New-Generation Thromboelastography

Comprehensive Evaluation of Citrated and Heparinized Blood Sample Storage Effect on Clot-Forming Variables

Joao D. Dias, PhD; Elaine I. Haney, BSc, MT; Blesy A. Mathew, MSc; Carlos G. Lopez-Espina, MSc; Adrian W. Orr, BSc, MBA; Mark A. Popovsky, MD

Objectives.—To establish the minimum time required for equilibration time and the maximum time for sample storage for all commercially available TEG tests for the new-generation TEG 6s and to determine how those times compare with the older generation TEG 5000.

Design.—Citrated and heparinized whole blood samples obtained from 20 healthy donors were analyzed for clot parameters at multiple time points for both the TEG 6s and the TEG 5000. Samples were activated with the citrated multichannel cartridge or the platelet-mapping cartridge in the TEG 6s or with recalcified kaolin in the TEG 5000.

Assessment of hemostasis with a thromboelastography (TEG) system has been used clinically for 50 years to identify, monitor, and guide treatment of coagulopathies in both laboratories and in point-of-care facilities.1 By individualizing goal-directed coagulation management, it is possible to reduce inappropriate blood transfusions and to stratify patients according to risk of bleeding and/or thrombotic complications.2,3 There is, consequently, an opportunity to achieve substantial cost savings with a goal-directed TEG program with improved patient outcomes. These outcomes have prompted several organizations to recommend the clinical use of viscoelastic hemostatic testing devices, such as TEG, in various clinical areas.4–7 The disadvantages of viscoelastic hemostatic assays have historically included a relatively high coefficient of variation, poorly standardized methodologies, and limitations on the specimen stability of native whole blood samples.8–10 In the pediatric setting, an advantage of the TEG is the relatively small sample volume needed, but a disadvantage is the difference in reference ranges between infants, especially newborns, and adults.10

A variety of assays containing different activators can be run on a TEG device, providing insights into different aspects of coagulation. Although the von Willebrand factor is not detected with a TEG, the effects of most whole blood elements (eg, coagulation factors, platelets, fibrinolytic factors, and inflammatory cells) are incorporated in the TEG analysis, encompassing clot formation and subsequent clot lysis. Thus, a TEG mirrors the cell-based model of hemostasis. In contrast, conventional coagulation tests (eg, prothrombin time, activated partial thromboplastin time) are performed using platelet-poor plasma samples and provide data on the time to initiation of fibrin formation but no data beyond that point.11 This lack of information has limited the value of plasma-based assays to correlate with bleeding in surgical procedures.12

Results.—All blood samples yielded TEG parameter results within reference ranges and had a tendency toward hypercoagulable profiles with increased storage time. Sample storage resulted in increased platelet inhibition with significant differences at 4 hours in the platelet-mapping cartridge (arachidonic acid percentage of inhibition, P = .002; adenosine diphosphate percentage of inhibition, P = .02).

Conclusions.—For nonemergent cases or in a central laboratory setting, all tests provided reliable results for up to 4 hours in the citrated multichannel cartridge and for 3 hours for platelet function information in the platelet-mapping cartridge. In emergent/urgent situations in which the sample needs to be run immediately, RapidTEG and functional fibrinogen tests may be preferred.

(Arch Pathol Lab Med. 2017;141:569–577; doi: 10.5858/arpa.2016-0088-OA)
The current viscoelastic hemostatic assay technologies—TEG (Haemonetics Corporation, Braintree, Massachusetts) and ROTEM (Tem International, Munich, Germany)—have been marketed for several years, but ease-of-use issues, labor, and time-intensive operations have limited more-widespread use in clinical practice and in large-scale clinical trials. Recently, a new point-of-care TEG system (TEG 6s, Haemonetics) was introduced. Building on the design of the original TEG 5000 (Haemonetics), up to 4 assays can be run simultaneously on the TEG 6s. This is achieved with one of the 2 microfluidic cartridges currently available. The TEG 6s global-hemostasis cartridge includes the traditional contact-activation kaolin test—the functional fibrinogen test—to access the contribution of fibrin to the overall clot strength, and the RapidTEG (rTEG) for faster results, with heparinase kaolin used to assess the effect of heparin on coagulation. However, traditional viscoelastic hemostatic assay tests are limited in their capacity to assess the bleeding risk of a patient who has taken antiplatelet drugs, thus requiring a separate platelet function test. The TEG PlateletMapping assay enables platelet function to be assessed through the P2Y12 or thromboxane pathway. Therefore, TEG analysis may be compared with methods specifically designed for monitoring platelet function. Light transmission aggregometry has been considered the gold standard assay but requires skilled technicians and lengthy and demanding preparation, and the results are subject to variability. Alternative methods are available with shorter turnaround times and more convenient sample preparations but still require a skilled operator. One example is the multiple-electrode aggregometry analyzer (Multiplate, Roche Diagnostics, Mannheim, Germany), a device that measures platelet aggregation by electrical impedance. In most clinical settings, platelet tests must be performed in addition to coagulation testing. Thromboelastography enables both coagulation rate and the effects of antiplatelet therapy to be assessed using one device. With a TEG, the need for a separate device specifically for platelet assessment can be eliminated.

Performing a TEG analysis in a central laboratory setting often requires transportation of the blood sample for more than 5 minutes, which is the upper limit for running native samples, and hence, storage of the blood sample in citrate or heparin becomes necessary. Citrate storage has been reported to influence the results of the TEG analysis, and it has been suggested that the blood sample should be equilibrated for a fixed period before the analysis is performed to standardize the results. Such an approach would impede the usefulness of the technology, which relies on displaying the result in real-time from the start of analysis to allow for earlier interventions than would be possible relying on conventional coagulation assays. A minimum wait time of 15 minutes after draw is the current recommendation from the manufacturer for the TEG 6s, with a maximum of 120 minutes allowable. The aim of this study was to establish the required waiting time before running the sample in the TEG system and the maximum waiting time the blood can be stored to provide meaningful results in the recently developed TEG 6s system for the complete suite of assays and how those times compare to the most commonly used assay in the TEG 5000.

**MATERIALS AND METHODS**

This study was conducted in accordance with the principles set forth in the US National Institutes of Health, Office of Human Research Protection, and was approved by an institutional review board. Written, informed consent was obtained from each subject before participation.

**TEG Analysis**

Thromboelastography measures the viscoelastic properties of a clot through all phases of hemostasis—from the enzymatic phase through the fibrinolytic phase. A mechanical representation of the mechanism of action is presented in Figure 1, A, for the TEG 5000 and Figure 1, B, for the TEG 6s. The various components of the classic TEG tracing are depicted in Figure 2, and a sample of the output from the new TEG 6s device is shown in Figure 3. The kaolin test generates a reaction time (R) parameter, which is measured in minutes and is the time elapsed from the initiation of the test until the onset of clotting provides enough resistance to produce a 2-mm amplitude reading on the TEG tracing. This parameter represents the initiation phase of coagulation related to the function of enzymatic clotting factors. The kinetic (K) parameter is a measurement of the time interval from R to the point at which fibrin cross-linking provides enough clot resistance to produce a 20-mm amplitude reading. The a angle is the angle formed by the slope of a tangent line, traced from the initiation of clotting (the split point) to the K time, and a central line is measured in degrees. The K time and the a angle denote the rate at which the clot strengthens and is representative of the thrombin cleaving the available fibrinogen into fibrin. The maximum amplitude (MA) indicates the point at which clot strength is greatest, measured in millimeters on the TEG tracing and reflects the maximal platelet-fibrin interaction via the glycprotein IIb-IIIa receptors. Lysis 30 (LY30) is the percentage of lysis 30 minutes after the MA is finalized. The LY30 measurement is based on the reduction of the tracing area that occurs between the time the MA is measured and 30 minutes after the MA is finalized.

The rTEG test incorporates both tissue factor and kaolin to generate the conventional kaolin parameters as well as the TEG activated clotting time (ACT) parameter. The ACT, which is measured in seconds, is the time from initiation of the test to the first clot formation. A prolonged TEG-ACT time indicates slower clot formation.

The kaolin TEG with heparinase incorporates heparinase into the standard kaolin TEG assay, eliminating the effect of heparin in the test sample. In the TEG functional fibrinogen test, the tissue factor is used for coagulation activation described classically as extrinsic, with the platelet function inhibited by ReoPro (abciximab, Eli Lilly and Company, Indianapolis, Indiana), a GPIIb/IIIa inhibitor, so the resulting contribution of the functional fibrinogen to clot strength can be viewed. By subtracting the functional fibrinogen contribution from the overall clot strength of the kaolin or rTEG, the specific contribution of the platelet function is extrapolated. The functional fibrinogen level is extrapolated from the functional fibrinogen MA value, which correlates with the Cluss fibrinogen method.

The TEG PlateletMapping assay uses 4 different channels: kaolin, activator F (ActF), adenosine diphosphate (ADP), and arachidonic acid (AA). In the ActF channel, reptilase and factor XIII are used to produce a clot with no platelet contribution. The presence of heparin inhibits the thrombin activity. The ADP and AA assays are performed with the ActF and are designed to measure the agonist-induced ADP or AA clot strength. Unlike standard kaolin TEG analyses, the absence of thrombin provides sensitivity to common antiplatelet agents, such as aspirin and clopidogrel, or to acquired platelet inhibition. The ADP and AA clot-strength measurements are compared with those from the kaolin and ActF to ascertain the response to each agonist.

**TEG 5000**

With the TEG 5000 device, the blood is contained within a cup and a stationary pin is immersed in the blood (Figure 1, A). The cup rotates left and right with an oscillatory...
Motion at a set speed through an arc of 4°45'. As the clot starts to form, it begins to bind to the cup and the pin, causing the pin to oscillate with the cup (Figure 2). The rate at which the movement of the pin increases is a function of the clot development. As coagulation progresses, the fibrin-platelet bonding increases the strength of the link between the cup and the pin, and increasing torque is applied to the pin. The strength of the fibrin-platelet bonding determines the magnitude of the pin’s motion, with strong clots moving the pin to a greater extent. Thus, the magnitude of the output is directly related to the strength of the clot. If lysis occurs, the fibrin-platelet bonding is reduced, and the movement of the pin is diminished. The extent of the pin’s rotation is converted into an electrical signal and then to a tracing that reflects the profile of the clot formation.

**Figure 1.** Geometry hemostasis analyzer principles of thromboelastography. A, Cross-section of the TEG 5000 cup and pin: $\theta_1(t)$ shows the rotation of the cup provided to the sample by the analyzer over time ($t$), and $\theta_2(t)$ shows the pin displacement measured over time ($t$). B, Cross-section of the TEG 6s measurement ring: $\omega_1(t)$ shows the sample vibration excitation provided to the sample by the analyzer over time ($t$), and $\omega_2(t)$ shows the sample drop movement over time ($t$) induced by the vibration.

**TEG 6s**

The TEG 6s system is a fully automated, 4-channel diagnostic instrument (Figure 1, B). The system is portable and employs multichannel, microfluidic cartridges, rendering it inherently insensitive to normal vibrations. An unmetered whole blood sample (approximately 0.4 mL) is pipetted into the entry port in the cartridge with a transfer pipette and is drawn into the cartridge under the instrument’s control. The sample is metered into 4 separate analysis channels. In addition to the aforementioned activators and reagents, the citrated multichannel cartridge channels contain prepacked calcium. Dried reagents present within each channel are reconstituted by movement under the control of the microfluidic valve and the bellows. After reconstitution, the sample is delivered to individual test cells at the terminus of each microchannel, where clotting is

**Figure 2.** Illustration of a thromboelastography (TEG) tracing and the accompanying parameters. The depiction of the TEG tracing and the parameters were measured throughout the lifetime of a clot. Abbreviations: $\alpha$, alpha angle; ACT, activated clotting time; $K$, coagulation time; LY30, percentage of lysis 30 min after MA; MA, maximum amplitude; $R$, reaction time.
monitored. Excess sample is directed into a waste chamber. Approximately 20 L of prepared sample is delivered to each cell, where a meniscus forms at the bottom of the cell (ring). The lower meniscus partially blocks light from a collimated light source measured by a photodiode. A piezoelectric actuator drives the blood sample through a vertically swept, sinusoidal motion profile. The input signal has a maximum amplitude of less than 10 μm, with frequencies in the 20 to 500 Hz range, which causes the sample to vibrate. The frequencies of the induced motion are then detected and analyzed with a photodetector. A fast Fourier transformation is performed on those frequency components to identify the resonant frequency at which the sample has the greatest amplitude because of the coagulation event. As clotting occurs, resonant frequencies increase. Those frequencies are converted to TEG equivalent units (in millimeters) using a mapping function to generate the TEG tracings. Parameters are then extracted from the tracing (Figure 3).

Before running samples for this study, each analyzer was verified within its calibration period. All TEG 6s cartridges used were verified to be within their expiration dates and were from released production lots. The citrated multichannel cartridge has 4 channels to perform the following assays: (1) kaolin (CK), (2) rTEG (CRT), (3) kaolin with heparinase (CKH), and (4) functional fibrinogen (CFF). The platelet-mapping cartridge also has 4 channels and performs a platelet-mapping assay containing the following 4 tests: (1) heparinized kaolin with heparinase (HKH), (2) ActF, (3) ADP, and (4) AA.

Study Design

The TEG 5000 studies were performed in 2006 with 20 healthy donors. Samples were obtained by antecubital venipuncture with a 21-gauge butterfly needle. A 5-mL discard tube was drawn first, followed by 4.5 mL in 3.2% citrate tubes (No. 366515, Becton Dickinson, Franklin Lakes, New Jersey). A stopwatch was started at the point at which the phlebotomist drew the last citrate tube and the most recent citrate tube was handed off to a second operator as the time zero sample. Subsequent samples were randomly selected from the other citrated tubes drawn to minimize any effect from the order of the draw, and were run at the following times after draw: 15, 30, 60, and 90 minutes and at 2 hours. The CK samples were run in quadruplicate for each time point. All operators were experienced TEG operators, and most of the testing was performed by a single operator. Samples were run following the manufacturer’s instructions for kaolin vial use.

| Test Completed | 8/10/2015 8:25 AM | CM Citrated KKH,RT,FF | HN-0308 |
|----------------|-------------------|-----------------------|--------|
| CK             | 6.4               | 1.7                   | 69.6   | 57.8   | 0.3   |
| CRT            | 116               | 0.7                   | 1.7    | 70.8   | 59.1  | 0.1   |
| CKH            | 5.9               | 1.7                   | 70.2   | 58.1   | 0.4   |
| CFF            | 0.7               | --                    | 62.4   | 17.7   | 0     | 323   |

Figure 3. A sample of results from the TEG 6s. Abbreviations: ACT, activated clotting time; angle, α angle; CFF, citrated blood sample activated by the functional fibrinogen test; CK, citrated blood sample activated with kaolin; CKH, citrated blood sample activated with kaolin and heparinase; CM, citrated multichannel cartridge; CRT, citrated blood sample activated with RapidTEG; FLEV, fibrinogen level; K, coagulation time; LY30, percentage of lysis 30 min after MA; MA, maximum amplitude; R, reaction time.
The TEG 6s analyses were performed in 2015 on the blood from 20 healthy donors with both TEG 6s assay cartridges—the Citrated Multichannel and the PlateletMapping—currently released. Samples were obtained by antecubital venipuncture with a 21-gauge butterfly needle. A 5-mL discard tube was drawn first, followed by 9 samples of 2.7-mL in 3.2% citrate tubes (No. 363083, Becton Dickinson), which was followed by 9 samples of 2.0-mL in 37-USP unit lithium-heparin tubes (No. 366664, Becton Dickinson). At the point at which the phlebotomist switched from citrate to heparin tubes, a stopwatch was started, and the most recent citrate and heparin tubes were handed off to a second operator as the time-zero sample. Subsequent samples were randomly selected from the other tubes drawn to minimize any effect from the order of draw and were run at the following times after the draw: 5, 10, 15, and 30 minutes and 1, 2, 3, and 4 hours. The TEG 6s cartridges were run in singlicate per the manufacturer’s instructions. All operators were experienced TEG operators, and most of the testing was performed by a single operator.

In both studies, before being run and after being filled with donor blood, Vacutainers were stored on their sides at room temperature. Just before running, each tube was gently inverted approximately 5 to 7 times. Separate tubes were collected for each time point.

Participants

For the TEG 5000, 20 healthy blood donors (9 men and 11 women) participated in the study. The mean (SD) age of donors was 36.4 (7.8) years, with the youngest being 19 and the oldest being 62.0 years. Nonsteroidal anti-inflammatory drug use was not relevant because only CK samples were run.

For the TEG 6s, 20 healthy blood donors (14 men and 6 women) participated in the study. The mean (SD) age of the donors was 45.4 (7.8) years, with the youngest being 34.8 years and the oldest being 57.0 years. Five of the donors had taken nonsteroidal anti-inflammatory drugs shortly before or at the time of testing.

Statistical Analysis

The Wilcoxon rank-sum test was used to compare between-group data from each time point against the reference time point (15 minutes). All statistical tests were 2-tailed and were paired with the reference time point (15 minutes). All statistical tests were generated with RStudio software (version 0.99.463 [2015], RStudio, Boston, Massachusetts).

Parameters were compared with the results obtained at 15 minutes because that is the manufacturer’s current recommendation for running samples.

**RESULTS**

**Effect of Citrated Blood Sample Storage in the TEG 5000 System**

All TEG 5000 parameters measured were within the reference range provided by the manufacturer for CK activated samples (Table 1). The R parameter was not affected statistically at any time point studied. There was no significant difference in any parameters between samples run immediately and those equilibrated for 15 minutes. Only the K parameter (P < .001) and the α angle (P < .001) were affected by the 2-hour storage time. The other CK parameters (R, MA, LY30) were not affected at that time point. All differences were less than the 15% variability threshold when compared with the 15-minute US Food and Drug Administration–approved time for semiquantitative devices.

**Effect of Citrated Blood Sample Storage in the TEG 6s System**

All measured clot variables tested on the TEG 6s were within the reference range provided by the manufacturer (Table 2). All samples trended toward a hypercoagulable status upon clot forming, and degradation parameters increased with sample storage time. The CRT and the CFF showed the least effect from increased storage times. The CK LY30 and the CRT ACT were not affected at any time point studied when compared with the 15-minute sample equilibration. Furthermore, all CRT parameters, except the CRT LY30 (P = .005), and all CFF and CKH parameters (K, α-angle, and LY30) were unaffected by sample storage time when samples were run immediately, compared with the manufacturer’s currently recommended 15-minute equilibration. In addition, all CK parameters, all CRT parameters, except the α-angle (P = .02) and the LY30 (P = .01), and all CKH parameters, except R (P = .02), were unaffected by blood sample storage for 4 hours, when compared with the manufacturer’s recommended 15-minute equilibration. Finally, differences were 15% or less for all clot parameters, except CRT LY30, when compared with the 15-minute US Food and Drug Administration–approved time. The CRT LY30 coefficient of variance was greater because of the small number used in the denominator when the coefficient of variance was calculated.

**Effect of Heparinized Blood Sample Storage in the TEG 6s System**

All TEG parameters measured were within the reference range provided by the manufacturer; a trend toward increasing platelet inhibition was observed with increasing

---

**Table 1. Mean Effect of Sample Storage Time on Clot Formation Variables in Citrated Recalcified Kaolin Blood With the TEG 5000 Thromboelastography System**

| Parameter | Time Point, mean (SEM), n = 20* |
|-----------|--------------------------------|
|           | 0 min | 15 min | 30 min | 60 min | 90 min | 120 min |
| CK R, min | 6.6 (0.2) | 6.5 (0.1) | 6.4 (0.1) | 6.2 (0.1) | 6.3 (0.1) | 6.2 (0.1) |
| CK K, min | 1.9 (0.1) | 1.9 (0.1) | 1.9 (0.1)* | 1.9 (0.1) | 1.8 (0.0)* | 1.7 (0.0)*** |
| CK angle, α | 64.8 (0.7) | 63.9 (0.6) | 64.7 (0.7)* | 64.8 (0.9) | 65.6 (0.5)* | 66.4 (0.6)*** |
| CK MA, mm | 61.4 (0.5) | 62.4 (0.6) | 61.7 (0.6) | 62.0 (0.6) | 62.1 (0.5) | 62.7 (0.5) |
| CK LY30, % | 1.8 (0.2) | 1.5 (0.2) | 1.9 (0.2)* | 1.6 (0.2) | 1.8 (0.2) | 1.7 (0.2) |

Abbreviations: angle, α angle; CK, citrated recalcified kaolin activated blood; K, coagulation time; LY30, percentage of lysis 30 minutes after MA was finalized; MA, maximum amplitude; R, reaction time.

* The 20 independent donors were measured in quadruplicate.

- *P < .05; **IP < .001.
storage time (Table 3). No parameters were affected by the heparinized blood treated with heparinase and platelet-mapping activators when compared with the 15-minute equilibration time results. No parameters, except HKH R time (P = .002 versus 15 minutes) and ActF MA (P = .001 versus 15 minutes), were significantly affected by sample storage time when the sample was run immediately. Heparinized blood samples could be stored up to 3 hours without significantly affecting the percentage of inhibition or aggregation in both the ADP and AA tests, when compared with the 15-minute equilibration time.

**DISCUSSION**

The use of viscoelastic testing is growing because of its demonstrated clinical value. However, the requirements and logistics of sample handling need to be better understood.

### Table 2. Mean Effect of Sample Storage Time on Clot Formation Variables in Citrated Recalcified Blood With the TEG 6s Thromboelastography System

| Parameter | 0 min | 5 min | 10 min | 15 min | 30 min | 60 min | 120 min | 180 min | 240 min |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| CK        |        |        |        |        |        |        |        |        |        |
| R, min    | 6.8 (0.2)* | 6.4 (0.1) | 6.5 (0.1) | 6.3 (0.1) | 6.1 (0.2) | 6.1 (0.2) | 6.0 (0.2) | 5.9 (0.1)** | 6.0 (0.1) |
| K, min    | 1.6 (0.1)** | 1.7 (0.1)** | 1.5 (0.1)** | 1.4 (0.0) | 1.4 (0.1) | 1.3 (0.1) | 1.4 (0.1) | 1.4 (0.1) | 1.4 (0.0) |
| Angle, °  | 69.4 (0.8)** | 68.6 (0.8)** | 70.5 (0.8) | 72.0 (0.3) | 71.7 (0.6) | 73.0 (0.6)** | 71.3 (0.8) | 71.0 (0.9) | 72.0 (0.4) |
| MA, mm    | 60.1 (0.7)** | 60.2 (0.7) | 60.8 (0.7) | 60.8 (0.7) | 61.2 (0.7)* | 60.7 (0.8) | 60.6 (0.7) | 61.0 (0.6) |        |
| LY30, %   | 0.5 (0.1) | 0.5 (0.2) | 0.5 (0.1) | 0.5 (0.1) | 0.4 (0.1) | 0.6 (0.2) | 0.5 (0.1) | 0.4 (0.1) | 0.3 (0.1) |

#### Table 3. Mean Effect of Sample Storage Time on Clot Formation Variables in Heparinized Blood With the TEG 6s Thromboelastography System

| Parameter | 0 min | 5 min | 10 min | 15 min | 30 min | 60 min | 120 min | 180 min | 240 min |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| HKH       |        |        |        |        |        |        |        |        |        |
| R, min    | 7.3 (0.2)** | 6.8 (0.2)** | 6.4 (0.2) | 6.4 (0.3) | 6.1 (0.2) | 5.7 (0.2)** | 5.2 (0.2)** | 5.1 (0.2)** | 4.9 (0.2)** |
| K, min    | 1.6 (0.0) | 1.7 (0.1) | 1.6 (0.1) | 1.6 (0.1) | 1.6 (0.1) | 1.5 (0.1) | 1.4 (0.1) | 1.4 (0.0)*** | 1.4 (0.1)** |
| Angle, °  | 69.1 (0.5) | 68.4 (0.6)* | 69.9 (0.6) | 69.3 (0.7) | 69.8 (0.6) | 70.9 (0.5)* | 72.0 (0.4)** | 72.1 (0.4)** | 72.5 (0.4)** |
| MA, mm    | 63.0 (0.6) | 62.5 (0.6) | 62.7 (0.7) | 62.6 (0.7) | 62.5 (0.7) | 62.3 (0.7) | 62.2 (0.7) | 61.6 (0.8)** | 61.5 (0.8)** |
| LY30, %   | 0.3 (0.1) | 0.3 (0.1) | 0.2 (0.1)* | 0.3 (0.1) | 0.4 (0.1) | 0.3 (0.1) | 0.3 (0.1) | 0.3 (0.1) | 0.3 (0.1) |
| ActF MA, mm | 9.0 (0.7)** | 9.1 (0.6)** | 9.3 (0.7) | 9.7 (0.6) | 10.0 (0.7) | 10.5 (0.7)** | 10.4 (0.7)** | 10.6 (0.7)** | 10.6 (0.8)** |

#### Table 4. Mean Effect of Sample Storage Time on Clot Formation Variables in Heparinized Blood With the TEG 6s Thromboelastography System

| Parameter | 0 min | 5 min | 10 min | 15 min | 30 min | 60 min | 120 min | 180 min | 240 min |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| ADP       |        |        |        |        |        |        |        |        |        |
| MA, mm    | 62.0 (0.7) | 61.9 (0.8) | 62.0 (0.8) | 61.5 (0.9) | 61.9 (0.9) | 62.1 (0.7) | 60.8 (0.8)* | 59.7 (0.8)* | 57.9 (1.2)** |
| Inhibition, % | 2.2 (0.7) | 2.2 (0.8)* | 2.3 (0.7) | 2.8 (1.0) | 2.3 (1.2) | 2.0 (0.5) | 3.4 (0.8) | 4.4 (1.0) | 7.8 (2.1)* |
| Aggregation, % | 97.8 (0.7) | 97.8 (0.8)* | 97.7 (0.7) | 97.2 (1.0) | 97.7 (1.2) | 98.0 (0.5) | 96.6 (0.8) | 95.6 (1.0) | 92.2 (2.1)* |

#### Table 5. Mean Effect of Sample Storage Time on Clot Formation Variables in Heparinized Blood With the TEG 6s Thromboelastography System

| Parameter | 0 min | 5 min | 10 min | 15 min | 30 min | 60 min | 120 min | 180 min | 240 min |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| AA        |        |        |        |        |        |        |        |        |        |
| MA, mm    | 54.0 (4.2) | 55.0 (4.2) | 54.94 (4.0) | 57.1 (3.5) | 57.9 (3.25) | 57.1 (3.3) | 55.8 (3.32)* | 53.6 (3.6)** | 52.3 (3.8)** |
| Inhibition, % | 20.0 (7.8) | 18.0 (8.2) | 17.1 (7.6) | 13.3 (6.81) | 11.9 (6.5) | 12.8 (6.73) | 14.9 (7.1) | 17.1 (7.6) | 19.3 (7.8)** |
| Aggregation, % | 80.0 (7.8) | 82.0 (8.2) | 82.3 (7.6) | 86.7 (8.61) | 88.1 (6.5) | 87.2 (6.73) | 85.1 (7.1) | 82.9 (7.6) | 80.7 (7.8)** |

Abbreviations: ACT, activated clotting time; angle, °; CFF, citrated functional fibrinogen; CK, citrated recalcified kaolin-activated blood; CKH, citrated recalcified kaolin-activated blood treated with heparinase; CRT, citrated recalcified kaolin and tissue factor activated blood; FLEV, functional fibrinogen level; K, coagulation time; LY30, percentage of lysis 30 minutes after MA was finalized; MA, maximum amplitude; R, reaction time. 

*P < .05; **P < .01; ***P < .001.

Abbreviations: AA, activator F and AA-activated blood; ActF, heparinized blood with activator F; ADP, activator F and ADP activated blood; angle, °; HKH, heparinized kaolin activated blood treated with heparinase; K, coagulation time; LY30, percentage of lysis 30 minutes after MA was finalized; MA, maximum amplitude; R, reaction time.

*P < .05; **P < .01; ***P < .001.
Many studies have been performed to investigate the influence of storage time on clot-forming variables, especially for the interval of 30 minutes to several hours after sampling, using either the citrated method and nonactivated or kaolin-activated samples. In practice, these studies were performed at different time points to understand the effects on the results caused by the need to transfer a blood sample to a central laboratory. In this study, we investigated the effect of sample storage on clot-forming variables comprehensively by running samples immediately after being drawn and after increasing storage times up to 4 hours in both citrated and heparinized-activated samples on all currently available TEG 6s cartridges. We also compared the results of blood sample storage time with the previous viscoelastic testing cup-and-pin technology (on the TEG 5000) with the new system with microfluidic cartridges and ultrasound (on the TEG 6s), which can be used either near where the patient is tested and in the centralized laboratory setting, to assess whether there was a need for a change in clinical practice between the technologies.

All results were within references range, independent of sample storage times up to 4 hours, with a trend toward hypercoagulable profiles for citrated blood samples and increased platelet inhibition for the heparinized blood samples with increased storage time, and there was a significant difference when tested 4 hours after blood was drawn. When running citrated samples, the tissue factor–activated RapidTEG and functional fibrinogen tests were the least affected of the clot-forming parameters by running immediately. Thus, in emergent situations, in which a citrated sample needs to be run immediately, reliable clot-forming parameters can be achieved by using a combination of different activators and parameters: CRT ACT (clotting time), CRT MA (clot strength), and CFF MA (fibrinogen contribution to the clot strength). The LY30 CK or LY30 CKH clot-degradation/lysis parameters were the most reliable for a citrated sample run immediately. However, because of the increased time needed for LY30 results of CK or CKH tests versus LY30 of the CRT test, the latter might still be preferred in emergent/urgent cases, although any differences found relative to the CK LY30 may not be clinically important. The identified combination of tests and parameters may be used with emergent/urgent cases and are in line with the most recently published TEG algorithms in trauma emergency care and are also now being investigated in a prospective, randomized clinical trial entitled Implementing Treatment Algorithms for the Correction of Trauma Induced Coagulopathy.30 Furthermore, we found that the platelet-mapping assay with heparinized blood samples could be run immediately in emergent/urgent cases and the sample could be stored up to 3 hours and still provide accurate platelet-inhibition and aggregation results for both the ADP or AA tests. When comparing results from the TEG 5000 to the TEG 6s in CK-activated blood samples, the TEG 6s was the least sensitive to the sample-storage effect, except when the sample was run immediately. The difference between the 2 technologies is that the cup-and-pin rotation torque is slightly less sensitive to the first fibrin filament formation, and this information should be considered when introducing the new technology to previous TEG 5000 users.

To our knowledge, this is the first systematic study to look at the effect of blood sample storage time on TEG variables in a large group, with different anticoagulation collection methods and with activators other than kaolin. In addition, this is the first study to evaluate the effect of sample storage time on clot-forming parameters with the TEG 6s system. Other studies have looked at the effects of citrate storage on TEG 5000 thromboelastography parameters. However, all those studies suffer from one or more shortcomings. Most of the studies had small samples sizes, and many authors used whole blood samples that were not activated, increasing the variability of the results. Furthermore, some authors compared citrated results to those obtained at time zero from noncitrated samples. However, blood without anticoagulation run immediately after venipuncture yields different results and requires its own set of reference ranges for the TEG 5000 system, which is different from citrated samples. Some studies drew a single blood sample and, subsequently, reopened and reran the previously tested blood sample tube. This yields results that are more hypercoagulable on the subsequent runs. In addition, it was not clear from most reported studies whether a discard tube of blood was drawn, the absence of which results in tissue factors contaminating samples, increasing the hypercoagulable status of the sample, which is magnified with storage time. Furthermore, it was not clear from most of the reported studies whether the blood tubes were allowed to fill completely by vacuum, which is important because underfilling can cause significant sample dilution and may yield falsely prolonged clotting times because of excess calcium–binding citrate being present. This effect depends on the citrate concentration, the tube size, and the test performed, but is more pronounced with 3.8% citrate tubes (not recommended) and small-volume (pediatric) collection tubes. Finally, in this study, only BD Vacutainer tubes (Becton Dickinson) were used for sample collection, as recommended by the manufacturer, which may explain some of the differences observed at time zero by Durila and colleagues, who used Greiner Vacuette tubes (Greiner Bio-One GmbH, Kremsmünster, Austria). We previously studied Greiner 3.2%-citrate Vacuette tubes (Greiner Bio-One, Frickenhausen, Germany) in the TEG 5000 system and found them to be acceptable. We also plan to validate the Greiner Vacuette and Sarstedt Monovette tubes (Sarstedt AG & Co, Nümbrecht, Germany) in the TEG 6s system.

To our knowledge, there are no published studies evaluating the effect of storage time on heparinized blood samples with the TEG platelet-mapping assay. The results of platelet-function measurements depend on many variables, such as the different assays and cutoff values and the diverse specific and unspecific stimulants used, as well as on preanalytic variables, such as the anticoagulants used, storage time, and temperatures. Variable prevalence of low-antiplatelet response to acetylsalicylic acid and thienopyridines, ranging between 1% to 45%, have been reported. One of the crucial first steps of sample preparation for all platelet function testing, including for the TEG platelet-mapping assay, is ex vivo anticoagulation. Platelets are stabilized in vivo by such mediators as nitric oxide, prostaglandin I2, prostacyclin, and endothelial ADPase to prevent pathologic thrombus formation. Because these modulators are absent under in vitro conditions, platelets become easily activated in a time-dependent manner, which influences hemostasis and augments artificially false-positive results from blood samples. A study of Multiplate (Roche Diagnostics, Mannheim, Germany) samples showed decreased aggregation over time, but those samples were collected in hirudin
Unfortunately, anticoagulants can influence platelet reactivity directly or indirectly. The platelet-mapping assay has been validated in both sodium heparin and lithium heparin tubes. According to published results, the results from area under the curve analysis of blood samples in lithium heparin tubes do not vary significantly between 30 minutes and 8 hours for the ADP activator or between 0 minutes and 8 hours for the AA activator. Our samples were drawn in lithium heparin tubes, and our findings are not in agreement with those data because we found that both the AA and ADP channels could be run immediately but should not be stored longer than 3 hours. The increased precision and accuracy of the TEG 6s might explain these differences because very small differences will be detected, even in a small body of samples, whereas systems with lower precision and accuracy need larger sample sizes to detect the same differences. In contrast, the measurement of the response to aspirin and clopidogrel by Multiplate was shown to vary as much as 26%. A limitation of this study is that healthy donors were used, and ideally, the study samples should include both healthy controls and samples from those with results that are not within reference range. The samples for the TEG 6s study included samples from subjects on nonsteroidal anti-inflammatory drugs, but other types of coagulopathy were not included. However, it was not feasible to include a variety of coagulopathy samples because of the need to collect multiple samples from the same patient, and especially from bleeding patients, where the number of phlebotomies needs to be minimized.

In conclusion, this study supports the standardization of TEG technology use by demonstrating that the TEG clot- and clot-degradation parameters are reliable when the sample is run immediately and when it has been stored for up to 4 hours in citrate or 3 hours in heparin. Although these findings require additional clinical validation, TEG technology, and especially the new TEG 6s technology, can be a valuable tool for investigating both coagulopathic and hypercoagulable disorders in emergency situations as well as for routine use.

References
1. Von Kaulla KN, Kaye H, von Kaulla E, Marchioro TL, Starzl TE. Changes in blood coagulation. Arch Surg. 1966;92(1):71–79.
2. Agarwal S, Johnson RI, Shaw M. Preoperative point-of-care platelet function testing in cardiac surgery. J Cardiothorac Vasc Anesth. 2015;29(2):333–341.
3. Gonzalez E, Moore EE, Moore HB, et al. Goal-directed hemostatic resuscitation of trauma-induced coagulopathy: a pragmatic randomized clinical trial comparing a viscoelastic assay to conventional coagulation assays [published online ahead of print December 28, 2015]. Ann Surg. 2016;263(6):1051–1059.
4. Kozek-Langenecker SA, Afshari A, Alhaladejo P, et al. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology [published correction appears in Eur J Anaesthesiol. 2014;31(4):247]. Eur J Anaesthesiol. 2013;30(6):270–382.
5. American Society of Anesthesiologists Task Force on Perioperative Blood Management. Practice guidelines for perioperative blood management: an update report by the American Society of Anesthesiologists Task Force on Perioperative Blood Management. Anesthesiology. 2015;122(2):241–275.
6. Whiting P, Al M, Westwood M, et al. Viscoelastic point-of-care testing to assist with the diagnosis, management and monitoring of haemostasis: a systematic review and cost-effectiveness analysis. Health Technol Assess. 2015;19(38):1–228, vii–viii.
7. Camazine MN, Hemmila MR, Leonard JC, et al. Massive transfusion policies at trauma centers participating in the American College of Surgeons Trauma Quality Improvement Program. J Trauma Acute Care Surg. 2015;78(6(suppl 1)):S48–S53.
8. Quarterman C, Shaw M, Johnson L, Agarwal S. Intra- and inter-centre standardisation of thromboelastography (TEG). Anaesthesia. 2014;69(8):881–890.
9. Jackson GN, Ashpole KJ, Yentis SM. The TEG vs the ROTEM thromboelastography/thromboelastometry systems. Anaesthesia. 2009;64(2):212–215.
10. Chen A, Tenya J. Global hemostasis testing thromboelastography: old technology, new application. Clin Lab Med. 2009;29(2):391–407.
11. Owen CA Jr. Historical account of tests of hemostasis. Am J Clin Pathol. 1990;93(4(suppl 1)):S3–S8.
12. Eckman MH, Erban JK, Singh SK, Kao GS. Screening for the risk for bleeding or thrombosis. Ann Intern Med. 2003;138(3):W15–W24.
13. Gurbel PA, Bilden KP, Tantry US, et al. First report of the point-of-care TEG: A technical validation study of the TEG-G6 system [published online ahead of print April 9, 2016]. Platelets. doi:10.1093/ptj/pyw000.
14. Sharma AD, Al-Achi A, Seccombe JF, Hummel R, Preston M, Behrend D. Does incorporation of thromboelastography improve bleeding prediction following adult cardiac surgery? Blood Coagul Fibrinolysis. 2014;25(6):561–567.
15. Reinhofer M, Brauer M, Franke U, Barz D, Marx G, Losche W. The value of rotation thromboelastometry to monitor disturbed perioperative haemostasis and bleeding risk in patients with cardiopulmonary bypass. Blood Coagul Fibrinolysis. 2008;19(3):212–219.
16. Sambu N, Curzen N. Monitoring the effectiveness of antiplatlet therapy: opportunities and limitations. Br J Clin Pharmacol. 2011;72(4):490–496.
17. Camenzind V, Bombeli T, Seifert B, et al. Citrate storage affects thromboelastography analysis. Anesthesiology. 2000;92(5):1242–1249.
18. Khurana S, Mattson JC, Westley S, O’Neill WW, Timmis GC, Sahian RD. Monitoring platelet glycoprotein IIb/IIIa-fibrin interaction with tissue factor-activated thromboelastography. J Clin Med. 1997;13(4):401–411.
19. Chavez JJ, Foley DE, Snider CC, et al. A novel thrombelastograph tissue factor/kaoassin assay of activated clotting times for monitoring heparin anticoagulation during cardiopulmonary bypass. Anesth Analg. 2004;99(5):1290–1294.
20. Harding SA, Mullett SV, Peachey TD, Cox DJ. Use of heparinase modified thromboelastography in liver transplantation. Br J Anaesth. 1997;78(2):175–179.
21. Harr JN, Moore EE, Ghahalyan A, et al. Functional fibrinogen assay: a reliable test? Blood Coagul Fibrinolysis. 2008;19(3):246–249.
22. Castellino FJ, Chapman MP, Donahue DL, et al. Traumatic brain injury causes platelet adenosine diphosphate and arachidonic acid receptor inhibition independent of hemorrhagic shock in humans and rats. J Trauma Acute Care Surg. 2014;76(5):1169–1176.
23. Bowbrick VA, Mkhaldiids DP, Stansby G. The use of citrated whole blood in thromboelastography. Anesth Analg. 2000;90(5):1086–1088.
24. White H, Zollinger C, Jones M, Bird R. Can Thromboelastography performed on kaolin-activated citrated samples from critically ill patients provide stable and consistent parameter? Int J Lab Hematol. 2010;32(2):167–173.
25. Vig S, Chitole A, Bevan DH, Halliday A, Dormandy J. Thromboelastography: a reliable test? Blood Coagul Fibrinolysis. 2001;12(7):555–561.
26. Johansson PI, Bochsen L, Andersen S, V;iuf D. Investigation of the effect of kaolin and tissue-factor-activated citrated whole blood, on clot-formation, as evaluated by thromboelastography. Transfusion. 2008;48(1):377–382.
27. Durula M, Lukas P, Bronsky J, Coacho vec J. Time impact on non-activated and kaolin-activated blood samples in thromboelastography. BMC Anesthesiol. 2015;15:50.
28. Zambruni A, Thalheimer U, Leandro G, Perry D, Burroughs AK. Thromboelastography with citrated blood: comparability with native blood, stability of citrate storage and effect of repeated sampling. Blood Coagul Fibrinolysis. 2004;15(1):103–107.
29. Wasowicz M, Srinivas C, Meineri M, Banks B, McCluskey SA, Karkouti K. Technical report: analysis of citrated blood with thromboelastography: comparison with fresh blood samples. Can J Anesth. 2008;55(5):284–289.
30. Johansson PI, Stensballe J, Oliveri R, Wade CE, Ostrowski SR, Holcomb JB. How I treat patients with massive hemorrhage. Blood. 2014;124(20):3052–3058.
31. Hill JS, Devenie G, Powell M. Point-of-care testing of coagulation and fibrinolytic status during postpartum haemorrhage: developing a thromboelastography-guided transfusion algorithm. Anaesth Intensive Care. 2012;40(6):1007–1016.
32. Adcock DM, Kressin DC, Marlar RA. Minimum specimen volume requirements for routine coagulation testing: dependence on citrate concentration. Am J Clin Pathol. 1998;109(5):595–599.
33. Chuang J, Sadler MA, Witt DM. Impact of evacuated collection tube fill volume and mixing on routine coagulation testing using 2.5-ml (pediatric) tubes. Chest. 2004;125(4):1262–1267.
34. Haemonetics. Timing of citrate samples [manufacturer letter]. Niles, IL: Haemonetics; 2008.
35. Breddin HK. Can platelet aggregometry be standardized? Platelets. 2005;16(3–4):151–158.
36. Kaiser AF, Neubauer H, Franken CC, Kruger JC, Mugge A, Meves SH. Which is the best anticoagulant for whole blood aggregometry platelet function testing?: comparison of six anticoagulants and diverse storage conditions. Platelets. 2012;23(5):359–367.

37. Khanna V, Holson A, Mikael R, Sambu N, Englyst N, Curzen N. Does the VerifyNow P2Y12 assay overestimate “therapeutic response” to clopidogrel?: insights using short thrombelastography. Thromb Haemost. 2014;111(6):1150–1159.

38. Ben-Dor I, Kleiman NS, Lev E. Assessment, mechanisms, and clinical implication of variability in platelet response to aspirin and clopidogrel therapy. Am J Cardiol. 2009;104(2):227–233.

39. Jin RC, Voetsch B, Loscalzo J. Endogenous mechanisms of inhibition of platelet function. Microcirculation. 2005;12(3):247–258.

40. Golanski J, Pietrucha T, Raj Z, Greger J, Watala C. Molecular insights into the anticoagulant-induced spontaneous activation of platelets in whole blood—various anticoagulants are not equal. Thromb Res. 1996;83(3):199–216.

41. Johnston LR, Larsen PD, La Flamme AC, Harding SA. Methodological considerations for the assessment of ADP induced platelet aggregation using the Multiplate® analyser. Platelets. 2013;24(4):303–307.

42. Glusa E. Platelet aggregation in recombinant-hirudin-anticoagulated blood. Haemostasis. 1991;21(suppl 1):1116–1120.

43. Haemonetics. Lithium heparin tubes validated for use with the Platelet-Mapping® assay on the TEG® hemostasis analyzer [manufacturer letter]. Niles, IL: Haemonetics Corporation; 2008.

44. Gerotziafas GT, Zarifis J, Bandi A, et al. Description of response to aspirin and clopidogrel in outpatients with coronary artery disease using multiple electrode impedance aggregometry. Clin Appl Thromb Hemost. 2012;18(4):356–363.