**Multicenter in vitro thromboelastography and thromboelastometry standardization**

Multicenter in vitro thromboelastography and thromboelastometry

Robert Goggs, BVSc, DACVECC, DECVECC, PhD; Antonio Borrelli,[1] DVM, PhD, MS; Benjamin M. Brainard,[1] DVM, DACVAA, DACVECC; Daniel L. Chan,[1] DVM, DACVN, DACVECC, DECVECC; Armelle de Laforcade,[1] DVM, DACVECC; Isabelle Goy-Thollot,[1] DVM, DECVECC; Karl E. Jandrey,[1] DVM, MS, DACVECC; Annemarie T. Kristensen,[1] DVM, PhD, DACVIM, DECVIM; Annette Kutter,[1] DVM, DECVAA; Clara B. Marschner,[1] DVM, PhD; Benoît Rannou[1] DMV, MSc, DACVP; Nadja Sigrist,[1] Dr.med.vet, DACVECC, DECVECC and Catherine Wagg,[1] DVM, DACVP

[1]These authors contributed equally to this study.

From the Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY (Goggs); the Department of Veterinary Science, University of Turin, Grugliasco, Italy (Borrelli); the University of Georgia (Brainard); the Department of Clinical Sciences and Services, Royal Veterinary College, London (Chan); the Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA (de Laforcade); the VetAgro Sup, Campus Vétérinaire de Lyon, Marcy l'Étoile, France (Goy-Thollot, Rannou); University of California, Davis, CA (Jandrey); the Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark (Kristensen, Marschner); Vetsuisse Faculty, University of Zurich, Zurich, Switzerland (Kutter, Sigrist); and the University of Calgary, Calgary, Canada (Wagg).

**Please address all correspondence and offprint requests to:** Dr. Robert Goggs, Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, 930 Campus Road, Ithaca, NY, 14850, USA. Email: r.goggs@cornell.edu

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**Running title:** Standardization of TEG and ROTEM

## Abbreviations

| | |
|---|---|
| CFT | clot formation time |
| CP | cryoprecipitate |
| CT | clotting time |
| CV | coefficient of variation |
| EX-TEM | proprietary tissue factor activated assay |
| FFP | fresh frozen plasma |
| FFP + CP | fresh frozen plasma with admixed cryoprecipitate |
| ICC | intraclass correlation coefficient |
| IN-TEM | proprietary ellagic acid activated assay |
| K time | clot formation time |
| MA | maximum amplitude |
| MCF | maximum clot firmness |
| PRP | platelet-rich plasma |
| R time | reaction time |
| ROTEM | thromboelastometry |
| TEG | thromboelastography |
| TF | tissue factor |

## Abstract

**Objective** – To establish and compare the repeatability and reproducibility of activated thromboelastography (TEG) and thromboelastometry (ROTEM) assays.

**Design** – Multicenter in vitro test standardization.

**Setting** – Veterinary academic centers.

**Animals** – Test samples were obtained from normal, healthy dogs. Sixty, identical 5 mL aliquots of canine platelet-rich plasma collected by apheresis, frozen in 6% dimethyl sulfoxide were tested initially. Sixty, identical 6 mL aliquots of canine fresh-frozen plasma with admixed cryoprecipitate were subsequently evaluated.

**Interventions** – None.

**Measurements and Main Results** – Frozen study samples, quality controls, reagents, and consumables were distributed to participating centers (7 TEG, 3 ROTEM). Thromboelastography centers analyzed study samples with kaolin and tissue factor activated assays; ROTEM centers ran proprietary ellagic acid activated and tissue factor activated assays. All machines underwent quality control prior to sample analysis. Within- and between-center coefficients of variation (CVs) were calculated and compared by Mann-Whitney tests and calculation of intraclass correlation coefficients. Within and between centers, individual parameters for both TEG and ROTEM assays were comparable. Both within-center and between-center CVs varied markedly (0.7 – 120.5% and 1.4 – 116.5%, respectively) with assay type, instrument, and parameter. Coefficients of variation for equivalent parameters were not significantly different between the 2 platforms. Intraclass correlation coefficients suggested moderate agreement between centers. In general, individual parameter CVs for platelet-rich plasma samples were lower in TEG centers, while CVs for canine fresh-frozen plasma with admixed cryoprecipitate samples were lower in ROTEM centers.

**Conclusions** – More variation within and between centers was identified than anticipated, but some parameters such as alpha angle were repeatable and reproducible. Sample types for future multicenter standardization efforts will require further optimization and may need to be adapted separately to each platform. Individual centers using viscoelastic tests for evaluation and management of clinical patients should take steps to minimize pre-analytical and analytical sources of variation.

**Keywords**: canine, dog, repeatability, reproducibility, thromboelastography, thromboelastometry

## Introduction

Patients with bleeding diatheses and thromboembolic disorders are frequently encountered in veterinary emergency and critical care practice. Optimizing the management of these animals requires identification of the cause and severity of their coagulation disorder, which may be multifactorial in origin. Ex vivo rotational viscoelastic tests of coagulation, namely thromboelastography (TEG) and thromboelastometry (ROTEM), provide a global assessment of hemostasis that integrates both the cellular and plasma components of the hemostatic system,[1] which may enhance understanding of the nature[2] and severity[3] of the coagulation disturbances seen in veterinary patients.[4] The TEG and ROTEM analysis systems employ a similar test principle and provide comparable, although not identical, results.[5,6] These tests are proposed to better reflect the cell-based model of hemostasis,[7] which may enable assessment of hemorrhage risk in the clinical setting better than routine plasma based assays.[3,8] Such monitoring is now recommended in select human perioperative scenarios.[9]

Within the veterinary TEG / ROTEM literature, various modifications of the basic assay have been employed and evaluated. Differences exist in 3 particular areas: control of pre-analytical variables such as anticoagulant type or blood collection system,[10,11] the method used to activate clot formation,[12] and modifications of the assay to enhance evaluation of specific hemostatic system components.[13-15] Previous work has highlighted the potential variation in data that can result from small changes in pre-analytical variables,[16] and through use of different activators.[17] It is likely that some of these differences are sufficient to alter interpretation of data obtained within the same clinical laboratory, which may lead to inappropriate diagnosis or alterations in patient management. In addition, variation between the assays used at different centers limits the comparability of published results, which limits the utility and impact of the veterinary TEG literature and hampers progress. As has been recently recognized in human medicine, standardization of TEG is a priority for the field.[18,19] To address this, the Partnership for ROtational ViscoElastic Test Standardization (PROVETS) collaboration was established from an international group of veterinary clinicians and investigators,[20] and was based on a similar project undertaken in human medicine.[21] The PROVETS collaboration devised evidence-based guidelines for assay performance and reporting,[22-27] in an attempt to improve consistency in assay conduct, interpretation, and reporting between centers.

Currently there is very little information on inter-assay variation in TEG in veterinary medicine. Experimental evaluation of assay reproducibility is fundamental to assay standardization and is essential for fair comparison of data obtained from different centers.[28] Assay comparability is required in order that data generated in 1 center can be used to diagnose and treat patients appropriately in other centers,[29] and so that research efforts conducted in different centers can collectively move the field forward. The process of evaluating coagulation

assay comparability is challenging, however, since obtaining and distributing standardized test material for hemostasis assays is difficult.[30] To achieve worldwide distribution typically requires the use of lyophilized or frozen material,[21] despite the fact that most hemostasis laboratories analyze whole blood or fresh plasma samples.

The PROVETS group sought to determine the comparability of the results obtained by different TEG centers and secondarily, whether results obtained from similar TEG and ROTEM assays were comparable. Specifically, our objectives were: to establish the intra- and inter-assay coefficients of variation (CVs) for the contact pathway and the tissue factor activated assays on the TEG and ROTEM platforms, and to compare the repeatability and reproducibility of both contact pathway and tissue factor activated assays on both platforms within and between centers. Based on the literature, it was hypothesized that CVs between centers would be significantly greater than those within centers for all assays;[21] that CVs of assays using different activators would not be significantly different within centers;[31] and that CVs for equivalent TEG and ROTEM assays would not be significantly different.[32]

## Materials and Methods

### Participating centers

Ten centers across 7 countries in North America and Europe were chosen from the PROVETS collaborating institutions based on their willingness and ability to perform sample evaluation, their access to suitable numbers of test machines and their demonstrated expertise in the field. These centers (alphabetized by country) were: University of Calgary, Calgary, Canada (TEG); University of Copenhagen, Frederiksberg, Denmark (TEG); VetAgro Sup, Lyon, France (ROTEM); University of Turin, Turin, Italy (ROTEM); University of Zurich, Zurich,

Switzerland (ROTEM); Royal Veterinary College, London, UK (TEG), Cornell University, Ithaca, NY (TEG); University of California, Davis, CA (TEG); University of Georgia, Athens, GA (TEG); Tufts University, Grafton, MA (TEG).

**Test material**

Two sets of samples were obtained and distributed to participating centers. Sample choice for the first iteration was modeled on a similar study in human medicine.[21] In the first iteration, 60 custom-made, identical 5 mL aliquots of canine platelet-rich plasma (PRP) collected by apheresis from a single canine platelet donor were obtained from a commercial veterinary blood bank.[a] At collection, the PRP had a platelet count of 700,000 cells/µL. Prior to freezing, 6% dimethylsulfoxide was added to the samples, which were then frozen at -80°C and sent to the coordinating center overnight on dry ice. These samples were maintained at -80°C prior to distribution to participating centers. The 60 samples were divided into groups of 5 aliquots of 5 mL each. These batches of samples were sent from the organizing location to each participating center on dry ice by express courier. Each center was notified in advance to expect the shipments. Each center assessed the samples to determine whether they were still completely frozen upon arrival. Upon receipt these samples were stored at -80°C until analysis. Samples were distributed to participating centers in April 2014 and all analyses were completed within 4 months of sample receipt at all participating centers.

In the second iteration, attempts were made to overcome limitations present in the samples from the first round of analyses. Platelets were eliminated from the test material and in an attempt to ensure clot formation time (K-time or CFT) would be consistently measurable, plasma samples with admixed cryoprecipitate were generated. To produce these custom samples,

480 mL of canine fresh frozen plasma (FFP; 2 x 240 mL units) and 210 mL of lyophilized canine cryoprecipitate (3 x 70 mL units) were obtained from the same commercial veterinary blood bank. Acid-citrate-dextrose-adenine was used as the anticoagulant for preparation of both FFP and cryoprecipitate. The FFP was thawed to room temperature and the cryoprecipitate reconstituted over 10 minutes at room temperature using FFP as the diluent. The resulting solution was aseptically filtered using a 100 µm filter,[b] to remove particulates and aggregates. Samples of FFP and the resulting plasma/cryoprecipitate mixture (FFP + CP) were collected and their fibrinogen concentrations measured by the Clauss method by use of a human thrombin reagent,[c] and a standard curve was derived from dilutions of a canine plasma standard.[d] The plasma/cryoprecipitate solution was then aliquoted into 6 mL aliquots in 15 mL conical bottom polypropylene tubes,[e] and frozen at -80°C, prior to shipping to participating centers as previously described for the PRP samples. The second set of samples were distributed to participating centers in February 2015 and all analyses were completed within 4 months of sample receipt at the participating centers.

**Consumables**

To ensure uniformity in reagent and plasticware batches, all study consumables were purchased by the organizing center, divided into center-specific consignments and then distributed to the participating locations. The (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid with bovine serum albumin (HEPES-BSA) buffer (2% BSA, 20 mM HEPES, 150 mM NaCl) required for the tissue factor (TF) assay on the TEG platform was made up at the organizing center as previously described.[33,f,g] This solution was aliquoted in 15 mL conical bottom tubes and frozen at -80°C prior to shipping to TEG centers. Where additional preparatory steps that could only be

performed locally were required, detailed instructions were supplied to each participating center to ensure solutions are produced correctly and to maximize uniformity (see supplemental data).

**Analyzers**

All instruments underwent maintenance checks within 30 days of analyzing study samples. For TEG, Level 1 and Level 2 quality control samples[h] were evaluated prior to analysis of study samples. Internal electronic quality control tests were conducted on all TEG machines prior to study sample testing. Sites with ROTEM analyzers ran ROTROL-N and ROTROL-P controls[i] to verify instrument performance prior to analyzing study samples.

**Assays**

At each center, a single operator analyzed all samples according to a specific study protocol distributed to each center with the consumables (see supplemental data). If multiple TEG machines were available, then any number of channels could be used simultaneously, provided each channel successfully passed both electronic and quality control tests. No ROTEM center had > 1 analyzer, thus a maximum of 4 channels were used for simultaneous ROTEM analyses. For both TEG and ROTEM a contact pathway activated assay (kaolin for TEG, proprietary ellagic acid activated assay [IN-TEM] for ROTEM) and tissue factor activated assay (non-proprietary for TEG, proprietary tissue factor activated assay [EX-TEM] for ROTEM) were performed. Manual pipetting was performed for TEG assays. Electronic pipetting was performed for ROTEM assays. Repeats were performed consecutively until plasma sample aliquots were used up or 2 hours had lapsed since samples were thawed. Fresh aliquots were thawed as necessary to complete the required replicates. Relevant in-built analysis software was used to

calculate values for 4 TEG variables: reaction time (R-time), K-time, clot formation angle (alpha angle) and maximum amplitude (MA); and 4 ROTEM variables: clotting time (CT), CFT, clot formation angle (alpha angle) and maximum clot firmness (MCF). Each test was run for 60 minutes or until MA/MCF was reached. Twenty repeats per assay were performed.[23] All assays were run at 37°C.

For TEG, aliquots of test plasma were recalcified with 0.2M $CaCl_2$ reagent at the time of analysis,[j] with calcium chloride being added to pre-warmed TEG cups prior to addition of the test sample. Proprietary kaolin-activated assays were performed as previously described.[12,k] To evaluate extrinsic pathway activation by TF, a solution of recombinant human TF with synthetic phospholipid,[l] in HEPES-BSA was used. In the first iteration, a final concentration of 1:50,000 was used per Wiinberg et al. 2005,[33] while in the second iteration a higher TF final concentration of 1:3,600 was used to increase the likelihood that the MA/MCF reached 20 mm. For ROTEM, aliquots of test plasma were recalcified using a proprietary 0.2M $CaCl_2$ in HEPES product,[m] with mixing using automatic pipette as recommended by the manufacturer. Contact pathway activation on the ROTEM platform was performed using a proprietary ellagic acid reagent,[n] while a proprietary recombinant TF and phospholipid product,[o] was used to activate the extrinsic pathway.[16]

**Statistical analyses**

Data were assessed for normality using the D'Agostino–Pearson test.[p] Within-center CVs were calculated for each of the key TEG/ROTEM variables for each of the analysis types using the following equation: CV = SD / Mean. Comparisons of CVs between TEG centers and separately between ROTEM centers were performed using 2-way ANOVA. In these analyses, the column

factor was defined as center, the row factor defined as the assay parameter (R-time, K-time, alpha angle, MA for TEG; CT, CFT, alpha angle and MCF for ROTEM). Coefficients of variation for the individual parameters were compared between analysis platforms using Mann–Whitney tests, with Bonferroni's correction for multiple comparisons. Between center CVs were calculated from the mean values derived for each variable from each center. Cronbach's alpha (C-α) and intraclass correlation coefficients (ICC) for absolute agreement using a 2-way mixed effects model were calculated to evaluate the degree of correlation between the values from the 7 TEG centers and from the values from the 3 ROTEM centers. Data were sorted in ascending value prior to calculation of C-α and the ICC values.[q] Values for C-α ≥ 0.9 were considered to represent excellent consistency, 0.7 ≤ C-α < 0.9 good consistency, 0.6 ≤ C-α < 0.7 acceptable consistency, 0.5 ≤ C-α < 0.6 poor consistency and < 0.5 unacceptable consistency.[34] Values for ICC values > 0.8 were considered to indicate near-perfect agreement, 0.7 – 0.8 strong agreement, 0.5 – 0.7 moderate agreement, 0.3 – 0.5 fair agreement, and < 0.3 poor agreement.[35]

## Results

### PRP-samples

For the first iteration of the study, data were available from 9 of the 10 centers including 6 TEG centers and 3 ROTEM centers. Data from many of the TEG and ROTEM variables were normally distributed, but some were not, hence the data are summarized in the figures by median, interquartile range, minimum-maximum. For the calculation of CVs, means and SDs were still calculated in order to use this established metric of assay variance. The distributions of the CVs were also non-parametric and hence were compared with Mann–Whitney tests. Samples

sent to 1 of the TEG centers (Center 2) thawed en route and although the assays were conducted, the resulting data were not used for analysis.

Values for the individual tracing variables within the TEG centers for the 2 different TEG assays are shown in Figure 1. Within the TEG centers, some parameters were more variable than others; CVs from the 20 repeated assays at each center are presented in Table 1. Within the TEG centers, the variables with the lowest CVs were alpha angle and MA for both kaolin and TF activated assays. Visual inspection of box and whisker plots suggests that in most centers R-time and K-time also had low variability, although their lower mean values produced high CVs.

Between TEG centers, the variables with the lowest CVs were alpha angle for kaolin-activated assays, and alpha angle and R-time for TF-activated assays (Table 2). There was no significant difference between the CVs for any of the TEG parameters for either the TF or kaolin activated assays, $P = 0.336$ and $P = 0.124$, respectively. Analysis of the C-α and ICC indices suggests that for both TF and kaolin assays, the R time and the MA had the highest consistency values, while R time had the highest ICC values suggestive of moderate agreement between TEG centers. Overall, the C-α values for the TEG assays using PRP samples varied between 0.30 – 0.95, while the ICC values varied between 0.15 – 0.65.

Values for the individual tracing variables within the ROTEM centers for the 2 different ROTEM assays are shown in Figure 2. The low MCF values precluded complete assessment of the CFT. Within the ROTEM centers, alpha angle had the lowest CVs for both the IN-TEM and EX-TEM assays.

Between ROTEM centers, the variables with the lowest CVs were CT and alpha angle for both IN-TEM and EX-TEM assays (Table 3). There was no significant difference between the CVs for any of the ROTEM parameters for either the EX-TEM or IN-TEM assays, $P = 0.425$ and

P = 0.414, respectively. Analysis of the C-α and ICC indices suggests that for both IN-TEM and EX-TEM assays, the MCF had the highest consistency values, while CT had the highest ICC values. Overall, the C-α values for the ROTEM assays using PRP samples varied between 0.49 – 0.96, while the ICC values varied between 0.05 – 0.78.

**FFP + CP samples**

The pooled FFP used for the reconstitution of the cryoprecipitate had a fibrinogen concentration of 5.20 µmol/L [177 mg/dL]. After addition of the cryoprecipitate, the FFP + CP samples created for the second iteration of the study had a fibrinogen concentration of 10.85 µmol/L [369 mg/dL]. For this part of the study, data were available from all 10 centers (7 TEG, 3 ROTEM). Within and between TEG centers, values for the individual variables for the 2 different TEG assays were more variable than was the case with the PRP samples (Figure 3). Within TEG centers, the MA values in the kaolin-activated assays and the R-time values in the TF assays had the lowest CVs. Between TEG center CVs were higher for most variables than was the case for the PRP samples, with the exception of MA, which was less variable with the FFP + CP samples than with the PRP samples. There was no significant difference between the CVs for any of the TEG parameters for either the TF or kaolin activated assays, P = 0.631 and P = 0.417 respectively. Overall, the C-α values for the TEG assays using FFP + CP samples varied between 0.56 – 0.97, while the ICC values varied between 0.37 – 0.76.

The analyses of the FFP + CP samples within and between ROTEM centers were less variable (Figure 4, Table 4). Within individual ROTEM centers, alpha angle and MCF had the lowest CVs for IN-TEM assays, while CT, alpha angle, and MCF all had low CVs with the EX-TEM assay. Between ROTEM centers, the variables with the lowest CVs were alpha angle and

MCF for both IN-TEM and EX-TEM assays. There was no significant difference between the CVs for any of the ROTEM parameters for either the EX-TEM or IN-TEM assays, $P = 0.932$ and $P = 0.566$, respectively. Analysis of the C-α and ICC indices suggests that for IN-TEM assays, the CT and alpha angle values were the most consistent and had the highest agreement between centers. For the EX-TEM assays, the MCF had the highest consistency and between-center agreement values. Overall, the C-α values for the ROTEM assays using FFP + CP samples varied between 0.26 – 0.97, while the ICC values varied between 0.19 – 0.96.

**Variation in TEG assays compared to ROTEM assays**

To test the hypothesis that the variability of equivalent TEG and ROTEM assays are not significantly different, we compared the CVs for the TEG assays to the CVs for the ROTEM assays. None of the CVs for the individual TEG variables (R time, K time, alpha angle, MA) were significantly different from the CVs for the corresponding individual ROTEM variables (CT, CFT, alpha angle, MCF) at $P < 0.05$, after adjustment for multiple comparisons.

**Discussion**

This study aimed to establish intra- and inter-assay CVs for contact pathway and TF-activated assays on both the TEG and ROTEM platforms; and to compare the repeatability (within center) and reproducibility (between center) of these assays within and between centers. Overall, the results suggest that the degree of variability both within and between centers was unacceptably high (CVs frequently > 10%).[21] The centers concurred on the measurement of the TEG/ROTEM parameters to variable extents depending on the parameter assessed with some individual assays in some centers being highly repeatable (kaolin TEG with PRP and FFP + CP, IN-TEM and EX-

TEM with FFP + CP), while others were much less consistent. It should be noted that there is little agreement on what constitutes acceptable within-center or between-center CVs for coagulation assays.[36] Since the CV is numerically dependent on the mean value, a 10% CV for MA might represent a standard deviation of ± 6mm if the mean is 60mm. Such a difference might affect interpretation. In contrast, a 10% CV for R time where the mean value is 2 minutes represents a SD of ± 0.2 minutes, which might not affect interpretation of R time. As such, the variation in each parameter may need to be interpreted individually.

The C-α parameter is a measure of internal consistency, that is, how closely related a set of items are as a group,[37] and is typically interpreted as a measure of reliability, where 1 represents perfect consistency between assay parameters. Based on this metric alone, most TEG and ROTEM parameters had good consistency between centers (exceptions were the K time for TEG and the CFT for ROTEM assays). This finding suggests the same assays in different locations are measuring similar aspects of the coagulation process. The lack of consistency with K time and CFT values may be due primarily to a number of tracings had an MA or MCF only just above 20 mm, inconsistently prolonging these clot formation time parameters.

The ICC represents the level of agreement between the centers and accounts for both inter- and intra-center variability or alternatively, an index for the reliability of different centers averaged together.[34] In general, the ICC values indicated fair to moderate agreement at best for the various parameters among TEG centers. For the ROTEM centers, particularly with FFP + CP samples, the calculated ICC values suggest strong agreement for IN-TEM CT and alpha angle and EX-TEM MCF, and fair or poor agreement for the remainder. This is consistent with the distributions apparent from Figures 2 and 4. It should be recognized that we had fewer ROTEM centers than TEG centers, which may have increased the variation seen between TEG centers.

There are various potential analytical and pre-analytical sources of the variation observed here.[38] We attempted to minimize sources of error we could control, including the analyzers, assay consumables, machine operators, and the samples. It is probable that viscoelastic coagulation testing and the hemostatic process ex vivo are inherently variable to some extent. There are likely to be differences between analysis platforms and assays that also contributed to variability. For instance, the TF assay for TEG is non-proprietary and requires user preparation, in contrast to the EX-TEM assay for the ROTEM platform, which is standardized by the manufacturer. The TEG system uses manual pipetting, while the ROTEM platform incorporates an automatic pipette.

Despite our efforts to minimize biologic variation, the source we consider most likely to be the cause of the variation is the nature of the samples tested. In order to assess the repeatability and reproducibility within and between centers, every center tested identical samples. To accomplish this on a worldwide scale, use of plasma-based samples was required since whole blood samples would have degraded in transit. The multicenter nature of the project necessitated samples that would not be altered by the freeze-thaw process required to send identical aliquots of a clinically relevant biologic material to collaborators in 7 countries. Previous work suggests that canine plasma is sufficiently stable when frozen, such that any impact on hemostatic analyses is minimal.[39,40] Use of plasma samples also allowed for analysis of multiple samples in a single run, minimizing between-run variation. The original intention was to provide centers with lyophilized plasma, since this sample type was used in human external quality assessments of TEG.[29,41] Ultimately, however, lyophilized canine plasma could not be obtained from a commercial animal blood bank. We therefore opted to use frozen plasma

samples. While these samples were more readily available, they were more challenging and costly to ship.

The first iteration of this project generated promising data from multiple centers, established the logistics for the multicenter international collaboration, and confirmed the potential of this approach. From these analyses, it appeared that the CVs from some assays and from some centers were acceptably low and that in many cases the results were similar. It was also clear that the variation in some centers was unacceptably high, however. Based on feedback from the test centers, much of this variability was inherent in the test samples since in some centers clots were visible in some of the samples, likely due to activation of platelets within the PRP and consequent aggregation. Based on these observations, the standardized test material was further refined. The presence of the platelets in the samples likely increased clot strength through their physical integration into the forming clot and by providing additional phosphatidylserine expressing surfaces for the assembly of coagulation factor complexes thereby accelerating clot formation.[42] It is likely that few of the platelets survived the freeze/thaw process despite the presence of the dimethylsulfoxide.[43,44] At the time of collection, our PRP samples contained a high number of platelets in an attempt to account for the anticipated loss due to the freeze/thaw process. The anecdotally reported pre-clotted samples that some centers received suggest that some platelet activation occurred during sample preparation. To avoid this in the second iteration, platelets were eliminated from the samples. The removal of platelets from the samples may have had unanticipated consequences. Removal of the platelet derived phospholipid may have reduced the surface area available for coagulation factor complex assembly, thus affecting the assays. The TF reagent used in the extrinsic TEG assay contains some synthetic

phospholipids and so to offset the elimination of the platelets, we increased the final TF reagent concentration in the TEG assays during the second part of the study.

For the second iteration of this study, we considered various alternative sample types. One potential alternative option was to use the lyophilized quality control materials supplied by the analyzer manufacturers. The study by Chitlur and others in 2011[21] reported reproducibility data from the College of American Pathologists that suggested excellent reproducibility across a large number of centers is possible for TEG using this type of lyophilized plasma sample. Although using lyophyilized plasma may have improved our consistency, the relevance of this approach to veterinary medicine was felt to be questionable since these quality control materials are not derived from canine, feline, or equine proteins. Fresh frozen plasma samples alone were not considered suitable. Typically, canine plasma-only TEG tracings have maximum amplitudes < 20 mm and hence do not enable measurement of K time on the TEG or CFT on the ROTEM platform.[39] Previous work suggested that a minimum fibrinogen concentration of 8.8 μmol/L [300 mg/dL] in the final sample is necessary to generate MCF of > 20 mm.[45] No commercial source of canine fibrinogen was available to enable production of hyperfibrinogenemic samples, and thus canine FFP + CP samples were produced to enhance the fibrinogen concentrations.[46] While these samples eliminated the platelet associated problems encountered in the first part of the study, they occasionally produced TEG and ROTEM tracings with MA / MCF values < 20 mm, and hence K time / CFT values were not universally available. Specific differences in the design of the analyzers may also contribute to differences in sensitivity. In the TEG assay, the cup rotates while the pin is stationary, while in ROTEM the pin is rotated within a stationary cup. These subtle differences might alter the torque generated within the system using platelet-poor samples. Future work in this field will require further optimization of sample types. Our

results suggest that optimization of a PRP-based sample type would enhance standardization of the TEG platform, since CVs were generally better for this sample type on the TEG platform, while FFP + CP based samples may be better suited to standardization of the ROTEM platform.

Despite the challenges we experienced, our results are comparable to those of the similar effort to standardize TEG and ROTEM assays in human medicine.[21] In that study, PRP, pooled plasma from healthy individuals, and factor VIII deficient plasma samples were evaluated on both TEG and ROTEM analyzers in multiple countries. That study reported CVs ranging from 3.12% – 59.98% depending on the combination of sample type, assay, parameter, and platform. The authors identified that significant between-center variation was present (CVs > 10%), but also concluded that this was in part due to sample type.

A recent human study compared the repeatability and reproducibility of TEG and ROTEM assays using whole blood collected from patients undergoing elective cardiac surgery.[47] Contact pathway assays (kaolin and IN-TEM) were performed and inter- and intra-operator CVs calculated. That study found that both inter- and intra-operator CVs were significantly lower for ROTEM assays than for TEG assays. Our study differed in design, being multicenter rather than single center and also analyzed plasma-based samples rather than whole blood. Our study demonstrated that the within-center CVs from the TEG centers were generally lower than those for ROTEM using the PRP samples, while the within-center CVs from the ROTEM centers were generally lower than those for the TEG centers using the FFP + CP samples. Given the finding with the PRP samples, this may be related solely to the sample type, but there may also be lower variability in the proprietary ROTEM EX-TEM assay compared with the user defined TF assay used for TEG. After adjustment for multiple comparisons (n = 15), none of the differences between CVs for the 2 platforms were significant, however.

The use of plasma samples does raise the important question of clinical relevance, since these assays are designed to analyze whole blood. Our aim was to determine assay repeatability and reproducibility, and our data suggest that the degree of variability observed was too high. Reassuringly, data from a human multicenter study determining whole-blood reference ranges for ROTEM suggests that CVs under 15% are feasible.[48] Worldwide standardization of TEG and ROTEM using whole blood cannot be achieved. However, it might be feasible to run whole blood samples in laboratories in separate centers in 1 geographic area, as was recently achieved in human medicine.[49] For veterinary medicine, this might be feasible where multiple TEG equipped centers exist, such as in a major metropolitan area.

Until such additional studies are undertaken, we can only recommend that individual centers take all possible steps to reduce variation within their own analyses, and that they scrutinize the data from other centers to ensure assays were meticulously performed. It is clear that the assays are sensitive to small degrees of analytic and biologic variation. The ability to discern subtle abnormalities is a potential strength, but clearly is also a potential weakness if suitable care and attention are not paid to sample collection, preparation, handling, and analysis. We therefore encourage users of viscoelastic tests to use the evidence-based guidelines developed for these assays[22] to minimize sources of variation and thereby maximize the diagnostic utility of these methods.

**Footnotes**

[a] Animal Blood Resources International, Stockbridge, MI

[b] Falcon Cell Strainer 100µm, BD Biosciences, Bedford, MA

[c] Fibrinogen (100 U/mL), Diagnostica Stago, Parsippany, NJ

[d] Cornell Comparative Coagulation Laboratory, Ithaca, NY

[e] Falcon Conical Tubes, BD Biosciences, Bedford, MA

[f] N-(2-Hydroxyethyl)piperazine-N-2-ethanesulfonic acid, Fisher Scientific, Waltham, MA

[g] Bovine serum albumin, Fisher Scientific, Waltham, MA

[h] Level I and Level II Controls, Hemonetics, Braintree, MA

[i] ROTROL N and ROTROL P, Tem Systems, Inc., Durham, NC

[j] Calcium chloride, Hemonetics, Braintree, MA

[k] Kaolin, Hemonetics, Braintree, MA

[l] Innovin, Siemens Medical Solutions USA, Malvern, PA

[m] STAR-TEM, TEM Systems, Inc., Durham, NC

[n] EX-TEM, TEM Systems, Inc., Durham, NC

[o] IN-TEM, TEM Systems, Inc., Durham, NC

[p] Prism 6.0, GraphPad Software Inc., La Jolla, CA

[q] IBM SPSS Statistics v21.0, IBM Corp., Armonk, NY

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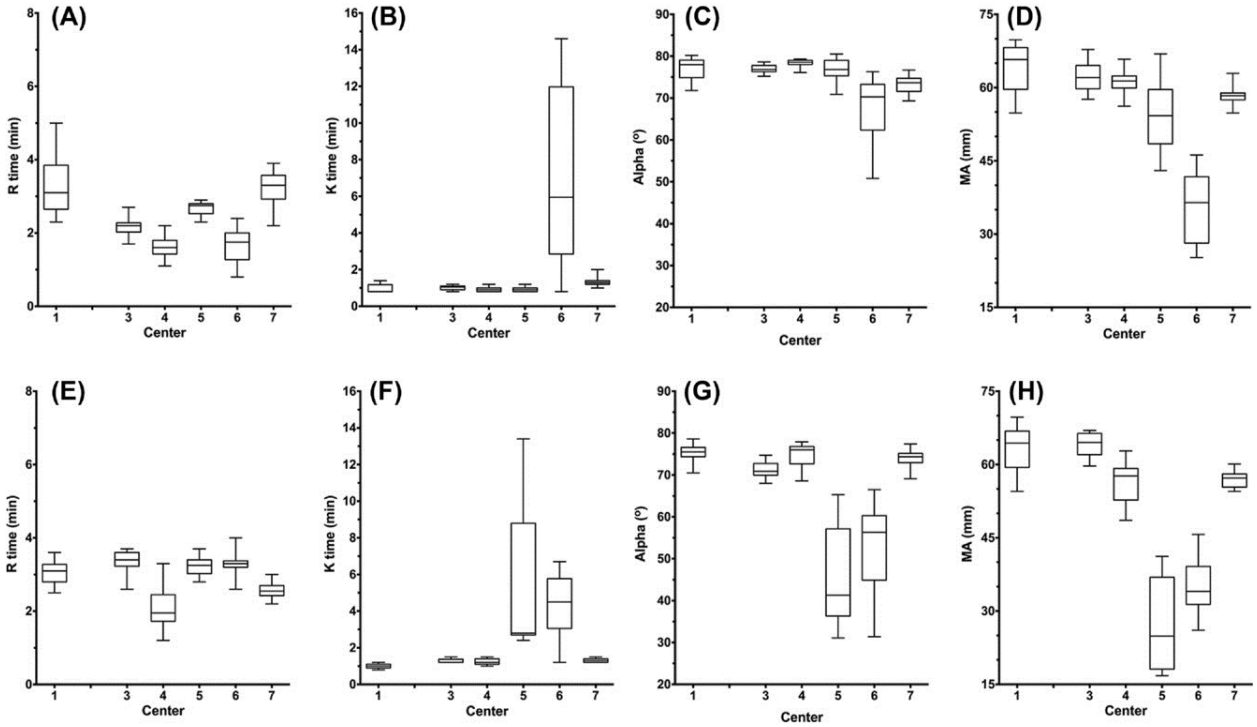
**Figures**

**Figure 1**

Box and whisker plots representing the median, interquartile range, and minimum-maximum values for the 4 principal TEG variables: R time, K time, alpha angle, and MA. Data were generated in 7 centers in 4 countries using platelet-rich plasma samples. Panels A – D represent data from the proprietary kaolin-activated TEG assay, which evaluates contact pathway activation, while panels E – H represent data from a tissue factor pathway activated assay. This assay, as previously reported,[33] uses recombinant human tissue factor (1:50,000 final dilution) to activate coagulation via the extrinsic pathway.




Alpha (°), alpha angle / clot formation angle; K time, clot formation time; MA, maximum amplitude; min, minutes; R time, reaction time; TEG, thromboelastography

**Figure 2**

Box and whisker plots representing the median, interquartile range and minimum-maximum values for the 4 principal ROTEM variables: CT, CFT, clot formation angle (alpha angle) and MCF. Data were generated from 3 centers in 3 countries using PRP samples. Panels A – D represent data from the IN-TEM, which evaluates contact pathway activation, while panels E – H represent data from the EX-TEM.

Alpha (°), alpha angle; CFT, clot-formation time; CT, clotting time; EX-TEM, proprietary tissue-factor assay; IN-TEM, proprietary ellagic acid activated assay; MCF, maximum clot firmness; min, minutes; PRP, platelet-rich plasma; ROTEM, thromboelastometry

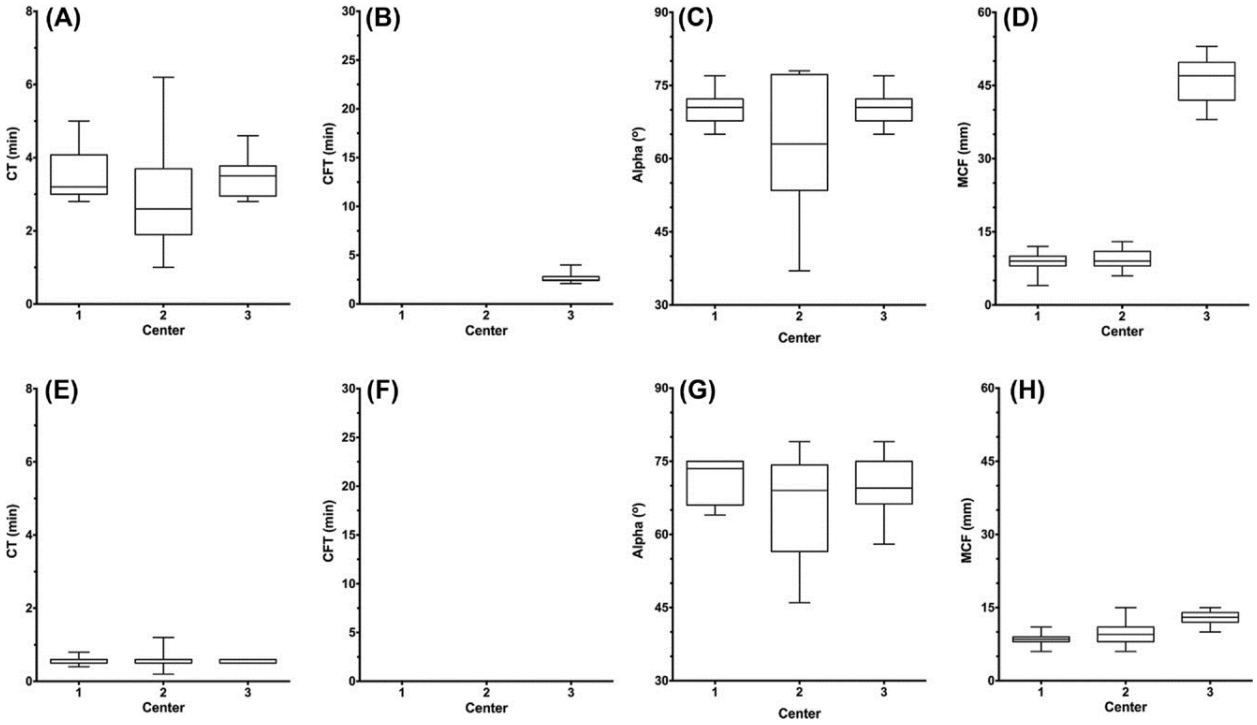


**Figure 3**

Box and whisker plots representing the median, interquartile range and minimum-maximum values for the 4 principal TEG variables: R time, K time, alpha angle, and MA. Data were generated in 7 centers in 4 countries using FFP samples with augmented fibrinogen concentrations. Panels A – D represent data from the proprietary kaolin-activated TEG assay that evaluates contact pathway activation, while panels E – H represent data from a customized tissue factor pathway activated assay. This assay also used recombinant human tissue factor to activate coagulation via the extrinsic pathway, but employed a lesser dilution (1:3,600 final TF concentration). Alpha (°), alpha angle / clot formation angle; FFP, fresh-frozen plasma; K time, clot formation time; MA, maximum amplitude; min, minutes; R time, reaction time; TEG, thromboelastography; TF, tissue factor

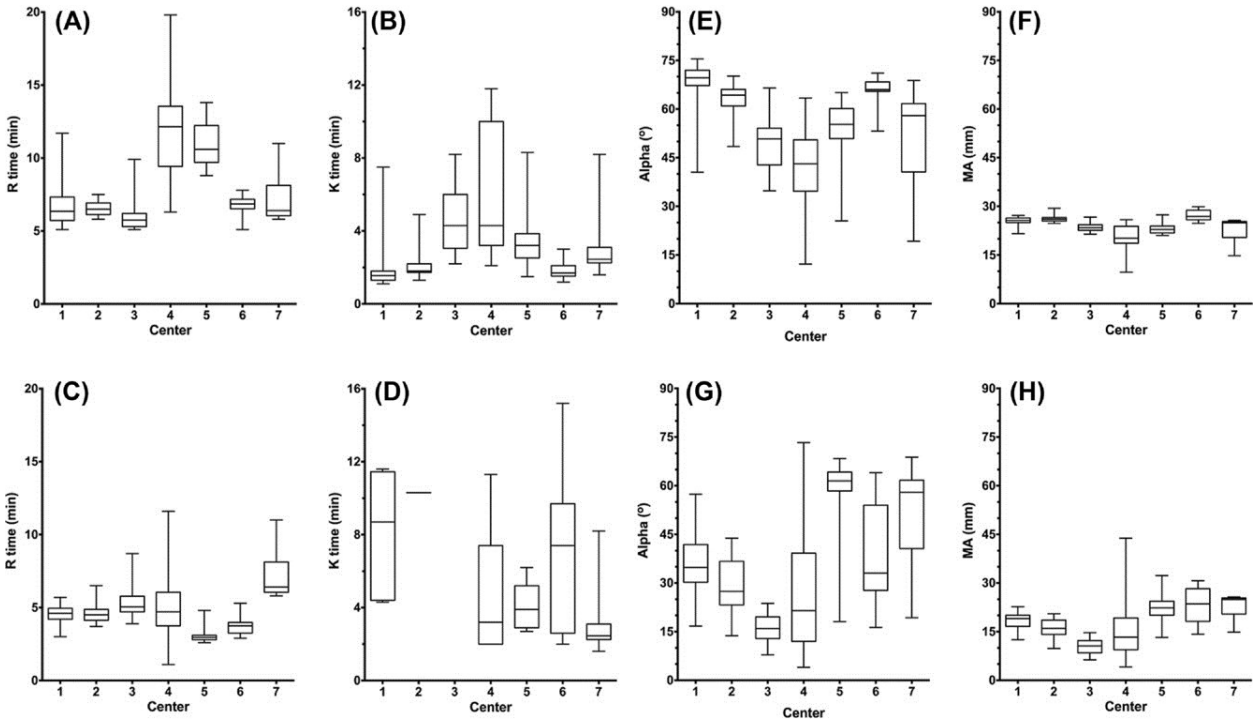
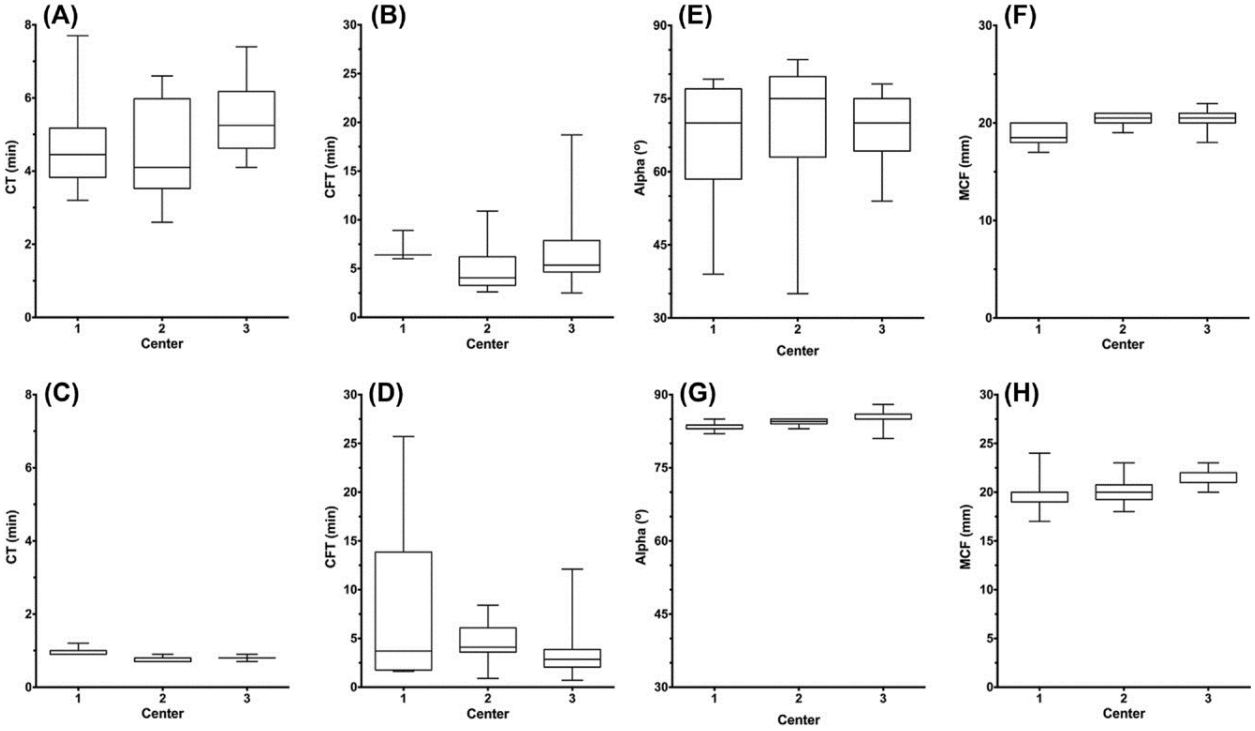


**Figure 4**

Box and whisker plots representing the median, interquartile range and minimum-maximum values for the 4 principal ROTEM variables: CT, CFT, alpha angle, and MCF. Data were generated in 3 centers in 3 countries using fresh-frozen plasma samples with augmented fibrinogen concentrations. Panels A – D represent data from the proprietary celite-activated assay (IN-TEM) which evaluates contact pathway activation, while panels E – H represent data from the proprietary tissue-factor assay (EX-TEM). Alpha (°), alpha angle; CFT, clot formation time; CT, clotting time; EX-TEM, proprietary tissue-factor assay; IN-TEM, proprietary ellagic acid activated assay; MCF, maximum clot firmness; min, minutes; PRP, platelet-rich plasma; ROTEM, thromboelastometry.



**Tables**

**Table 1.** Within-center CVs for TEG assays (%), rounded to the nearest whole number. For the PRP analyses, data for Center 2 were not analyzed because the samples thawed in transit.

C1 – C7, Center 1 to Center 7; CVs, coefficients of variation; FFP + CP, fresh frozen plasma with admixed cryoprecipitate; K time, clot formation time; MA, maximum amplitude; PRP, platelet-rich plasma; R time, reaction time; TEG, thromboelastography; TF, tissue factor.

| | | PRP | | | | | | | FFP + CP | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Test** | **Parameter** | **C1** | **C2** | **C3** | **C4** | **C5** | **C6** | **C7** | **C1** | **C2** | **C3** | **C4** | **C5** | **C6** | **C7** |
| Kaolin | R time | 24 | | 12 | 20 | 6 | 31 | 14 | 22 | 7 | 20 | 30 | 13 | 10 | 23 |
| | K time | 23 | | 12 | 12 | 14 | 73 | 18 | 73 | 36 | 37 | 57 | 43 | 27 | 66 |
| | Alpha angle | 3 | | 1 | 1 | 3 | 12 | 3 | 11 | 8 | 17 | 31 | 18 | 8 | 30 |
| | MA | 8 | | 5 | 4 | 13 | 20 | 3 | 5 | 4 | 5 | 20 | 7 | 6 | 16 |
| TF | R time | 9 | | 9 | 28 | 8 | 8 | 9 | 14 | 14 | 23 | 53 | 16 | 18 | 23 |
| | K time | 11 | | 8 | 14 | 77 | 36 | 8 | 44 | - | - | 89 | 31 | 61 | 66 |
| | Alpha angle | 3 | | 2 | 4 | 26 | 19 | 2 | 28 | 29 | 28 | 75 | 18 | 38 | 30 |
| | MA | 8 | | 4 | 8 | 34 | 16 | 3 | 13 | 18 | 22 | 69 | 19 | 23 | 16 |

**Table 2.** Summary TEG assay data and between-center CVs for TEG assays (%). Coefficients of variation have been rounded to the nearest whole number. For the PRP analyses, data for Center 2 were not analyzed because the samples thawed in transit.

C-α, Cronbach's alpha; CVs, coefficients of variation; FFP + CP, fresh frozen plasma with admixed cryoprecipitate; ICC, intraclass correlation coefficient; K time, clot formation time;

MA, maximum amplitude; min, minutes; PRP, platelet-rich plasma; R time, reaction time; TEG, thromboelastography; TF, tissue factor.

| | | PRP | | | | | FFP + CP | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Test** | **Parameter** | **Mean** | **SD** | **CV (%)** | **C-α** | **ICC** | **Mean** | **SD** | **CV (%)** | **C-α** | **ICC** |
| Kaolin | R time (min) | 2.4 | 0.7 | 31 | 0.94 | 0.60 | 8.0 | 2.3 | 29 | 0.85 | 0.50 |
| | K time (min) | 2.0 | 2.3 | 117 | 0.30 | 0.15 | 3.3 | 1.5 | 47 | 0.79 | 0.60 |
| | Alpha angle (°) | 75.0 | 4.2 | 6 | 0.84 | 0.58 | 56.3 | 9.5 | 17 | 0.96 | 0.76 |
| | MA (mm) | 55.9 | 10.6 | 19 | 0.94 | 0.47 | 24.1 | 2.4 | 10 | 0.85 | 0.56 |
| TF | R time (min) | 2.9 | 0.5 | 17 | 0.95 | 0.65 | 4.7 | 1.3 | 27 | 0.81 | 0.47 |
| | K time (min) | 2.4 | 1.9 | 80 | 0.45 | 0.28 | 6.1 | 2.7 | 45 | 0.56 | 0.37 |
| | Alpha angle (°) | 65.9 | 12.6 | 19 | 0.77 | 0.33 | 37.0 | 14.5 | 39 | 0.93 | 0.53 |
| | MA (mm) | 50.5 | 15.4 | 31 | 0.93 | 0.31 | 18.6 | 4.7 | 25 | 0.97 | 0.57 |

**Table 3**. Summary ROTEM assay data and between-center CV for ROTEM assays (%). Coefficients of variation have been rounded to the nearest whole number. Some tracings failed to reach MCF > 20mm and hence values for CFT for some assays were not available. Coefficients of variation could not be calculated for these assays, indicated by (-).

C-α, Cronbach's alpha; CFT, clot formation time; CT, clotting time; CV, coefficients of variation; EX-TEM, proprietary tissue factors activated assay; FFP + CP, fresh frozen plasma with admixed cryoprecipitate; ICC, intraclass correlation coefficient; IN-TEM, proprietary ellagic acid activated assay; MCF, maximum clot firmness; min, minutes; PRP, platelet-rich plasma; ROTEM, thromboelastometry.

| | | PRP | | | | | FFP + CP | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Test** | **Parameter** | **Mean** | **SD** | **CV (%)** | **C-α** | **ICC** | **Mean** | **SD** | **CV (%)** | **C-α** | **ICC** |
| IN-TEM | CT (min) | 3.3 | 0.3 | 9 | 0.71 | 0.68 | 4.9 | 0.5 | 10 | 0.97 | 0.92 |
| | CFT (min) | 2.6 | - | - | - | - | 6.3 | 1.3 | 21 | 0.67 | 0.19 |
| | Alpha angle (°) | 66.9 | 3.8 | 6 | 0.49 | 0.49 | 68.7 | 1.6 | 2 | 0.97 | 0.96 |
| | MCF (mm) | 21.3 | 21.3 | 100 | 0.89 | 0.05 | 19.8 | 1.0 | 5 | 0.92 | 0.67 |
| EX-TEM | CT (min) | 0.6 | 0.0 | 7 | 0.78 | 0.78 | 0.9 | 0.1 | 12 | 0.87 | 0.35 |
| | CFT (min) | - | - | - | - | - | 5.3 | 2.2 | 43 | 0.26 | 0.23 |
| | Alpha angle (°) | 69.1 | 3.2 | 5 | 0.90 | 0.42 | 84.4 | 1.2 | 1 | 0.82 | 0.46 |
| | MCF (mm) | 10.3 | 2.3 | 22 | 0.96 | 0.56 | 20.3 | 0.9 | 5 | 0.93 | 0.76 |

**Table 4.** Within center coefficients of variation (CV) for thromboelastometry (ROTEM) assays (%), rounded to the nearest whole number. Abbreviations are as follows: C1-C3, Center 1 to Center 3; CFT, clot formation time; CT, clotting time; EX-TEM, proprietary tissue factors activated assay; FFP + CP, fresh frozen plasma with admixed cryoprecipitate; IN-TEM, proprietary ellagic acid activated assay; MCF, maximum clot firmness; PRP, platelet-rich plasma. Some tracings failed to reach MCF >20mm and hence values for CFT for some assays were not available. Coefficients of variation could not be calculated for these assays, indicated by (-).

| | | PRP | | | FFP + CP | | |
|---|---|---|---|---|---|---|---|
| **Test** | **Parameter** | **C1** | **C2** | **C3** | **C1** | **C2** | **C3** |
| IN-TEM | CT | 19 | 51 | 17 | 28 | 28 | 17 |
| | CFT | - | - | 17 | 22 | 47 | 61 |
| | Alpha angle | 5 | 24 | 5 | 17 | 17 | 11 |
| | MCF | 25 | 22 | 11 | 6 | 3 | 6 |
| EX-TEM | CT | 18 | 31 | 7 | 8 | 8 | 7 |
| | CFT | - | - | - | 121 | 43 | 71 |
| | Alpha angle | 7 | 18 | 8 | 1 | 1 | 2 |
| | MCF | 15 | 21 | 12 | 8 | 6 | 4 |