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Performance characteristics of thromboelastometry assays using incompletely filled and prolonged stored samples

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Abstract

Background: Thromboelastometry (TEM) is often used to guide transfusion therapy in patients with massive bleeding. The effect of testing incompletely filled samples and those stored for a prolonged time at 4°C was investigated.

Methods: Whole blood samples were collected from 15 healthy blood donors and were pooled according to ABO group. From these pools aliquots were taken and diluted to produce final whole blood: citrate buffer ratios ranging from 90:10 (fully filled sample) to 40:60 (extremely under filled samples). These samples were then tested by EXTEM, INTEM, and FIBTEM on calibrated ROTEM *delta* machines. Separately, the four samples at 90:10 dilution were kept at 4°C for 16-20 hours and then retested on the ROTEM machines.

Results: All of the samples at the 90:10 and 80:20 (half-filled sample) whole blood:citrate buffer dilutions demonstrated ROTEM parameters within their respective reference ranges, although the samples from the 80:20 dilution tended to demonstrate slightly longer or slower times, depending on each ROTEM parameter, compared to the completely filled samples. All of the samples with more dilute whole blood to citrate buffer ratios (i.e., 70:30 to 40:60) yielded abnormal TEM results. The TEM results for the 90:10 dilution samples exposed to 16-20 hours of storage at 4^{o}C were within the reference intervals.

Conclusion: Completely and half-filled samples, and completely filled samples after prolonged cold storage, produced normal ROTEM results. Tubes that are less than half-filled should not be used for ROTEM testing.

Key words: ROTEM, testing, incompletely filled, parameters, storage

Introduction

Thrombelastometry (TEM) and thrombelastography (TEG) are whole blood functional clotting tests used to identify coagulopathies using real time graphic depiction of viscoelastic changes in the blood.[1] While TEM/TEG were originally used in transplant and cardiac surgery, both were later introduced in traumatology and intensive care medicine for the development of personalized transfusion strategies for patients with coagulopathies.[2-4] As part of a large, regional tertiary care hospital's patient blood management program, TEM in the form of ROTEM *delta* (TEM International GmbH, Munich, Germany) was implemented at Odense University Hospital (OUH; Odense, Denmark) in January 2016.

At the OUH, the ROTEM protocol requires TEM testing to be initiated within 10 minutes of sample collection. In order to demonstrate hyperfibrinolysis, the ROTEM total run time is 60 minutes. However, a short turnaround time is important for accurate blood component guidance in severely bleeding patients,[5] thus, ROTEM results are reported in real time to the clinicians at the OUH from the beginning of the test.

The blood samples for ROTEM at OUH are collected in tubes with a final whole blood:citrate buffer ratio of 90:10. Ideally, the test tube should be filled with whole blood according to this specification. However, attaining this ratio can be a challenge in patients who are critically ill with low blood pressure or in situations in which the blood sampling is rushed. In case of an insufficient filling of the tube the blood sample will be relatively more diluted by the citrate buffer as the tubes contain 10 percent volume pre-added Na_3citrate buffer, which might affect the performance of the TEM test.

The objectives of this study were to determine if TEM results are valid if the citrate containing test tube is inadequately filled with the patient's blood and if the blood sample is exposed to prolonged cold storage.

Materials and methods

Whole blood dilution experiments

Two blood samples from 15 healthy volunteer blood donors were collected in 3 mL vacutainer tubes containing 0.3 mL 0.109 M Na_3Citrate buffer (BD Vacutainer 9NC 0,109M, Buffer Na_3Citrate, ref. 363048, lot no.9014675). The samples were pooled by ABO group in plastic cylinders with 3-4 donors from each ABO group (**Figure 1**). Six aliquots from each of the four pools were pipetted (Biohit automatic pipette) into empty tubes and Na_3Citrate buffer was added to each tube to produce final whole blood:buffer ratios ranging from 90:10 (standard) to 40:60 (extremely diluted) corresponding to 90, 80, 70, 60, 50, and 40 percent patient blood in citrate-buffer test tube by volume (Table 1). The 90:10 samples were tested within 90 minutes of collection, all other samples within four hours of collection, and all were maintained at room temperature from the time they were collected until testing. TEM analysis was performed as described below.

Prolonged cold storage experiments

After the dilution testing was completed as described above, the 90:10 samples were stored at 4^{o}C for 16-20 hours, an arbitrarily decided time range. Following storage, TEM testing was repeated, and the pre- and post-cold storage TEM parameters were compared.

TEM testing protocol

Using blood from healthy blood donors hyperfibrinolysis was not expected, therefore all TEM experiments were performed with a 30 minute run time for EXTEM, INTEM, and FIBTEM on calibrated ROTEM® *delta* machines using cup-sets (Cup & Pin Pro lot no. M18072) and reagents for EXTEM, INTEM, and FIBTEM

(Startem lot no. 20738005, Ex-tem lot no. 42222301, In-tem lot no. 42191401, Fib-tem 42212601) according the manufacturer's directions.

TEM results are presented as follows: the clotting time (CT) is the time (seconds) from start of the TEM until clot initiation. Clot formation time (CFT) is the time (seconds) from clot initiation until a clot firmness of 20 mm is reached. Maximum clot firmness (MCF) is the maximum amplitude of the ROTEM curve.[6] Additionally, the α-angle was recorded for comparison reasons; this is the angle of the tangent to the curve where clot firmness changes from 0 to 20 mm and is thus related to the CFT (**Figure 2**).

Ethics and data safety

The study's protocol was approved by the Danish Data Protection Agency [no. 19/16273). Data collection, storage and handling were done according to the legislation and European Union's general data protection regulation.

Results

Donor demographics

The ABO groups and genders of the 15 blood donors whose samples were used in this study are demonstrated in **Figure 1**.

Whole blood dilution experiments

All of the samples at the 90:10 and 80:20 whole blood to citrate buffer dilutions demonstrated ROTEM parameters within their respective reference ranges, although the samples from the 80:20 dilution (simulating a half filled sample) tended to demonstrate slower times, depending on each TEM parameter, compared to the completely filled samples (**Table 2**).

All of the samples with more dilute whole blood to citrate buffer ratios (i.e., 70:30 to 40:60) yielded highly abnormal TEM results. Representative data for the 70:30 dilutions are shown in **Table 2**.

Prolonged cold storage experiments

The TEM results for the 90:10 dilution samples exposed to 16-20 hours of storage at 4^{o}C were within the reference intervals (**Table 2**).

The four results at different dilutions, one result from each of the ABO groups, were combined and are reported together as the median and range. Similarly for the prolonged storage experiments, the four results, one from each of the four ABO groups at the 90:10 dilution, are also reported together as the median and range (**Table 2**).

Discussion

The TEM testing results using samples with a whole blood:buffer ratio of 90:10, reflecting a completely filled sample, were within the reference intervals, as were the 80:20 samples that reflects a half-filled sample, although the latter tended to demonstrate slightly longer or shorter TEM parameter times compared to the fully filled sample. Samples that were more dilute produced invalid TEM results due to severe hypocoagulability. In addition, the 90:10 samples that underwent prolonged cold storage produced TEM values that were within the reference range for each parameter. Based on these findings, the policy for accepting samples for TEM testing at OUH will be modified to permit the acceptance of samples that are at least half filled with the patient's whole blood; samples that are less than half filled (i.e., more diluted than 80:20 whole blood:citrate buffer) will be rejected. Despite these results, the new policy will not permit the testing of samples that are received in the laboratory >10 minutes after they were drawn in order to keep the results as relevant as possible for managing a bleeding patient.

Blood samples for TEM testing at this hospital are collected in tubes containing 10 percent by volume trisodium citrate. The citrate binds with ionized calcium (Ca^{2+}) in the patient's plasma, and as Ca^{2+} is necessary for both the intrinsic, extrinsic, and common coagulation pathways, its chelation will primarily reduce platelet activation and aggregation in the collection tube. Hence an increase in the amount of citrate buffer relative to the amount of whole blood collected will not only cause a volume dependent dilution of the clotting factors, but it will also lead to a relatively lower plasma Ca^{2+} level in the tube, which will impede clotting.

Comparing the results of TEM testing performed on completely filled samples (90:10) to the results for the more diluted samples there was a tendency for the INTEM values to the most preserved, i.e., the least amount of change noted between completely filled and under filled samples. The greatest relative

difference between the different samples was noted in the FIBTEM measurements, with EXTEM demonstration an intermediate amount of difference between the samples (Table 2). Likewise INTEM appeared as more robust to prolonged storage than EXTEM and FIBTEM.

Volunteer blood donors were used instead of patients in order to avoid any illness or medication use that might have influenced the TEM test. The samples were pooled in order to minimize inter-donor variability in terms of coagulation factor and platelet concentrations; however, the result of this pooling also eliminated the ability to detect small inter-donor differences in the TEM results. Thus, there might be some donors whose TEM results might be more affected than the median results demonstrated here if their samples are submitted with even a modestly increased dilution above 90:10. Furthermore, the small number of samples tested precluded a formal statistical analysis of the differences between the medians of each TEM parameter at each dilution.

The decision to administer transfusions to a patient with massive bleeding is initially made on clinical grounds, and the TEM results that become available later on in the resuscitation are used to guide the number and volume of blood components and hemostatics that are administered to the patient. Normal TEM results are compatible with whole blood equivalent transfusion, while TEM results outside reference ranges may cause a changed ratio of blood components. The current study showed a dilution dependent trend towards a lower coagulability; hence a hypocoagulopathy will be detected also in case of under filled tubes although it may appear as a more significant coagulopathy than it actually is. Furthermore this study showed that TEM testing performed on non-coagulopathic samples that were at least half filled still fell within or close to the normal reference range. Thus TEM testing on these samples can be used to guide the rescucitation.

That the TEM results on half-filled samples were still within the references ranges in this study was consistent with the results from a previous study where blood was drawn from 12 healthy volunteers into citrate buffer containing tubes and then diluted with an irrigation fluid composed of 2.7% sorbitol and 0.54% mannitol.[7] Results showed that for dilution levels of up to 30% volume irrigation fluid most TEM results were within the reference range, whereas at a dilution of 40% most were not within the reference range.[7] Both studies were also consistent in showing a dilution-depended trend towards hypocoagulobilty even in dilution levels with normal TEM results. A different study exposed whole blood from five healthy volunteers to cold temperature, acidification, and dilution with crystalloid fluids simulating resuscitation induced coagulopathy and found that all three conditions impaired the TEM analysis with hypothermia inducing the greatest impairment.[8]

While the trend towards more hypocoagulability as effect of *in vitro* dilution of whole blood is demonstrated in more studies reporting the results of changes in hemostasis,[7-8] few have reported normal range results.[7] The current study is unique in creating dilution only with citrate buffer, the same diluent as is found the sample collection tubes used clinically, and so reproducing a condition with under filled tubes.

Conclusions

Using samples from healthy blood donors, it was demonstrated that samples diluted to a whole blood:anticoagulant citrate buffer ratio as low as 80:20, corresponding to a half-filled test tube, produced TEM results that were within the reference range of each parameter. Further dilution of the whole blood rendered the samples unsuitable for TEM testing. It was also demonstrated that the TEM results were within the reference range for each parameter in samples that had undergone prolonged cold temperature storage. Based on these data, the hospital policy will change to permit the acceptance of half-filled samples

for TEM testing, but samples received in the laboratory more than 10 minutes after sample collection will not be accepted in order to ensure relevant results are provided to those managing bleeding patients.

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Figure legends

Figure 1. Number of blood donors by ABO group and gender.

Figure 2. INTEM from sample with 80:20 blood:citrate buffer ratio. Results for CT (1) and CFT (2) are measured in seconds (x-axis), for MCF (3) in millimeters (y-axis) and the α-angle as shown (4).

Figure 1

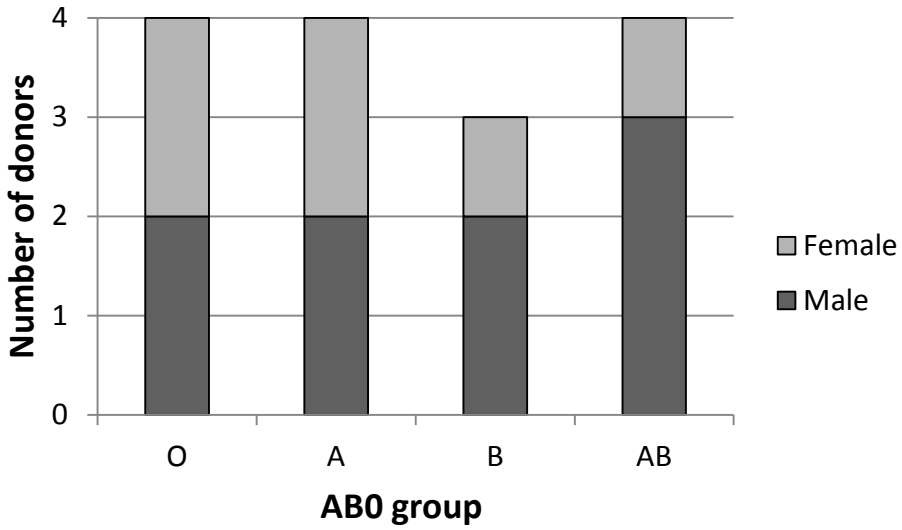

Figure 2

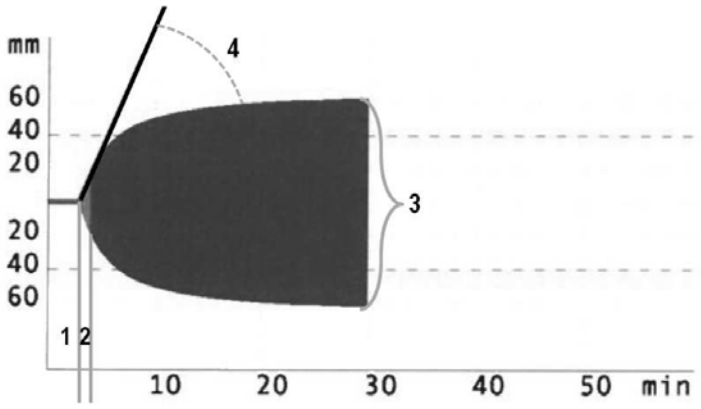

Table 1

a) Test tube #	b) Blood sample volume pipetted to test tube (volume of whole blood/volume of buffer mL)	c) Buffer pipetted to test tube as diluent	d) Blood:buffer ratio	e) Simulated blood sample size in 3 mL tubes
I	3.00 mL (2.70/0.30 mL)	0.00 mL	90:10	3.00 mL
II	2.67 mL (2.40/0.27 mL)	0.33 mL	80:20	1.50 mL
III	2.33 mL (2.10/0.23 mL)	0.67 mL	70:30	1.00 mL
IV	2.00 mL (1.80/0.20 mL)	1.00 mL	60:40	0.75 mL
V	1.67 mL(1.50/0.17 mL)	1.33 mL	50:50	0.60 mL
VI	1.33 mL (1.20/0.13 mL)	1.67 mL	40:60	0.50 mL

Table 1. Test tube filling: For each ABO group six test tubes (a) were filled equal to different blood:buffer ratios (d) by adding specified volumes from the pooled blood samples collected in 10 percent volume citrate buffer (b) and of supplemental citrate buffer (c).
As blood samples were collected in completely filled 3 mL tubes containing 10 percent volume pre-added Na_3Citrate buffer, the blood sample volume pipetted into test tubes had a blood and buffer content as listed (b).

Table 2

			Dilution study				Prolonged cold store		Reference range
No. samples			4	4		4	4		
Whole blood: citrate ratio			90:10	80:20		70:30	90:10		
Storage temp			RT	RT		RT	4˚C		
ROTEM channel /parameter					**Δ% 80:20 vs. 90:10**		**Δ% 70:30 vs. 90:10**	**Δ% 4˚C vs. RT**	
	EXTEM	CT	61 (55-66)	64 (58-85)	5.7	968 (429-1,275)	1,391	62 (59-68)	3.8	38-79
		CFT	68 (66-86)	95 (73-99)	25	849	1,186	99 (89-112)	36	34-159
		α	76 (72-76)	71 (70-75)	-4.0	19	-75	73 (68-73)	-4.6	63-83
		MCF	65 (60-66)	62 (61-65)	-3.0	17 (4-30)	-74	62 (58-63)	-3.9	50-72
	INTEM	CT	175 (164-182)	191 (174-227)	6.4	1,315 (927-1,527)	649	174 (163-190)	-0.6	100-240
		CFT	66 (59-70)	71 (63-93)	12	427 (358-496)	558	80 (78-89)	25	30-110
		α	77 (76-78)	76 (71-77)	-1.9	35 (32-38)	-55	74 (72-75)	-3.9	70-83
		MCF	61 (57-63)	62 (59-65)	2.4	24 (7-42)	-58	59 (57-61)	-2.4	50-72
	FIBTEM	CT	57 (50-60)	59 (53-106)	8,0	1,795 (1,745-1,795)	3,053	59 (50-64)	-0.1	38-62
		α	68 (62-72)	65 (63-67)	-3.0	-	-	69 (58-62)	-1.1	-

	MCF	13 (11-16)	13 (9-13)	-9.1	-	-	13 (10-15)	-3.1	9-25

Table 2. ROTEM results for 90:10, 80:20 and 70:30 whole blood:citrate ratios and for 90:10 ratio cold stored samples. Data are given as median (range), except for Δ% data, where only median is reported. For 70:30 ratio data, n = 4 only for CT, for other parameters n = 1-3. If none of the four pools tested produced a measureable result this is listed as "–". RT= room temperature.