Technical note

Thromboelastography: a reliable test?

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(Received 12 September 2000; revised 12 March 2001; accepted 19 March 2001)

The thromboelastograph (TEG), a measure of global haemostasis, is routinely used during cardiac and hepatic surgery to optimize blood product selection and usage. It has recently been suggested that it may also be a useful tool to screen patients with hypercoagulable states. Limited published data on performance characteristics has led to speculation regarding its consistency and, therefore, validity of the results. This study was designed to assess the effect of stability of blood samples prior to testing, repeated sampling, intra- and inter-assay variability using the native, celite, tissue factor (TF) and Reopro-modified TEG. Analysis of native and celite samples after storage over 90 min showed a period of instability up to 30 min. Thereafter, all parameters between 30 and 90 min were stable [*P* = not significant (NS)]. When the same sample was repeatedly assayed, both native and celite TEG parameters showed a significant change towards hypercoagulability ($P < 0.01$), whereas the TF and Reopro-modified TEG showed no change. Intra- and inter-assay variability on samples tested after 30 min showed excellent reproducibility for all parameters (*P* = NS). The data suggest that the TEG is a useful tool in haemostasis but requires a formal standard operating procedure to be adopted that takes into account the initial period of sample instability. *Blood Coagul Fibrinolysis* 12:555–561 © 2001 Lippincott Williams & Wilkins.

Keywords: thromboelastography, performance data, stability, hypercoagulability

Introduction

Hartert first described the thromboelastograph (TEG) as a global test of blood coagulation over 50 years ago [1]. The instrument allows a rapid assessment of coagulation and fibrinolysis from a single sample of native whole blood, anticoagulated citrated recalcified whole blood or activated whole blood. As the TEG produced an overall impression of coagulation rather than precise quantitative analysis of individual contributing factors, it remained largely a research tool in haematology. However, the role of the TEG is more diverse, with some haematologists advocating it as a screening tool for hypercoagulable defects [2]. The TEG has been investigated extensively by anaesthetists in cardiac and hepatic surgery [3–5]. Where used clinically, it has allowed appropriate selection and usage of blood and blood products during and following cardiac bypass and hepatic transplantation, resulting in a significant reduction in the number of blood transfusions [6–12]. Its popularity has also led to an increase in the number of publications over the past 10 years.

The principle components of the TEG are a cylindrical cup and a pin (Fig. 1). A warmed cup oscillates for 10 s through an angle of 4° 45′ with a pin freely suspended in the cup by a torsion wire. Blood is added and a torque is first transmitted as the clot forms linking the cup and pin together, increasing as the clot strengthens and decreasing as the clot lyses. The clot's physical properties, i.e. rate of formation, clot strength and stability, are dependent on the interaction of fibrinogen, platelets and plasma proteins, and this process produces a characteristic trace that historically has been visually classified as normal, hypocoagulable or hypercoagulable.

The CTEG® model 3000 (Haemoscope Corp, Skokie, Illinois, USA) now consists of a bench-top instrument comprising a dual-channel Coagulation Analyser (TEG®) and TEG® analytical software.

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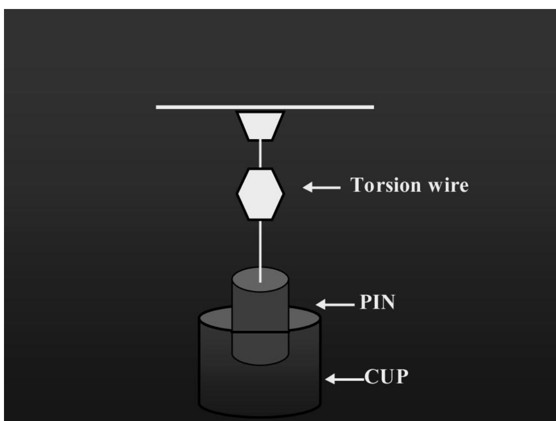

Figure 1. Principles of thromboelastography.

The software allows eight samples to be analyzed independently and automatically calculates the TEG parameters and computes a coagulation index; a computer-derived classification of the traces.

There are four main TEG parameters that are measured (Fig. 2). The reaction time (*R*) is the distance from the start of the sample run to the point of first significant clot formation with an amplitude of 2 mm, and it is measured in millimetres. The TEG trace advances at a rate of 2 mm/min. *R* is prolonged by anticoagulants (warfarin/heparin), coagulation factor deficiencies and decreased with coagulation inhibitor deficiencies. The *K* value is the distance (mm) taken to achieve clot strength with an amplitude of 20 mm. This becomes prolonged by anticoagulants (warfarin/heparin). The alpha angle (α) reflects the kinetics of clot formation, and is increased in hypercoagulable conditions and decreased by anticoagulation. The angle is measured in degrees of arc from the point of divergence to the maximum tangent drawn to the curve. The maximum amplitude (MA) is a measurement of maximum clot strength, and is contributed to by fibrinogen and platelet activity and measured in millimetres.

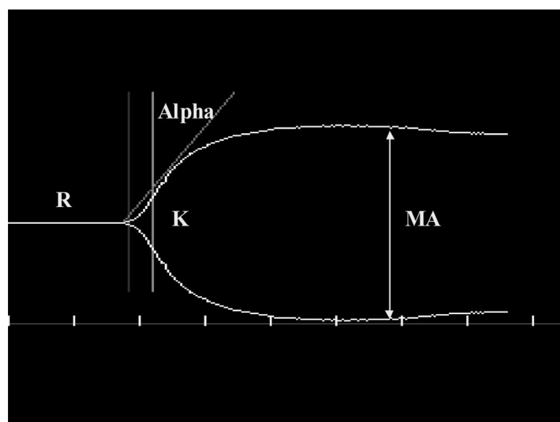

Figure 2. Thromboelastograph trace showing parameter measurements.

Although samples may be assayed from native whole blood, which is often used as the gold standard, this is not practical for testing carried out remote from the TEG due to the blood coagulation process. In the majority of studies, blood is collected into tri-sodium citrated anticoagulant, allowing testing to be carried out in the laboratory.

The assay can also be manipulated *in vitro* by the addition of celite or tissue factor as activators to allow a more rapid analysis by shortening the *R* time. Celite (silica particles or diatomaceous earth) shortens the coagulation time by acting as a contact surface activating factor XII and platelets.

An interesting concept is the Reopro-modified TEG assay. Reopro (Eli Lilly, Centocor, Malvern, Pennsylvania, USA) is an antibody fragment that inhibits clot retraction and abolishes platelet aggregation by binding to platelet fibrinogen receptors GPIIb/IIIa. The addition of Reopro to a sample therefore abolishes platelet function and clot strength, and the resulting MA is therefore thought to be proportional to functional fibrinogen [13–15]. TEG assays may also be run on platelet-rich and platelet-poor plasma.

Despite this apparent diversity of applications, the very limited published commercial data on performance with regard to assay precision or inter-assay variability has led to speculation regarding its use in a routine laboratory environment.

The purpose of this study was to evaluate the performance of the TEG used in a controlled laboratory environment. We therefore studied the effect of repetitive sampling on TEG trace parameters, stability of blood samples prior to TEG analysis, and intra-assay and inter-assay variability.

Methods

To enable these effects to be assessed three TEG machines were utilized during the study, enabling analysis on six channels simultaneously.

Blood sampling

Venepuncture was carried out with a 21-gauge needle using minimum stasis directly into 4.5 ml Vacutainer bottles containing 3.2% (0.105 mol/l) sodium citrate (Becton Dickinson Vacutainer Sys-

tems, Rutherford, New Jersey, USA). Blood was transported to the laboratory within 5 min.

Statistical analysis

A repeated-measure analysis of variation was performed and the intraclass coefficient *r* was calculated. Where appropriate, the significance of difference between variables was tested by a two-tailed *t* test for means. Intra-sample variation was estimated by the coefficient of variation.

Assays

The following thromboelastographic assays were carried out.

- *Recalcified native blood (RN).* Twenty microlitres of 0.2 mol/l $CaCl_2$ was pipetted into the warm TEG cup. The citrated blood was gently inverted to ensure mixing of the sample and then 340 μl citrated blood pipetted into the TEG cup.
- *Recalcified native blood with Celite (RC).* Twenty microlitres of 0.2 mol/l $CaCl_2$ were pipetted into the warm TEG cup. The celite vials were warmed to room temperature. The citrated blood was gently inverted to ensure mixing of the sample and then 1 ml citrated blood pipetted into 1% celite vials (Haemoscope Corp.). This vial was then gently inverted to allow mixing. Then 340 μl citrated blood with celite was pipetted into the TEG cup.
- *Recalcified native blood with tissue factor (RTF).* Ten microlitres of tissue factor (Ortho RecombiPlastin® Hemoliance; Ortho Diagnostic Systems Inc., Tritan, New Jersey, USA) and 20 μl of 0.2 mol/l $CaCl_2$ was pipetted into the warm TEG cup. The citrated blood was gently inverted to ensure mixing of the sample and 330 μl citrated blood pipetted into the TEG cup.
- *Recalcified native blood with tissue factor and Reopro (RTFR).* Ten microlitres of tissue factor, 5 μl Reopro and 20 μl of 0.2 mol/l $CaCl_2$ were pipetted into the warm TEG cup. The citrated blood was gently inverted to ensure mixing of the sample and 330 μl citrated blood pipetted into the TEG cup.

For each test, the disposable cups and pins were placed into the TEG and allowed to warm to 37°C for 10 min. Patient details were then entered into the computer program. After the addition of the sample, the pin was then gently raised and lowered into the cup five times, finishing in the down position. TEG monitoring was started by holding down the start button for 5 s. The sample was then carefully topped with mineral oil and the chamber cover closed.

Stability of blood samples prior to TEG analysis

Eight samples of blood were taken at one sitting from each of 10 subjects recruited. RN and RC TEG analysis was then carried out consecutively at 5, 15, 30, 40, 50, 60, 75 and 90 min after sample collection.

The effect of repeated sampling on TEG parameters

A single sample was taken from each of 10 subjects. This sample was analysed at 45, 90, 135 and 180 min, and analysis was carried out for RN, RC, RTF and RTFR samples.

Intra-assay variability

Six samples of blood were taken at one sitting from each of 10 subjects recruited. TEG analysis was carried out simultaneously on each of the six samples from the same subject on six channels after a delay of 30 min from sampling based on the already reported data. Analysis was carried out for both RN and RC sampling.

Inter-assay variability

Ten patients and 10 controls were recruited to be TEG analysed at two separate time points ranging from 48 h to 6 months. Analysis was carried out for both RN and RC sampling.

Results

Stability of blood samples prior to TEG analysis

When stability of the sample was studied, storage of a RN sample prior to analysis appeared to shorten the *R* parameter significantly between sampling and 30 min (Table 1). All RN parameters were then stable between 30 and 90 min. However, the storage of native blood, which was then activated by celite, did not appear to cause any significant change in the parameters over 90 min (Table 2).

The effect of repeated sampling on TEG parameters

Repeated sampling of a single sample of RN blood caused the *R* and *K* parameters to decrease while MA and α parameters were greater, leading to an increasingly hypercoagulable trace (Fig. 3). Analysis of variation showed a significant change for the parameters at 45 and 180 min, most markedly for the *R* parameter ($P < 0.0001$). The period of most stability for all parameters was between 45 and 135 min (Table 3). It is obviously difficult to separate the effect of time and repeated sampling in this analysis. Repeated sampling of the RC blood sample again appeared to activate the sample,

Table 1. Statistical analysis of effect of storage of native citrated samples

	ANOVA	*t* Test			
	Analysis of variation between all runs 1–8	10 min versus 15 min	10 min versus 30 min	10 min versus 90 min	30 min versus 90 min
R	0.0001*	0.0459	0.0026*	0.0027*	0.6912
K	0.0353	0.228	0.0878	0.1086	0.3557
Maximum amplitude	0.1785	0.3894	0.7066	0.4131	0.2235
α	0.1364	0.328	0.1397	0.091	0.4488

ANOVA, Analysis of variance. *Significant *P* value. Shaded areas indicate period of stability.

Table 2. Statistical analysis of effect of storage of celite citrated samples

	ANOVA	*t* Test			
	Analysis of variation between all runs 1–8	10 min versus 15 min	10 min versus 30 min	10 min versus 90 min	30 min versus 90 min
R	0.2132	0.4465	0.6415	0.0736	0.2695
K	0.3746	0.5911	0.4754	0.2569	0.8723
Maximum amplitude	0.0394	0.1459	0.4892	0.3516	0.1651
α	0.607	0.0496	0.1547	0.2818	0.6783

ANOVA, Analysis of variance. Shaded areas indicate period of stability.

Table 3. Statistical analysis of the effect of repeated analysis of a single native citrated blood sample

	ANOVA	*t* Test				
	All runs	45 min versus 180 min	90 min versus 180 min	45 min versus 90 min	90 min versus 135 min	135 min versus 180 min
R	< 0.0001*	< 0.0001*	0.022	0.011	0.053	0.133
K	0.01*	0.009*	0.065	0.797	0.164	0.003*
Maximum amplitude	0.008*	0.033	0.021	0.382	0.102	0.164
α	0.038	0.068	0.114	0.299	0.199	0.402

ANOVA, Analysis of variance. *Significant *P* value. Shaded areas indicate period of stability.

with all parameters appearing more hypercoagulable. Analysis of variation between all runs showed highly significant changes for *R*, *K* and α ($P < 0.0001$). The period of most stability with no significant change in the parameters was up to 135 min (Table 4). It was interesting to note that all parameters for both tissue factor-activated and Reopro samples did not differ significantly from time of sampling to 180 min after sampling. This appeared to show that neither storage nor repeated sampling altered the RTF or RTFR TEG assays (Tables 5 and 6).

Intra-assay variability

The results of the presented data were taken into consideration when intra-assay variability was performed. RN samples were assayed 30 min after sampling as this appeared to be the most stable time period and, for uniformity, celite samples were then also run after a delay of 30 min. For RN samples,

Table 4. Statistical analysis of the effect of repeated analysis of a single celite citrated blood sample

	ANOVA	*t* Test				
	All runs	45 min versus 180 min	90 min versus 180 min	45 min versus 90 min	90 min versus 135 min	135 min versus 180 min
R	0.0001*	< 0.0001*	0.0044*	0.83	0.04	0.009*
K	< 0.0001*	< 0.0001*	< 0.0001*	0.59	0.01	0.005*
Maximum amplitude	0.02	0.07	0.0021*	0.89	0.03	0.22
α	< 0.0001*	0.0001*	0.0004*	0.28	0.02	0.02

ANOVA, Analysis of variance. * Significant *P* value. Shaded areas indicate period of stability.

Table 5. Statistical analysis of the effect of repeated analysis of a single tissue factor-activated citrated blood sample

	ANOVA	*t* Test				
	All runs	45 min versus 180 min	90 min versus 180 min	45 min versus 90 min	90 min versus 135 min	135 min versus 180 min
R	0.29	0.24	0.59	0.22	1.00	0.73
K	0.61	0.59	0.34	0.17	1.00	0.59
Maximum amplitude	0.73	0.76	0.74	0.92	0.46	0.32
α	0.03	0.21	0.07	0.02	0.51	0.41

ANOVA, Analysis of variance.

Table 6. Statistical analysis of the effect of repeated analysis of a single tissue factor-activated and Reopro-modified citrated sample

	ANOVA	*t* Test				
	All runs	45 min versus 180 min	90 min versus 180 min	45 min versus 90 min	90 min versus 135 min	135 min versus 180 min
R	0.27	0.20	0.22	0.09	0.23	0.72
K	Not measurable					
Maximum amplitude	0.11	0.20	0.27	0.37	0.19	0.85
α	0.58	0.30	0.15	1.00	0.58	0.58

ANOVA, Analysis of variance.

this produced percentage coefficients of variation (%CVs) of 13 and 14 for *R* and *K*, respectively. MA and α showed less variation with %CVs of 5 and 4.4% respectively. For RC samples, %CVs showed little variability with MA and α at 3.8 and 4.5% respectively. *R* and *K* parameters showed more variability with %CVs of 17% (Table 7). Coefficients of variation for all parameters tested during Reopro- and tissue factor-manipulated samples were < 1% (Tables 5 and 6).

Inter-assay variability

Laboratory use of this data with single vial sampling after a storage delay of 30 min allowed excellent inter-assay variability for all RN and RC parameters (P = not significant) (Table 8).

Discussion

Historically, the thromboelastograph has been set up and run ‘anywhere, anyhow’, with poor standardization causing a negative bias towards research carried out using it. There is also very limited published commercial data on performance with regard to assay precision or inter-assay variability that again led to scepticism. Recently, there have been two fundamental changes to the instrument that could allow standardization of results.

Table 7. Statistical analysis of intra-assay variability of native and celite citrated samples

	Analysis of variation within assay runs		% Coefficient of variation	
	Native	Celite	Native	Celite
R	0.06	0.11	13	17.1
K	0.06	0.18	14.1	17.3
Maximum amplitude	0.62	0.27	5	3.8
α	0.17	0.55	4.4	4.5

Data presented as the *P* value.

Table 8. Results of inter-assay variability for native and celite sampling

	Native	Celite
R	0.91	0.46
K	0.94	0.75
Maximum amplitude	0.64	0.44
α	0.91	0.23

Data presented as the *P* value.

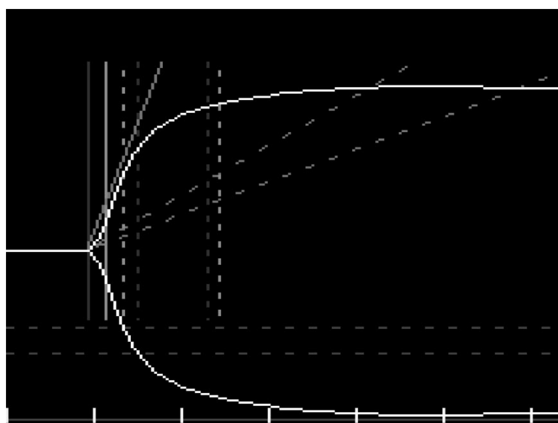

Figure 3. Thromboelastograph trace showing hypercoagulability.

The initial traces produced by the machine were on heated, activated paper and were interpreted subjectively. The addition of an electro-mechanical transducer converts the oscillations to electrical signals, allowing monitoring by a customized software package (Haemoscope Corp) that is then monitored on-line with an IBM-compatible personal computer. The torque produced by the pin is plotted as a function of time and allows an objective interpretation of the traces. Second, TEG cups and pins initially used were formed from stainless steel and became worn and scratched with use. This caused a potential for a variation in the assay result as the surface provided an activation medium for the coagulation pathway. Disposable plastic cups and pins, for one use only, have now replaced these, again increasing the standardization.

Our results indicate that an initial period of 30 min is required after blood collection for the anticoagulated native blood to achieve stability and to equilibrate. Celite samples appear more robust to this effect as the activation of the sample with celite causes a shortening of the *R* and *K* parameters, which could compensate for any inherent changes of the sample. Repeated sampling and agitation of the native and celite samples appears to cause activation and causes an apparent progressively hypercoagulable effect on these traces. RT and RTFR TEG analyses do not appear to show this effect. This can partly be explained by the almost immediate activation of the clotting process following the addition of tissue factor, therefore nullifying any inherent activation of the sample. This in effect 'fixes' the *R* parameter, allowing an almost constant MA and α parameter. However, this rapidity of result generation may be at the cost of losing valuable information on the global coagulation process.

The factors highlighted in this paper are important if the TEG is to be used as a screening tool for hypercoagulability as inappropriate handling could lead to false-positive hypercoagulable traces. Based on our findings, we adopted a formal standard operating procedure that takes into account the period of sample stability and handling. Native citrated TEG analysis should be carried out between 30 and 90 min. Celite citrated TEG analysis is more robust and could be carried out between 10 and 90 min post-sampling. Repeated analysis from the same vial of blood appears to cause a hypercoagulable effect on native and celite TEG parameters, and should be avoided. Both tissue factor- and Reopro-modified TEG analysis produces results that are repeatable regardless of immediate handling. The use

of these guidelines allows the production of acceptable and reproducible results.

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