Effect of some preanalytical variables on some screening tests of coagulation: a single center experience

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Background
Preanalytical circumstances are significant in laboratory assessment. Prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen (Fbg), measurements are basic coagulation tests used to evaluate variations of coagulation systems.

Aim
Investigate to what extent the storage temperature and time influence the results of routine coagulation tests (PT, APTT, and Fbg). It included a total of 120 participants were assayed for different storage temperatures.

Patients and methods
The platelet poor plasma was assayed for baseline values for PT, APTT, and Fbg on Sysmex 1500 apparatus, and then it was assayed for different storage temperature and time.

Results
The PT results showed no significant difference when compared with the baseline when samples were kept at 4°C for 12 h storage and at −20°C for 12 h. The APTT results showed significant difference when compared with the baseline at all temperatures. The Fbg results showed no significant difference when compared with the baseline when the sample were kept at 4 and −20°C for 24 h.

Conclusion
It is not recommended that the PT samples in normal persons be stored at room temperature but can be stored at 4 or −20°C for 12 h. APTT samples in normal persons cannot be stored up to 12 h at any temperature. Fbg samples in normal persons cannot be stored up to 24 h at any temperature. So estimation of APTT and Fbg must be done as early as possible.

Keywords:
activated partial thromboplastin time, fibrinogen, hemostasis, preanalytical variables, prothrombin time

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Introduction

Coagulation tests and factor measurements have been widely applied in clinical practice. Preanalytical conditions are very important in the laboratory assessment of hemostatic and coagulation systems [1].

Preanalytical variables including specimen collection, anticoagulant type and concentration, filling status of the sampling tube, transportation, centrifugation, as well as storage and assay method can all affect coagulation test and factor analysis results [2].

Activated partial thromboplastin time (APTT), fibrinogen (Fbg), prothrombin time (PT), and thrombin time measurements are routine coagulation tests used to assess pathological alterations of hemostatic and coagulation systems to guide clinical therapy [1].

In addition, the PT and international normalized ratio are used to monitor oral anticoagulant therapy for reducing the risk of thromboembolic events and minimizing the incidence of bleeding complications [3].

The Clinical and Laboratory Standards Institute (CLSI) H21-A5 [4] recommended that specimens should be analyzed within 24 h for PT and 4 h for APTT and other assays if stored at room temperature (RT) (25°C). However, they have not recommended a storage time for refrigerated storage (2–8°C) [1].

Effects of storage as regards duration and temperature were studied by some authors [5–8]. Zhào *et al.* [6] and Yao *et al.* [5] found that separated plasma can be stored for 24 h at RT and at 4°C without affection of the results of PT, thrombin time, and Fbg. The same authors found that the result of APTT can be acceptable up to 8 h.

Alesci *et al.* [7] found that freezing at −20°C affects the PT and APTT, while Foshat *et al.* [8] found that

storage of plasma sample at −20°C causes no change in PT and APTT for up to 2 weeks.

The large number of specimens received can lead to a delay in sample testing in the clinical laboratory, so we need to establish the acceptable storage temperature and time procedures.

Aim

Investigate to what extent storage temperature and time influence the results of routine coagulation tests (PT, APTT, and Fbg) and whether any changes caused by delayed analysis result in a clinically relevant difference.

Patients and methods

Study area

This study was carried out between January 2017 and January 2018 in the Thrombosis and Hemostasis Laboratory of Clinical Pathology Department, Assiut University Hospital.

Ethical approval

This study was carried out after an ethics committee of faculty of medicine approval. The patients were recruited into the study after giving informed consent for using the test result to be included in this study and using the residual part of the sample for further testing.

Study population

The study included 120 persons who are apparently healthy with no history of bleeding tendency and those not receiving any medication (especially Marevan (GlaxoSmithKline NZ Limited, Downtown, Auckland, New Zealand), heparin) and free from chronic liver diseases, chronic kidney disease, disseminated intravascular coagulation, or congenital coagulation factor deficiencies.

A total number of 120 participants were divided as follows:

For determination of prothrombin time

Group I included 40 apparently normal healthy persons (not receiving any medication).

For determination of activated partial thromboplastin time

Group II included 40 apparently normal healthy persons (not receiving any medication).

For determination of fibrinogen

Group III included 40 apparently normal healthy persons (not receiving any medication).

Sample collection and storage

Blood sample was delivered into tri sodium citrate BD tubes (3.2%) at a ratio of 1: 9 (anticoagulant: blood). The centrifugation process was carried out within 2 h of blood sample collection. Centrifugation was done for 10 min at 4000 rpm and between 18 and 25°C. Platelet poor plasma was assayed for baseline values for PT, APTT, and Fbg on Sysmex 1500 apparatus (Siemens Healthcare Diagnostics inc, Erlangen, Germany), and then it was divided into three aliquot groups:

The first one was processed at RT (18–25°C) which were verified by a data logger, the second at 4°C and the third one at −20°C; all groups were processed as follows:

(1) PT after 12 and 24 h storage for those which were kept at RT, 4 and −20°C and 1 week storage duration for those which was kept at 4 and −20°C.
(2) APTT after 12 h storage duration.
(3) Fbg after 24 h storage for those which were kept at RT, 4 and −20°C and 1 week storage duration for those which were kept at 4 and −20°C.

Samples after 12 h storage were processed at an emergency laboratory on the same model of Sysmex 1500 apparatus with the same methodology and adjusted by comparability sample and quality control system.

Statistical analysis

Data were analyzed using SPSS version 20 (SPSS version 20: IBM Corporation, Armonk, New York, U.S). Data were represented as mean, SD, median and range. The results following storage for 12 and 24 h and 1 week were compared with the baseline results by using paired *t*-tests as the data was normally distributed. *P* value was considered significant if it was less than 0.05, moderately significant if less than 0.01, and highly significant if less than 0.001.

To assess the stability of the coagulation tests and Fbg, the percentage changes compared with the baseline results were calculated as [(result at storage time X − result at the baseline)/result at the baseline].

If the number of individuals with a greater than 10% change was less than 25% of the total sample number, the effect of the given preanalytical variable was termed moderate, whereas if more than 25% of the samples had a greater than 10% change' the effect was deemed large [5].

Graphs were produced by using Microsoft Excel version 2010 (Excel version 2010: Microsoft, One Microsoft Way, Redmond, Washington, U.S).

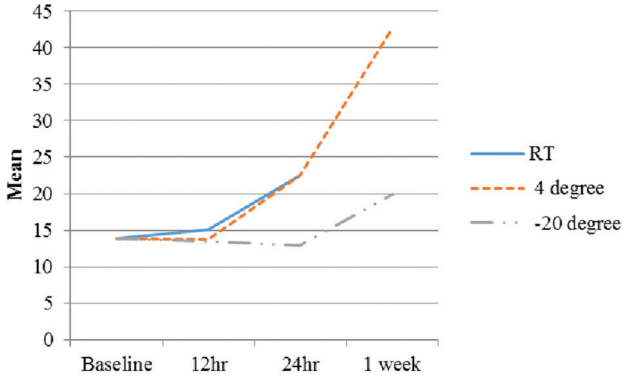
Results

Results of prothrombin time determination

Group I (Table 1 and Fig. 1)

(1) The baseline sample results of PT determination of group I ranged from 11.9 to 15.5 s with mean ± SD equal to 13.8 ± 0.82 s.
(2) PT results of the group I after 12 h storage:
   (a) At RT, it ranged from 11.1 to 25.6 s with mean ± SD equal to 15.07 ± 2.69 s.
   (b) At 4°C, it ranged from 9.2 to 18.8 s with mean ± SD equal 13.7 ± 2.35 s.
   (c) At −20°C, it ranged from 10.3 to 17.9 s with mean ± SD equal to 13.46 ± 1.69 s.
(3) PT results from the group I after 24 h storage:
   (a) At RT, it ranged from 11.6 to 83.4 s with mean ± SD equal to 22.49 ± 13.69 s.
   (b) At 4°C, it ranged from 10.4 to 22.3 s with mean ± SD equal to 22.49 ± 13.69 s.
   (c) At −20°C, it ranged from 10.3 to 17.1 s with mean ± SD equal to 12.95 ± 1.63 s.
(4) PT samples from group I after 1 week storage:
   (a) At 4°C, it ranged from 13 to 94.8 s with mean ± SD equal to 42.77 ± 75.99 s.
   (b) At −20°C, it ranged from 10.2 to 67.8 s with mean ± SD equal to 19.99 ± 15.39 s.

Figure 1



Prothrombin time measurements over time of the studied population in group I.

The previous results showed no significant difference (when compared with the baseline) when samples were kept at 4°C for 12 h storage ($P = 0.8$) and at −20°C for 12 h ($P = 0.256$). This means the samples can be kept without changes at 4 and −20°C for 12 h.

PT samples in the normal group I showing more than 10% change (Table 2).

(1) After 12 h:
   (a) At RT there were 15 cases out of 40 representing 37.5%
   (b) At 4°C there were 10 cases out of 40 representing 25%.
   (c) At −20°C there were five cases out of 40 representing 12.5%.
(2) After 24 h:
   (a) At RT there were 29 cases out of 40 representing 72.5%.
   (b) At 4°C there were 12 cases out of 40 representing 30%.
   (c) At −20°C there were four cases out of 40 representing 10%.
(3) After 1 week:
   (a) At 4°C there were 38 cases out of 40 representing 90%.
   (b) At −20°C there were 15 cases out of 40 representing 37.5%.

The percentage of change of more than 10% was less than 25% of the total number of samples is a clinically relevant change occurring at −20°C after 12 and 24 h.

Table 1 Prothrombin time measurements over time of the studied population in normal persons (group I)

Normal I	Prothrombin time (s)					
	RT	$P^{\#}$	4°C	$P^{\#}$	−20°C	$P^{\#}$
Baseline						
Mean±SD	13.8±0.82	-	13.8±0.82	-	13.8±0.82	-
Median (range)	13.8 (11.9-15.5)		13.8 (11.9-15.5)		13.8 (11.915.5)	
After 12 h storage						
Mean±SD	15.07±2.69	0.001**	13.7±2.35	0.800	13.46±1.69	0.256
Median (range)	14.25 (11.1-25.6)		13.6 (9.2-18.8)		13.4 (10.3-17.9)	
After 24 h storage						
Mean±SD	22.49±13.69	<0.001**	22.49±13.69	<0.001**	12.95±1.63	0.004*
Median (range)	18.85 (11.6-83.4)		14.05 (10.4-22.3)		12.95 (10.3-17.1)	
After 1 week storage						
Mean±SD	ND	-	42.77±75.99	0.018*	19.99±15.39	0.013*
Median (range)			27.2 (13-94.8)		13.9 (10.2-67.8)	

ND, not done; RT, room temperature. $^{\#}P$ value by using the paired *t*-test. *Moderate significance. **Highly significance.

Results of activated partial thromboplastin time

Group II (Table 3 and Fig. 2)

(1) The immediate results (baseline) of APTT of group II ranged from 23.3 to 40.2 s with mean ± SD equal to 32.48 ± 4.95 s.
(2) APTT results of the group II after 12 h storage:
   (a) At RT, it ranged from 27.5 to 162.8 s with mean ± SD equal to 45.4 ± 24.69 s.
   (b) At 4°C, it ranged from 14.1 to 52.4 s with mean ± SD equal to 36.37 ± 6.67 s.
   (c) At −20°C, it ranged from 26.3 to 46 s with mean ± SD equal to 36.02 ± 5.11 s.

The previous results showed a significant difference (when compared with baseline). This means the samples should be measured within 2 h from sample taking.

APTT samples in group II (40 normal persons) showing more than 10% change (Table 4).

(1) At RT there were 34 cases out of 40 representing 85%.
(2) At 4°C there were 29 cases out of 40 representing 72.5%.
(3) At −20°C there were 23 cases out of 40 representing 57.5%.

The percentage of change of more than 10% was less than 25% of the total number of samples which is a clinically irrelevant change occurring at all groups at all temperatures (Table 4).

Results of fibrinogen

Group III (Table 5 and Fig. 3)

The immediate results (baseline) of Fbg of group III ranged from 2 to 4 g/l with mean ± SD equal to 2.55 ± 0.77 g/l.

Table 2 Percentage of change in prothrombin time measurement over time in group I (40 normal persons)

Normal I	Samples with >10% change [*n* (%)]		
	RT	4°C	−20°C
After 12 h storage	15 (37.5)	10 (25)	5 (12.5)
After 24 h storage	29 (72.5)	12 (30)	4 (10)
After 1 week storage	ND	38 (90)	15 (37.5)

ND, not done; RT, room temperature.

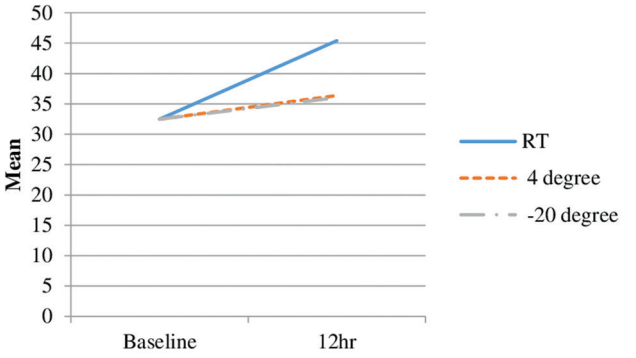
(1) Fbg results from group III after 24 h storage:
   (a) At RT, it ranged from 0.1 to 4 g/l with mean ± SD equal to 1.89 ± 0.89 g/l.
   (b) At 4°C, it ranged from 1.4 to 4.5 g/l with mean ± SD equal to 2.31 ± 0.69 g/l.
   (c) At −20°C, it ranged from 0.1 to 4 g/l with mean ± SD equal to 2.21 ± 0.78 g/l.
(2) Fbg results from group III after 1 week storage:
   (a) At 4°C, it ranged from 0.1 to 3.7 g/l with mean ± SD equal to 1.89 ± 0.91 g/l.
   (b) At −20°C, it ranged from 0.9 to 5.7 g/l with mean ± SD equal to 2.12 ± 0.84 g/l.

The previous results showed no significant difference (when compared with the baseline) when the samples were kept at 4°C for 24 h storage (P = 0.146) and at −20°C for 24 h (P = 0.053). This means the samples can be kept without change at 4 and −20°C for 24 h.

Fbg samples in normal group III showing more than 10% change (Table 6).

(1) After 24 h:
   (a) At RT there were 23 cases out of 40 representing 57.5%.
   (b) At 4°C there were 17 cases out of 40 representing 45.5%.
   (c) At −20°C there were 18 cases out of 40 representing 45%.

Figure 2



Partial thromboplastin time measurements over time of the studied population in group II.

Table 3 Activated partial thromboplastin time measurements over time of the studied population in the group II

Normal II	Activated partial thromboplastin time (s)					
	RT	$P^{\#}$	4°C	$P^{\#}$	−20°C	$P^{\#}$
Baseline						
Mean±SD	32.48±4.95	-	32.48±4.95	-	32.48±4.95	-
Median (range)	33.2 (23.3-40.2)		33.2 (23.3-40.2)		33.2 (23.3-40.2)	
After 12 h storage						
Mean±SD	45.4±24.69	0.002**	36.37±6.67	0.004**	36.02±5.11	0.002**
Median (range)	39.4 (27.5-162.8)		37.3 (14.1-52.4)		36.5 (26.3-46)	

RT, room temperature. $^{\#}P$ value by using paired *t*-test. **Highly significance.

(2) After 1 week:
   (a) At 4°C there were 20 cases out of 40 representing 50%.
   (b) At −20°C there were 22 cases out of 40 representing 55%.

The percentage of change of more than 10% was less than 25% of the total number of samples which is a clinically irrelevant change occurring at all temperatures.

Discussion

Detailed guidelines for correct labeling, patient positioning, phlebotomy technique, volume collection, tube mixing, inspection for hemolysis or clotting, and order of blood draws have been developed to ensure that the preanalytical factors do not compromise samples prior to processing [9].

Van Geest-Daalderop *et al.* [10] proposed that if the number of individuals with a greater than 10% change was less than 25% of the total sample number, the effect should be termed moderate and clinically relevant. However, Feng *et al.* [1] suggested that the imprecision may have a greater impact on the results than the changes in stability studies.

This study evaluated the preanalytical variables of temperature and duration of storage on the stability and validity of assay results for PT, APTT, and Fbg.

In this study, the PT of normal participant samples cannot be kept at RT without deterioration. This finding disagree with Wang *et al.* [11] and Saghir *et al.* [12] who found that estimation of the PT can be delayed for 4 h at RT. Also this result disagrees with Zhao *et al.* [6], Kemkes-Matthes *et al.* [13], and Rao *et al.* [14] who declare that PT can be estimated without change after 24 h at RT.

However, the PT samples of normal person can be kept at 4°C for up to 12 h without deterioration. This finding slightly agree with Wang *et al.* [11] and van Geest-Daalderop *et al.* [10] who found that PT samples can be stored for up to 6 h, as cooling prevent the samples from deteriotion which cope with cold activation. However, Rao *et al.* [14] and Zhao *et al.* [6] found that estimation of the PT can be delayed for 24 h at 4°C.

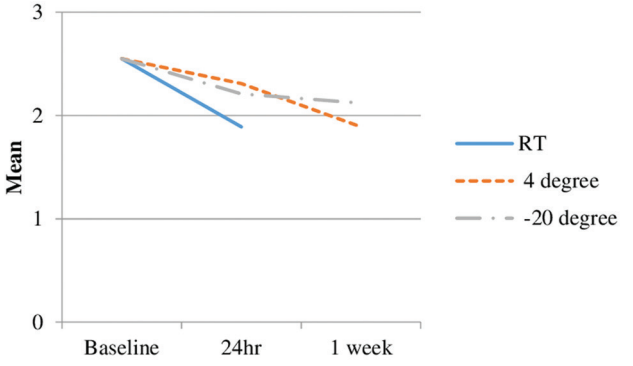
About freezing, the samples of PT of normal participants were kept at −20°C without deterioration and gave the result of PT without significant change for 12 h. However, Woodhams *et al.* [15] concluded that PT (allowing for 10% variation) in normal citrated plasma samples were stable for up to 3 months if frozen at −24°C or below, and stable for at least 18 months if frozen at −74°C. They found significant prolongation of PT during storage for up to 24 months. Additionally, they found that the freezing process (freezing at −74°C

Table 4 Percentage of change in activated partial thromboplastin time measurement over time in the studied population

APTT after 12 h storage	Samples with>10% change [*n* (%)]		
	RT	4°C	−20°C
Normal II (*n*=40)	34 (85)	29 (72.5)	23 (57.5)

APTT, activated partial thromboplastin time; RT, room temperature.

Figure 3



Fibrinogen measurements over time of the studied population in group III.

Table 5 Fibrinogen measurements over time of the studied population in the group III

Normal III	Fibrinogen (g/l)					
	RT	*P*#	4°C	*P*#	−20°C	*P*#
Baseline						
Mean±SD	2.55±0.77		2.55±0.77		2.55±0.77	
Median (range)	2 (2-4)		2 (2-4)		2 (2-4)	
After 24 h storage						
Mean±SD	1.89±0.89	<0.001**	2.31±0.69	0.146	2.21±0.78	0.053
Median (range)	1.8 (0.1-4)		2 (1.4-4.5)		2.05 (0.1-4)	
After 1 week storage						
Mean±SD	ND	-	1.89±0.91	<0.001**	2.12±0.84	0.019*
Median (range)			2 (0.1-3.7)		2 (0.9-5.7)	

ND, not done; RT, room temperature. #*P* value by using paired *t*-test. *Moderate significance. **Highly significance.

Table 6 Percentage of change in fibrinogen measurement over time in studied normal population III (40 normal persons)

Fibrinogen normal III	Samples with >10% change [*n* (%)]		
	RT	4°C	−20°C
After 24 h storage	23 (57.5)	17 (45.5)	18 (45)
After 1 week storage	ND	20 (50)	22 (55)

ND, not done; RT, room temperature.

and storage at −24°C vs. freezing at −24°C and storage at −24°C) was not responsible for the changes in stability. But Alesci *et al.* [7] found that freezing and storage strongly influenced PT and showed that the changes in PT were smaller in samples stored at −70°C compared with −20°C after 1, 2, 3, and 4 months of storage. The CLSI guidelines indicate that storage at −20°C is acceptable for samples processed within 2 weeks given that other collection and temperature monitoring standards are followed.

In this study, APTT of the normal participant samples cannot be kept at RT, 4 and −20°C after 12 h storage. This means the samples should be measured within 2 h from the sample taking. This finding agrees with Saghir *et al.* [12] as they recommended measuring within 2 h from the sample taking. But Oddoze *et al.* [16] declare that APTT can be estimated without change after 6 h at RT and 4°C. However, Rao *et al.* [14] found that the estimation of APTT can be delayed for 12 h at RT and 4°C.

About freezing, Woodhams *et al.* [15] concluded that APTT (allowing for 10% variation) in normal citrated plasma samples were stable for up to 3 months if frozen at −24°C or below, and stable for at least 18 months if frozen at −74°C. They found significant prolongation of APTT. Additionally, they found that the freezing process (freezing at −74°C and storage at −24°C vs. freezing at −24°C and storage at −24°C) was not responsible for changes in stability. But Alesci *et al.* [7] found that freezing and storage strongly influenced APTT assays and showed that the changes in APTT were smaller in samples stored at −70°C compared with −20°C after 1, 2, 3, and 4 months of storage.

CLSI H21-A5 has recommended that specimens can be analyzed for PT within 24 h if stored at RT. However, this study results are against this recommendation due to having small sample size and the differences may be due to several factors, which can affect the stability of coagulation factors such as the automated machines and different type of reagents [12].

In this study, Fbg of normal participant samples cannot be kept at RT without significant change. This finding agrees with Toulon *et al.* [17] who declare that Fbg results obtained after 4 and 6 h storage were significantly different from those obtained after less than 2 h storage, whereas all other comparisons failed to demonstrate any significant difference. However, this result disagrees with Kemkes-Matthes *et al.* [13] who have reported that Fbg can be reliably tested after storage for 8 h at RT, and Feng *et al.* [1] demonstrated that plasma samples tested for Fbg determination could be safely stored for up to 24 h at 25°C.

Although there was clinically relevant change (in 45.5% of samples), yet Fbg samples can be kept at 4°C for up to 24 h without significant change. This agrees with Zhao *et al.* [6] and Feng *et al.* [1] who found that the estimation of Fbg can be delayed for up to 24 h at 4°C. This result is against Piccione *et al.* [18] who found that the Fbg concentration decreased 8 and 24 h after storage at 8°C. The decrease induced by the storage was minimal but statistically significant.

About freezing, the samples were kept at −20°C without deterioration and gave result of Fbg without significant change for 24 h although there was clinically relevant change (in 45% of samples). This disagrees with Woodhams *et al.* [15] who concluded that Fbg (allowing for 10% variation) in normal citrated plasma samples were stable for up to 3 months if frozen at −24°C or below, and stable for at least 18 months if frozen at −74°C. They found no relevant changes of Fbg during storage for up to 24 months. Additionally, they found that the freezing process (freezing at −74°C and storage at −24°C vs. freezing at −24°C and storage at −24°C) was not responsible for the changes in stability. Alesci *et al.* [7] found that freezing and storage weakly influenced Fbg assays and showed that the changes in Fbg were smaller in samples stored at −70°C compared with −20°C after 1, 2, 3, and 4 months of storage.

Piccione *et al.* [18] demonstrated that this variation could be due to conformational changes of Fbg triggered by refrigeration resulting in an altered precipitation tendency. The final turbidity of a fibrin clot generated from previously refrigerated Fbg appears to be greater than the turbidity of a fibrin clot formed from fresh plasma.

Conclusion

The optimum time for storage the PT samples in normal persons (not receiving any medication) at both 4 and −20°C is within 12 h and it is not recommended to be stored at RT. APTT samples in normal persons (not receiving any medication) cannot be stored for up to 12 h at any temperature. Fbg samples in normal persons (not receiving any medication) cannot be stored for up to 24 h at any temperature. So

estimation of APTT and Fbg must be done as early as possible.

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Conflicts of interest
There are no conflicts of interest.

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