine practice [2]. By providing a complete kit of reagent and controls, the STG®-DrugScreen application of the ST Genesis simplifies the evaluation of the thrombin generation in patients treated with anticoagulants in the routine laboratory. This is made possible thanks to the standardized, well-controlled and validated methodology of the ST Genesis. The ST Genesis is derived from CAT principle, but not strictly equivalent. Yet, as already shown by others [20], there is a direct correlation between the two systems under comparable triggering conditions. This study is not intended to provide the clinical performance of the STG®-DrugScreen application to predict any clinical events, but it aims at providing the analytical performances of the system in the routine setting and at evaluating the stability of the samples once conserved at -70°C or below.

The precision of the ST Genesis has been evaluated using the data from the daily calibration and the value of the two levels of control and reference plasma (i.e. the STG®-QualiTest Low DS and STG®-QualiTest Norm DS and STG®-RefPlasma DS, respectively) over a 15 months period. Two batches of reagents were provided by the manufacturer during the course of this study. Main results revealed that the coefficient of variation of the different parameters were always <10% and for the majority of these results the CV was even <5% (► **Table 1**). As previously reported [10], this study also demonstrates that the use of a reference plasma reduced the inter-experiment variability. Regarding the stability of the samples, our results demonstrate that freezing has no impact on most thrombin generation parameters except the time-to-peak, especially in samples containing apixaban, dabigatran or rivaroxaban. Once frozen, our data suggest that the plasma is stable during at least 11 months, except for LMWH samples for which the maximal duration storage is 6 months.

As mentioned above, this study has limitations. First, this is a monocentric study and thus, the inter-laboratory performance of the ST Genesis has not been assessed. Secondly, results of external quality assurance program have not been included in this validation as the current thrombin generation EQA program are not designed for thrombin generation assessment of anticoagulant treatment [21] and thus the high tissue factor activity of the STG®-DrugScreen trigger may not be comparable to that of the other reagents and methodologies. Finally, the study was not powered to drive any conclusions regarding the impact of anticoagulants on thrombin generation since only peak plasma samples were collected for this part of the study. Only trends can be drawn on the overall effect of the different anticoagulant agents and further works are necessary to define the minimal concentration of anticoagulants that would yield results outside the reference range.

Reference plasma

Many previous publications recommended the use of a reference plasma to reduce the inter-laboratory variability [6, 7, 9, 10]. The choice of providing a reference plasma within the STG®-DrugScreen kit agrees with the literature since it has been reported that the use of local plasma (i.e. a reference plasma performed at the laboratory facilities) were generally unable to improve the inter-laboratory variability or the CV is even worsen after normalization [6, 8, 10]. The choice of a commercially available and certificated plasma is therefore more appropriate than using local plasma. On the other hand, it has been reported a certain heterogeneity between the different reference plasma which may impede the benefit of the use of such reference plasma [6]. Therefore, the best approach would be to use a reference plasma specifically dedicated for the application. This strategy improves the between batch variation. In this study, we report relative differences between batches from 0 to 24% when results are not normalized (► **Table 1 – see results of the controls**). Once results are normalized most of the parameters show better inter-batch CVs (except for the peak height with the STG®-QualiTest Low DS), demonstrating the efficiency of this approach. The choice of a freeze-dried plasma can be challenged since it has been reported that these plasmas have higher thrombin generation capacity than frozen plasma due to multiple pre-analytical reasons [6, 22]. In any case, the conditions in which these reference plasmas are produced are probably far away from the best recommendations of blood samples collection for thrombin generation testing and this is due to the fact that these reference plasmas have to be produced at a large scale. Thus, one solution may be to assess the response of the commercial reference plasma to a smaller pool of plasma from healthy donors collected in ideal conditions and then to apply a correction factor to the commercial plasma to compensate for these differences. In the package insert of the STG®-DrugScreen kit, an assay value of the reference plasma is provided by the manufacturer to correct the reference plasma activity. The results of this study demonstrate that this approach is efficient since our healthy subjects, collected in ideal conditions for this application, were close to 100% or a ratio of 1 depending on the parameter (► **Table 5**). In addition, the difference between the two batches of the STG®-RefPlasma DS was $\leq 5\%$ for all TGT parameters demonstrating that the use of a correction factor specific to each batch of reagent and reference plasma is useful to reduce the batch-to-batch variation (► **Table 1**).

Quality controls

Interestingly, it has been reported that an internal quality control (IQC) is essential to produce consistent results for thrombin generation [23]. However, in the era of thrombin generation, the confusion between an IQC and a reference is often made. Namely, most studies have proposed to normalize the results according to a reference plasma [6, 7, 9, 10, 23], which is not the same as true control plasma. To comply with local legislation, regulations, guidelines or standards issued by relevant bodies, at least two levels of controls is recommended. Thus, this is of upmost importance for thrombin

generation to propose such different level of controls to allow the implementation of thrombin generation into routine testing. This will ensure the technique is under control for both normal and abnormal sample analysis.

Over our series of 97 measurements of the reference plasma and the quality controls, we demonstrate that the CVs of the normalized results were always below 10% and for the majority of the parameters were even below 5%. These inter-assay precision results are in line with the intra- and inter-assay reproducibility data provided by the manufacturer in the insert-sheet of the STG®-DrugScreen kit (► **Supplementary material - Table 2**). All these data demonstrate that the STG®-DrugScreen performed on the ST Genesia is a precise thrombin generation application which will allow thrombin generation to be performed routinely to assess patients treated with anticoagulants. All the requirements are now met to implement this technique in the routine laboratory.

Impact of freezing and stability of the samples

A previous study evaluating the impact of pre-analytical parameters on the measurement of circulating microparticles with thrombin generation revealed that plasmas are stable for 1 year at -80°C [24]. Apart from this information, few has been done to assess the stability of plasma samples for thrombin generation testing. In this previous study of Lacroix et al., the authors assessed the stability of thrombin generation using the CAT system for the measurement of the procoagulant activity of circulating microparticles with the MP-reagent from Thrombinoscope bv, a reagent that does not contain tissue factor to trigger the coagulation. It has to be noted that this is a different setting than the one assessed in this study because the STG®-DrugScreen reagent contains tissue factor at concentration higher than 5pM. This higher tissue factor concentration provides robustness to the analytical procedure. Indeed, in another study of Loeffen et al., thrombin generation triggered with tissue factor at a concentration of 5pM has not demonstrated to be impacted by the two centrifugation protocols tested nor by the temperature at which the plasma is stored until analysis (4°C, room temperature or 37°C) [8].

Freezing has no effect on the majority of the thrombin generation parameters using the STG®-DrugScreen application, except for time-to-peak (► **Table 2**). Once frozen, plasma samples are stable during at least 11 months except for LMWH samples for which an impact is seen after 6 months (► **Table 3 & ► Figure 2**). A previous report also demonstrates that LMWH samples are not stable beyond 6 months [25]. This may be explained by the release of PF-4 from residual platelets. Namely, according to the CLSI document H21-A5, heparin neutralization may occur in sample not collected on CTAD tubes due to the release of PF4 from platelets [26]. It is possible that residual platelets released PF4 during storage and thus neutralize heparin. This is consistent with the increase of the peak observed. For the other subgroups, all parameters were stable which is in correlation with the findings of Woodhams et al. [27] who demonstrated that all plasma coagulation

proteins were stable for at least 18 months once stored at -74°C. If all the coagulation proteins are stable at -74°C, it can be expected that a global test such as thrombin generation, which relies on these coagulation proteins, can also be stable for a such long period. Our results confirm this assumption for a period of 11 months for healthy subjects and 10 months for anticoagulated patients.

Normal range definition

In this study, 42 samples from healthy donors were tested to provide grounds for the definition of a normal for the STG®-DrugScreen application on the ST-Genesia. All these samples were normal according to PT and aPTT screening. The small correction between absolute and normal value is certainly due to the fact that all these 42 samples were tested on the same day with the first batch of reagents (i.e. batch 201797). For all the parameters the inter-individual variation was always below 20% and was below 10% for the lag time and the time-to-peak. When comparing to the results obtained in patients treated with an anticoagulant, the 10th-90th percentile range (► **Table 4**) could serve as a basis for the classification of normal and abnormal plasma. It could be proposed that samples below the 10th percentile (for peak height and ETP) and above the 90th percentile (for lag time and time-to-peak) may represent samples in which anticoagulant therapy is suspected. However, further studies in larger cohorts of patients taking anticoagulants are needed before adopting this assumption.

Usefulness of thrombin generation to assess anticoagulated patients

Tailoring drug dosages to fit the needs of individual patients could be helpful to make old and new drugs more effective and safer [2]. Preliminary observations showed that thrombin generation testing is affected by all anticoagulant drugs and therefore it could be the candidate assay [18, 19, 28-31]. The test has been found to be very sensitive to all kind of anticoagulants [13-16, 18, 32, 33] and may best represent the inter-individual response than just exploring plasma drug concentrations [28]. In addition of considering the inter-individual response to an antithrombotic drug, thrombin generation testing is also able to explore in more details the impact of anticoagulants on the coagulation process. Namely, depending the type of drug, our results confirmed that-the fingerprint of the thrombin generation differs, revealing their different pharmacodynamics [17, 18, 32, 34] (► **Table 5**). This is of particular importance since bleeding or thrombosis have been reported within the “on-therapy” range, demonstrating that the drug level alone may not be sufficient to identify those who are more at risk [35]. However, further investigation on patients who bleed or who have recurrent thrombosis while on a fixed dose of anticoagulants is needed to show the benefit of thrombin generation and provide cut-offs for bleeding and thrombotic complications. Thrombin generation has also been reported as an informative tool to document

on antidote administration in polytrauma models with direct implication for patient care [36]. This is particularly important as it may help in adjusting the dose of prothrombin complex concentrate to administer [37]. The ST Genesia will provide a step forward to this aim in allowing thrombin generation to be done routinely with reduced inter-experiments and inter-laboratory variations. In this way, it will be easier to recruit patients, design multicenter studies and provide data for the establishment of reliable and evidence-based thresholds.

CONCLUSIONS

This is the first study presenting performance data of the ST Genesia, a new benchtop analyzer for thrombin generation testing. The study aimed at evaluating the performances of the STG®-DrugScreen application, a kit of reagents designed for the quantitative determination of thrombin generation in plasma from patients treated with an anticoagulant. Results revealed an excellent inter-experiment precision thanks to the standardized methodology, the excellent reproducibility of the analyzer and the use of the reference plasma included in the kit. Stability study revealed that plasma samples are stable for at least 11 months after freezing and storage at -70°C or lower, except for those containing LMWH which have to be tested within 6 months. Freezing has no impact of the majority of thrombin generation parameters except on time-to-peak. Thus, our results support the hypothesis that the ST Genesia may facilitate the implementation of thrombin generation in the routine laboratory, and by this way, it may offer a serious input in the individual screening of patients requiring anticoagulants. However, multicentric studies are required to assess the inter-laboratory reproducibility of the ST Genesia and confirm its usefulness for standardizing thrombin generation testing. In addition, the STG®-DrugScreen application of the ST Genesia should be part of the propositions of external quality assurance programs.

Authorship

Among the authors, JD and FM designed the study; CD, MG, JB performed the analyses; JD, CD, MG and JB collected data; JD, LM and CB analyzed and interpreted data; JD, LM and CB performed statistical analysis; JD, LM, CB, MdS, BD, ASD, JMD, ASL, SL and FMU wrote the manuscript.

Conflicts of interests

The study was financed by Diagnostica Stago group. Among the authors, J. Douxfils is CEO and founder of QUALIblood s.a. and reports personal fees from Diagnostica Stago, Roche, Roche Diagnostics and Daiichi-Sankyo, outside the submitted work. F. Mullier reports institutional fees from Diagnostica Stago, Werfen, Nodia, Sysmex and Bayer. He also reports speaker fees from Boehringer Ingelheim, Bayer Healthcare and Bristol-Myers Squibb-Pfizer, all outside the submitted work. The other authors have no conflicts of interest to disclose.

REFERENCES

- 1 Macfarlane RG, Biggs R. A thrombin generation test; the application in haemophilia and thrombocytopenia. *Journal of clinical pathology*. 1953; **6**: 3-8.
- 2 Tripodi A. Thrombin Generation Assay and Its Application in the Clinical Laboratory. *Clinical chemistry*. 2016; **62**: 699-707. 10.1373/clinchem.2015.248625.
- 3 Hemker HC, Willems GM, Beguin S. A computer assisted method to obtain the prothrombin activation velocity in whole plasma independent of thrombin decay processes. *Thromb Haemost*. 1986; **56**: 9-17.
- 4 Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoort R, Lecompte T, Beguin S. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003; **33**: 4-15. 10.1159/000071636.
- 5 Kintigh J, Monagle P, Ignjatovic V. A review of commercially available thrombin generation assays. *Res Pract Thromb Haemost*. 2018; **2**: 42-8. 10.1002/rth2.12048.
- 6 Dargaud Y, Luddington R, Gray E, Lecompte T, Siegemund T, Baglin T, Hogwood J, Regnault V, Siegemund A, Negrier C. Standardisation of thrombin generation test--which reference plasma for TGT? An international multicentre study. *Thrombosis research*. 2010; **125**: 353-6. 10.1016/j.thromres.2009.11.012.
- 7 Dargaud Y, Luddington R, Gray E, Negrier C, Lecompte T, Petros S, Hogwood J, Bordet JC, Regnault V, Siegemund A, Baglin T. Effect of standardization and normalization on imprecision of calibrated automated thrombography: an international multicentre study. *British journal of haematology*. 2007; **139**: 303-9. 10.1111/j.1365-2141.2007.06785.x.
- 8 Loeffen R, Kleinegris MC, Loubele ST, Pluijmen PH, Fens D, van Oerle R, ten Cate H, Spronk HM. Preanalytic variables of thrombin generation: towards a standard procedure and validation of the method. *Journal of thrombosis and haemostasis : JTH*. 2012; **10**: 2544-54. 10.1111/jth.12012.
- 9 Dargaud Y, Wolberg AS, Luddington R, Regnault V, Spronk H, Baglin T, Lecompte T, Ten Cate H, Negrier C. Evaluation of a standardized protocol for thrombin generation measurement using the calibrated automated thrombogram: an international multicentre study. *Thrombosis research*. 2012; **130**: 929-34. 10.1016/j.thromres.2012.07.017.
- 10 Perrin J, Depasse F, Lecompte T, French-speaking CATg, under the aegis of G, French-speaking CATg, French-speaking CATgaiFuos. Large external quality assessment survey on thrombin generation with CAT: further evidence for the usefulness of normalisation with an external reference plasma. *Thrombosis research*. 2015; **136**: 125-30. 10.1016/j.thromres.2014.12.015.
- 11 Spronk HM, Dielis AW, De Smedt E, van Oerle R, Fens D, Prins MH, Hamulyak K, ten Cate H. Assessment of thrombin generation II: Validation of the Calibrated Automated Thrombogram in platelet-poor plasma in a clinical laboratory. *Thromb Haemost*. 2008; **100**: 362-4. 10.1160/th08-03-0141.
- 12 Gerotziakas GT, Elalamy I, Depasse F, Perzborn E, Samama MM. In vitro inhibition of thrombin generation, after tissue factor pathway activation, by the oral, direct factor Xa inhibitor rivaroxaban. *Journal of thrombosis and haemostasis : JTH*. 2007; **5**: 886-8. 10.1111/j.1538-7836.2007.02429.x.
- 13 Douxfils J, Chatelain B, Chatelain C, Dogne JM, Mullier F. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. *Thromb Haemost*. 2016; **115**: 368-81. 10.1160/TH15-05-0415.
- 14 Douxfils J, Chatelain C, Chatelain B, Dogne JM, Mullier F. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. *Thromb Haemost*. 2013; **110**: 283-94. 10.1160/TH12-12-0898.
- 15 Douxfils 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. *Thrombosis research*. 2012; **130**: 956-66. 10.1016/j.thromres.2012.09.004.
- 16 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**: 985-97. 10.1160/TH11-11-0804.
- 17 Bloemen S, Zwaveling S, Douxfils J, Roest M, Kremers R, Mullier F. The anticoagulant effect of dabigatran is reflected in the lag time and time-to-peak, but not in the endogenous thrombin potential or peak, of thrombin generation. *Thrombosis research*. 2018; **171**: 160-6. 10.1016/j.thromres.2018.10.005.
- 18 Dale B, Eikelboom JW, Weitz JI, Young E, Paikin JS, Coppens M, Whitlock RP, Connolly SJ, Ginsberg JS, Hirsh J. Dabigatran attenuates thrombin generation to a lesser extent than warfarin: could this explain their differential effects on intracranial hemorrhage and myocardial infarction? *Journal of thrombosis and thrombolysis*. 2013; **35**: 295-301. 10.1007/s11239-012-0857-9.
- 19 Hacquard M, Perrin J, Lelievre N, Vigneron C, Lecompte T. Inter-individual variability of effect of 7 low molecular weight antithrombin-dependent anticoagulants studied in vitro with calibrated automated thrombography. *Thrombosis research*. 2011; **127**: 29-34. 10.1016/j.thromres.2010.07.024.
- 20 Siguret V, Foulon G, Abdoul J, Carlo A, Lecompte T, Gouin-Thibault I. PB584/Thrombin Generation Analysis with a New Automated System (ST-Genesia): Inter-series Performances during DRIVING Study and Comparison with CAT System. *Res Pract Thromb Haemost*. 2018; **2**: 285. 10.1002/rth2.12125.
- 21 Kluft C, Meijer P. External quality assessment for thrombin generation tests: an exploration. *Seminars in thrombosis and hemostasis*. 2010; **36**: 791-6. 10.1055/s-0030-1265296.

- 22 Rodgers SE, Wong A, Gopal RD, Dale BJ, Duncan EM, McRae SJ. Evaluation of pre-analytical variables in a commercial thrombin generation assay. *Thrombosis research*. 2014; **134**: 160-4. 10.1016/j.thromres.2014.04.010.
- 23 Bagot CN, Leishman E. Establishing a reference range for thrombin generation using a standard plasma significantly improves assay precision. *Thrombosis research*. 2015; **136**: 139-43. 10.1016/j.thromres.2015.04.020.
- 24 Lacroix R, Judicone C, Poncelet P, Robert S, Arnaud L, Sampol J, Dignat-George F. Impact of pre-analytical parameters on the measurement of circulating microparticles: towards standardization of protocol. *Journal of thrombosis and haemostasis : JTH*. 2012; **10**: 437-46. 10.1111/j.1538-7836.2011.04610.x.
- 25 Rojnuckarin P, Akkawat B, Juntiang J. Stability of plasma anti-Xa activity in low-molecular-weight heparin monitoring. *Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis*. 2010; **16**: 313-7. 10.1177/1076029609336853.
- 26 Adcock Funk DM, Hoefner D, Kottke-Marchant K, Marlar RA, Szamosi DI, Warunek DJ. H21-A5: Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline — Fifth Edition. In: Institute CaLS, ed., 2008.
- 27 Woodhams B, Girardot O, Blanco MJ, Colesse G, Gourmelin Y. Stability of coagulation proteins in frozen plasma. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. 2001; **12**: 229-36.
- 28 Rigano J, Ng C, Nandurkar H, Ho P. Thrombin generation estimates the anticoagulation effect of direct oral anticoagulants with significant interindividual variability observed. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. 2018; **29**: 148-54. 10.1097/MBC.0000000000000678.
- 29 Chowdary P, Adamidou D, Riddell A, Aghighi S, Griffioen A, Priest P, Moghadam L, Kelaheer N, Huq FY, Kadir RA, Tuddenham EG, Gatt A. Thrombin generation assay identifies individual variability in responses to low molecular weight heparin in pregnancy: implications for anticoagulant monitoring. *British journal of haematology*. 2015; **168**: 719-27. 10.1111/bjh.13193.
- 30 Radulovic V, Hyllner M, Ternstrom L, Karlsson M, Bylock A, Hansson KM, Baghaei F, Jeppsson A. Sustained heparin effect contributes to reduced plasma thrombin generation capacity early after cardiac surgery. *Thrombosis research*. 2012; **130**: 769-74. 10.1016/j.thromres.2011.12.025.
- 31 al Dieri R, Alban S, Beguin S, Hemker HC. Thrombin generation for the control of heparin treatment, comparison with the activated partial thromboplastin time. *Journal of thrombosis and haemostasis : JTH*. 2004; **2**: 1395-401. 10.1111/j.1538-7836.2004.00798.x.
- 32 Robert S, Ghiotto J, Pirotte B, David JL, Masereel B, Pochet L, Dogne JM. Is thrombin generation the new rapid, reliable and relevant pharmacological tool for the development of anticoagulant drugs? *Pharmacol Res*. 2009; **59**: 160-6. 10.1016/j.phrs.2008.12.003.
- 33 Siriez R, Evrard J, Dogne JM, Pochet L, Gheldof D, Chatelain B, Mullier F, Douxfils J. Betrixaban: Impact on Routine and Specific Coagulation Assays-A Practical Laboratory Guide. *Thromb Haemost*. 2018; **118**: 1203-14. 10.1055/s-0038-1657772.
- 34 Bloemen S, Hemker HC, Al Dieri R. Large inter-individual variation of the pharmacodynamic effect of anticoagulant drugs on thrombin generation. *Haematologica*. 2013; **98**: 549-54. 10.3324/haematol.2012.073601.
- 35 Sennesael AL, Larock AS, Douxfils J, Elens L, Stillemans G, Wiesen M, Taubert M, Dogne JM, Spinewine A, Mullier F. Rivaroxaban plasma levels in patients admitted for bleeding events: insights from a prospective study. *Thrombosis journal*. 2018; **16**: 28. 10.1186/s12959-018-0183-3.
- 36 Honickel M, Braunschweig T, Rossaint R, Stoppe C, Ten Cate H, Grottke O. Reversing Dabigatran Anticoagulation with Prothrombin Complex Concentrate versus Idarucizumab as Part of Multimodal Hemostatic Intervention in an Animal Model of Polytrauma. *Anesthesiology*. 2017; **127**: 852-61. 10.1097/ALN.0000000000001856.
- 37 Neal MD, Levy JH. Precision Correction of Coagulopathy or Prothrombin Complex Concentrates?: Reversal Options for Dabigatran following Trauma. *Anesthesiology*. 2017; **127**: 744-6. 10.1097/ALN.0000000000001857.

TABLES

Table 1: Coefficient of variation of the different quality controls over 2 batches of STG®-DrugScreen kit. Results are presented as CV expressed in percentage. For the 2 levels of controls, results are presented as absolute or normalized (against STG® -RefPlasma DS). The use of a reference plasma titrated by a manufacturer reduces the inter-batch variation. Difference in % represents the relative difference between the two batches of reagents and controls for each TGT parameter.

		STG®-QualiTest Norm DS			STG®-QualiTest Low DS			STG®-RefPlasma DS			Mean coefficient of variation
		Mean value	Standard deviation	Coefficient of variation	Mean value	Standard deviation	Coefficient of variation	Mean value	Standard deviation	Coefficient of variation	
Lag Time (min – absolute / ratio - normalized)											
Absolute	All (n=97)	0.87 min	0.06 min	6.53%	1.05 min	0.12 min	11.03%	0.95 min	0.05 min	5.11%	7.56%
	Batch 201797 (n=37)	0.93 min	0.04 min	4.03%	1.19 min	0.05 min	3.97%	0.98 min	0.05 min	5.25%	4.42%
	Batch 202028 (n=60)	0.84 min	0.04 min	4.36%	0.96 min	0.03 min	3.14%	0.93 min	0.03 min	3.38%	3.63%
	Difference in %	11%			24%			5%			
Normalized	All (n=97)	0.92	0.04	4.71%	1.10	0.09	8.47%				6.59%
	Batch 201797 (n=37)	0.95	0.04	3.83%	1.21	0.05	4.22%				4.03%
	Batch 202028 (n=60)	0.91	0.04	4.42%	1.04	0.04	3.69%				4.06%
	Difference in %	4%			16%						
Time-to-peak (min – absolute / ratio – normalized)											
Absolute	All (n=97)	1.91 min	0.08 min	4.07%	2.14 min	0.17 min	7.85%	2.13 min	0.07 min	3.26%	5.06%
	Batch 201797 (n=37)	1.97 min	0.07 min	3.60%	2.33 min	0.10 min	4.25%	2.17 min	0.08 min	3.61%	3.82%
	Batch 202028 (n=60)	1.86 min	0.05 min	2.60%	2.02 min	0.05 min	2.28%	2.11 min	0.05 min	2.50%	2.46%
	Difference in %	6%			15%			3%			
Normalized	All (n=97)	0.90	0.02	2.70%	1.00	0.06	6.19%				4.45%
	Batch 201797 (n=37)	0.91	0.02	2.09%	1.08	0.03	2.55%				2.69%
	Batch 202028 (n=60)	0.89	0.02	2.58%	0.96	0.03	2.56%				2.57%
	Difference in %	2%			13%						
Peak Height (nM – absolute / % - normalized)											
Absolute	All (n=97)	480.1 nM	22.6 nM	4.71%	215.9 nM	13.3 nM	6.17%	472.5 nM	18.6 nM	3.95%	4.94%
	Batch 201797 (n=37)	501.8 nM	16.5 nM	3.30%	206.2 nM	10.1 nM	4.89%	485.8 nM	17.0 nM	3.50%	3.90%
	Batch 202028 (n=60)	466.7 nM	13.7 nM	2.93%	221.9 nM	11.5 nM	5.16%	464.3 nM	14.5 nM	3.12%	3.74%
	Difference in %	8%			7%			5%			
Normalized	All (n=97)	101.6%	2.9%	2.81%	45.7%	3.2%	6.94%				4.88%
	Batch 201797 (n=37)	103.2%	2.2%	2.08%	42.4%	1.4%	3.30%				2.69%
	Batch 202028 (n=60)	100.5%	2.8%	2.75%	47.8%	2.0%	4.15%				3.45%
	Difference in %	3%			11%						

Endogenous Thrombin Potential (ETP) (nM.min – absolute / % - normalized)											
Absolute	All (n=97)	1531 nM.min	96 nM.min	6.26%	523 nM.min	22 nM.min	4.27%	1722 nM.min	72 nM.min	4.19%	4.91%
	Batch 201797 (n=37)	1607 nM.min	65 nM.min	4.07%	524 nM.min	19 nM.min	3.53%	1736 nM.min	64 nM.min	3.67%	3.76%
	Batch 202028 (n=60)	1483 nM.min	80 nM.min	5.40%	522 nM.min	25 nM.min	4.69%	1714 nM.min	76 nM.min	4.45%	4.85%
	Difference in %	8%			0%			1%			
Normalized	All (n=97)	88.9%	4.9%	5.56%	30.4%	1.3%	4.18%				4,87%
	Batch 201797 (n=37)	92.6%	3.7%	3.95%	30.2%	1.1%	3.47%				3,71%
	Batch 202028 (n=60)	86.6%	4.2%	4.85%	30.5%	1.4%	4.55%				4,70%
	Difference in %	7%			1%						

Table 2: Impact of freezing on thrombin generation parameters. Wilcoxon matched-pairs signed rank test have been performed to compare the impact on freezing (i.e. at D0 on fresh and at D+1 once the samples have been frozen). When considering all results (n=29) freezing has an impact on lag time and time-to-peak. Results are also stratified by treatment but the number of samples is too small to drive any conclusion.

Fresh (D0) versus frozen (D1)	Passed normality testing?	Mean difference (D1-D0)	Wilcoxon matched-pairs signed rank test or paired t-test (p-value)	Significant?
	All results (n=29)			
Lag time†	yes†	-0.08 min	0.7179†	no
Peak height	no	3.29 nM	0.096	no
Time-to-peak	no	-0.15 min	0.020	yes
ETP†	yes†	1 nM.min	0.9702†	no
	Apixaban (n=4)			
Lag time	N too small	-0.09 min	0.375	no
Peak height		19.38 nM	0.250	no
Time-to-peak		-0.24 min	0.250	no
ETP		-4 nM.min	0.875	no
	Dabigatran (n=3)			
Lag time	N too small	-0.25 min	0.250	no
Peak height		7.53 nM	0.500	no
Time-to-peak		-0.23 min	0.500	no
ETP		27 nM.min	0.250	no
	Healthy (n=6)			
Lag time	N too small	-0.02 min	>0.999	no
Peak height		-20.17 nM	0.688	no
Time-to-peak		-0.03 min	>0.999	no
ETP		-116 nM.min	0.438	no
	LMWH (n=5)			
Lag time	N too small	-0.00 min	>0.999	no
Peak height		2.78 nM	0.625	no
Time-to-peak		-0.09 min	0.474	no
ETP		28 nM.min	0.313	no
	Rivaroxaban (n=5)			
Lag time	N too small	-0.21 min	0.063	no
Peak height		14.77 nM	0.125	no
Time-to-peak		-0.53 min	0.063	no
ETP		79 nM.min	0.188	no
	Vitamin K antagonists (n=6)			
Lag time	N too small	-0.02 min	0.750	no
Peak height		4.79 nM	0.313	no
Time-to-peak		-0.04 min	0.313	no
ETP		22 nM.min	0.219	no

† For these analyses, a paired t-test was used as the data passed the normality testing using the Shapiro-Wilk test.

Table 3: Stability of plasma samples once frozen at -80°C. The table summarizes all the parameters of the linear regressions obtained

Parameter		Linear regression										
		Slope	SE	95%CI	Slope significantly non-zero?	Intercept	SE	95% CI	Adjusted RL (%)		Time at which RL is reached*	
Lag time	all	0.414	0.898	-1.358 to 2.185	no	0.005	5.219	10.290 to 10.300	-99.995	to 100.005	11 months	
	apixaban	-3.210	3.243	-9.903 to 3.483	no	20.180	19.250	-19.540 to 59.910	-79.820	to 120.18	10 months	
	dabigatran	-1.412	1.853	-5.340 to 2.516	no	6.077	11.150	-17.560 to 29.720	-93.923	to 106.08	10 months	
	healthy	2.165	1.316	-0.504 to 4.834	no	-17.280	8.207	-33.920 to -0.632	-117.280	to 82.72	11 months	
	LMWH	-0.383	2.028	-4.520 to 3.753	no	-9.564	11.810	-33.830 to 14.320	-109.564	to 90.436	10 months	
	rivaroxaban	1.453	2.485	-3.603 to 6.509	no	-0.721	13.190	-27.560 to 26.120	-100.721	to 99.279	10 months	
	VKA	2.216	2.069	-1.994 to 6.426	no	9.868	11.590	-13.710 to 33.450	-90.132	to 109.87	10 months	
Time-to-peak	all	-1.596	0.769	-3.113 to -0.078	yes	-3.735	4.471	-12.560 to 5.086	-103.735	to 96.265	11 months	
	apixaban	-0.031	2.089	-4.334 to 4.271	no	2.476	12.190	-22.640 to 27.590	-97.524	to 102.746	10 months	
	dabigatran	-3.204	3.012	-9.589 to 3.181	no	-21.720	18.13	-60.140 to 16.710	-121.720	to 78.280	10 months	
	healthy	-1.465	1.197	-3.892 to 0.9618	no	-17.370	7.463	-32.510 to 2.238	-117.37	to 82.630	11 months	
	LMWH	-3.527	1.870	-7.342 to 0.2870	no	-3.726	10.890	-25.930 to 18.480	-103.726	to 96.274	10 months	
	rivaroxaban	-0.9155	1.585	-4.140 to 2.309	no	0.691	8.412	-16.420 to 17.810	-99.309	to 100.691	10 months	
	VKA	-0.055	1.737	-3.592 to 3.483	no	7.920	9.870	-12.180 to 28.020	-92.080	to 107.920	10 months	
ETP	all	2.453	0.794	0.886 to 4.019	yes	-4.123	4.615	-13.230 to 4.980	-104.123	to 95.877	11 months	
	apixaban	2.873	1.989	-1.191 to 6.937	no	-4.348	11.690	-28.470 to 19.770	-104.348	to 95.652	10 months	
	dabigatran	-1.850	2.721	-7.618 to 3.918	no	17.650	16.380	-17.060 to 52.360	-82.350	to 117.65	10 months	
	healthy	3.015	1.097	0.7903 to 5.240	yes	-6.215	6.842	-20.090 to 7.661	-106.215	to 93.785	11 months	
	LMWH	5.053	2.586	-0.221 to 10.330	no	-12.630	15.050	-43.330 to 18.060	-112.630	to 87.37	10 months	
	rivaroxaban	3.280	2.321	-1.441 to 8.002	no	-9.106	12.320	-34.170 to 15.950	-109.106	to 90.894	10 months	
	VKA	1.697	7.429	-2.515 to 2.882	no	1.697	7.429	-13.420 to 16.810	-98.303	to 101.7	10 months	
Peak height	all	3.749	1.051	-1.675 to 5.822	yes	-10.310	6.108	-22.360 to 1.745	-110.310	to 89.69	11 months	
	apixaban	-0.064	2.204	-4.612 to 4.484	no	-20.860	13.080	-47.860 to 6.136	-120.860	to 79.14	10 months	
	dabigatran	0.952	2.244	-3.805 to 5.708	no	11.470	13.500	-17.150 to 40.100	-88.530	to 111.47	10 months	
	healthy	3.252	0.682	1.870 to 4.634	yes	7.126	4.250	-1.494 to 15.750	-92.874	to 107.13	11 months	
	LMWH	10.950	4.141	2.506 to 19.400	yes	-16.530	24.100	-65.690 to 32.630	-116.530	to 83.47	6 months	
	rivaroxaban	1.657	2.245	-2.911 to 6.224	no	-22.670	11.920	-46.910 to 1.573	-122.670	to 77.33	10 months	
	VKA	2.904	1.591	-0.334 to 6.141	no	-10.460	8.912	-28.590 to 7.673	-110.460	to 89.54	10 months	

* When the regression slope is not statistically significant ($p > 0.05$) from zero, this indicates parameter is not changing as a function of time and the maximum storage time is equal to T_{MAX} (last point of the linear regression).
When the regression slope is statistically significant ($p \leq 0.05$) from zero, and the on-sided 95% confidence limit of the regression line does not intersect the adjusted rejection limit at T_{MAX} , the maximum storage time is equal to T_{MAX} .
When the regression slope is statistically significant ($p \leq 0.05$) from zero, and the one-sided 95% confidence limit of the regression line intersects the adjusted rejection limit before reaching T_{MAX} , the maximum storage time is determined as this intersection.

Abbreviations: CI: Confidence Interval; ETP: Endogenous Thrombin Potential; LMWH: Low Molecular Weight Heparin; RL: Rejection Limit; SE: Standard Error; VKA: Vitamin K Antagonists

Table 4: Normal range definition of the STG®-DrugScreen application on the ST Genesis system using plasma sample from 42 healthy individuals. All samples were tested on the same day, explaining the few differences in term of variability between absolute and normalized results.

Healthy (n=42)*	Lag time absolute	Peak Height absolute	Time-to-peak absolute	ETP absolute	Lag time normalized (ratio)	Peak Height normalized	Time-to-peak normalized (ratio)	ETP normalized
Mean	0.93 min	456.20 nM	2.03 min	1418 nM	1.16	97%	0.98	83%
Standard deviation	0.09 min	56.35 nM	0.16 min	270 nM	0.10	12%	0.08	15%
Coefficient of variation (%)	9.8%	12.4%	7.9%	19.1%	9.6%	12.1%	7.9%	18%
Minimum	0.73 min	355.90 nM	1.80 min	1027 nM	0.81	75%	0.87	56%
Maximum	1.22 min	571.40 nM	2.69 min	1993 nM	1.33	120%	1.29	118%
10% Percentile	0.82 min	380.20 nM	1.87 min	1122 nM	0.90	81%	0.90	68%
90% Percentile	1.00 min	538.90 nM	2.17 min	1851 nM	1.11	115%	1.29	109%

Abbreviations: ETP: Endogenous Thrombin Potential

Table 5: Thrombin generation results at T0 of all patients and healthy donors included in the stability study. Results of the intensity of anticoagulation (expressed as ng/mL for DOACs, IU/mL for LMWH and INR for VKA) are provided.

Patient n°	Lag time absolute	Peak Height absolute	Time-to-peak absolute	ETP absolute	Lag time normalized (ratio)	Peak Height normalized	Time-to-peak normalized (ratio)	ETP normalized	Anticoagulant ng/mL or IU/mL or INR
Healthy 1	0.96 min	561.90 nM	2.12 min	1903 nM.min	0.98	113%	0.96	105%	
Healthy 2	1.11 min	382.90 nM	2.37 min	1263 nM.min	1.12	77%	1.08	69%	
Healthy 3	1.00 min	395.00 nM	2.36 min	1509 nM.min	1.01	80%	1.07	83%	
Healthy 4	0.95 min	488.20 nM	2.01 min	1844 nM.min	0.96	98%	0.91	101%	
Healthy 5	1.14 min	410.00 nM	2.64 min	1589 nM.min	1.15	83%	1.20	87%	
Healthy 6	1.06 min	427.10 nM	2.38 min	1716 nM.min	1.07	86%	1.08	94%	
Apixaban 1	2.48 min	153.90 nM	4.69 min	1295 nM.min	2.62	31%	2.23	77%	307 ng/mL
Apixaban 2	1.90 min	112.40 nM	6.79 min	1214 nM.min	2.01	23%	3.23	72%	269 ng/mL
Apixaban 3	2.77 min	247.90 nM	4.82 min	1437 nM.min	2.83	46%	2.19	74%	158 ng/mL
Apixaban 4	2.14 min	169.20 nM	4.29 min	995 nM.min	2.27	33%	2.08	56%	195 ng/mL
Dabigatran 1	2.71 min	325.30 nM	3.84 min	1150 nM.min	2.64	69%	1.74	68%	89 ng/mL
Dabigatran 2	2.22 min	401.50 nM	3.35 min	1361 nM.min	2.22	87%	1.50	83%	51 ng/mL
Dabigatran 3	2.64 min	312.80 nM	3.66 min	1015 nM.min	2.86	68%	1.78	60%	187 ng/mL
LMWH 1	1.87 min	43.83 nM	3.81 min	260 nM.min	1.93	8%	1.81	14%	1.37 IU/mL
LMWH 2	1.41 min	331.70 nM	2.81 min	1179 nM.min	1.45	64%	1.34	65%	0.35 IU/mL
LMWH 3	1.33 min	334.00 nM	2.54 min	1092 nM.min	1.39	69%	1.25	64%	0.35 IU/mL
LMWH 4	1.38 min	259.70 nM	2.79 min	879 nM.min	1.41	57%	1.27	49%	0.52 IU/mL
LMWH 5	1.35 min	122.70 nM	3.49 min	661 nM.min	1.51	27%	1.64	40%	1.26 IU/mL
Rivaroxaban 1	2.47 min	177.90 nM	7.01 min	1695 nM.min	2.49	37%	3.23	101%	229 ng/mL
Rivaroxaban 2	3.33 min	93.67 nM	9.67 min	965 nM.min	3.65	20%	4.61	58%	197 ng/mL
Rivaroxaban 3	2.88 min	191.30 nM	6.29 min	1454 nM.min	3.12	38%	3.01	82%	218 ng/mL
Rivaroxaban 4	3.28 min	146.10 nM	7.35 min	1187 nM.min	3.43	30%	3.62	70%	296 ng/mL
Rivaroxaban 5	1.95 min	106.40 nM	8.03 min	1064 nM.min	2.12	23%	3.90	63%	300 ng/mL
VKA 1	2.06 min	104.80 nM	3.43 min	451 nM.min	2.01	22%	1.55	27%	2.57
VKA 2	2.14 min	109.80 nM	3.35 min	391 nM.min	2.24	23%	1.65	23%	3.31
VKA 3	2.87 min	137.90 nM	4.56 min	525 nM.min	3.01	29%	2.16	29%	1.86
VKA 4	2.39 min	118.70 nM	3.74 min	451 nM.min	2.44	26%	1.71	25%	2.91
VKA 5	2.10 min	91.17 nM	3.26 min	260 nM.min	2.14	20%	1.49	15%	2.68
VKA 6	1.82 min	130.90 nM	3.36 min	459 nM.min	2.04	29%	1.60	27%	1.79

Abbreviations: DOACs: Direct Oral Anticoagulants (i.e. apixaban, dabigatran, rivaroxaban); ETP: Endogenous Thrombin Potential; INR: International Normalized Ratio; IU: International anti-Xa units; LMWH: Low Molecular Weight Heparin; VKA: Vitamin K Antagonist

Figures

Figure 1: Stability of the different thrombin generation parameters. The 100% rejection limit (RL) has been defined as the maximal allowable error (MAE). For the ETP, the MAE was 20% and for the lag time, time-to-peak and the peak height, the MAE was 18% based on the data from the precision study. The Y-intercept was used to determine the adjusted RL (upper adjusted RL = 100 + Y-intercept – lower adjusted RL = -100 + Y-intercept). The 95%CI of the linear regression (the blue hashed zone) do not cross the adjusted RL which means that the samples are stable over the analyzed period (i.e. 11 months for healthy subject and 10 months for anticoagulated patients).

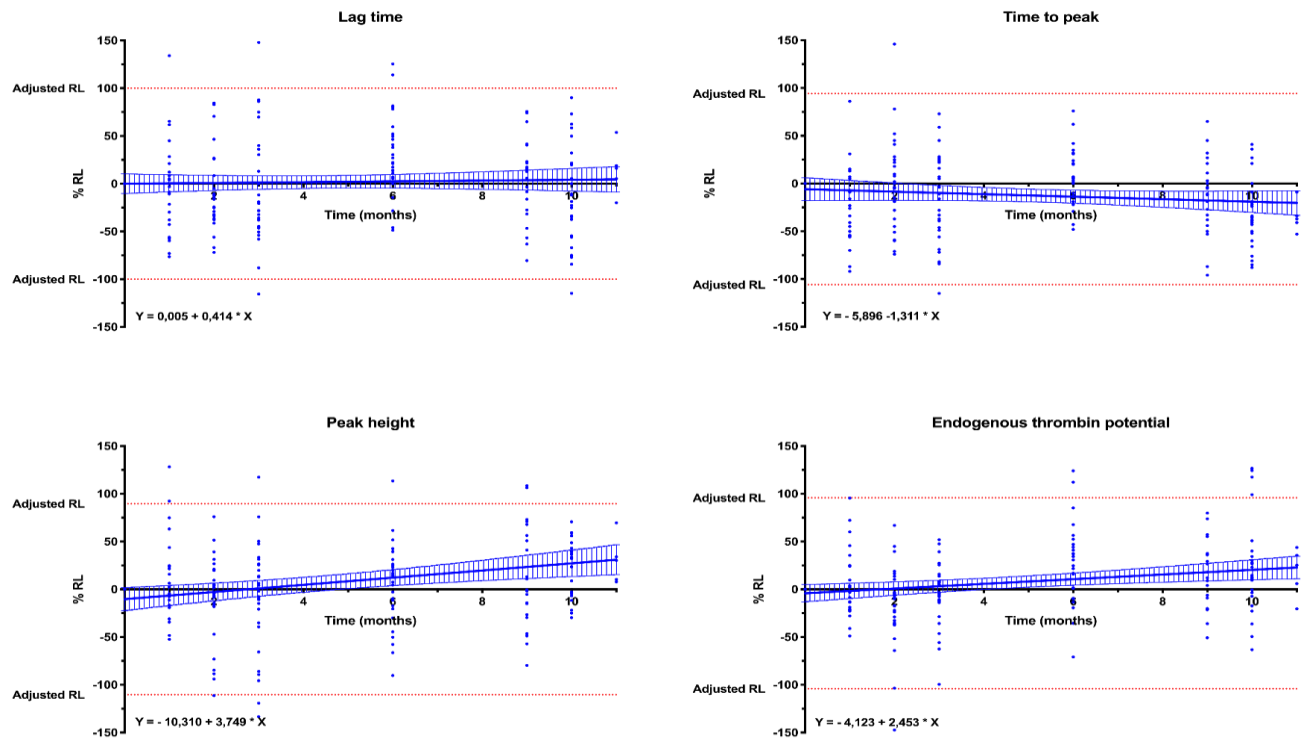


Figure 2: Stability results for samples from patients treated with low molecular weight heparin. The upper 95%CI limit of the linear regression crosses the adjusted rejection limit (RL) after 6 months determining the maximal stability of the samples.

